Human temperature regulation during exercise in the heat: effects of the menstrual cycle and ambient thermal profile

Tze-Huan Lei BSpEx, MPhEd

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School of Sport, Exercise and Nutrition
Massey University
Palmerston North
New Zealand

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ABSTRACT

Behavioural thermoregulation is the most effective means with which we regulate our body temperature at rest and during exercise. Yet, research into behavioural thermoregulation during exercise is still at an emergent stage, as it has not included females, or investigated different thermal profiles. In particular, limited studies are available to describe the behavioural and physiological differences between dry and humid heat for both sexes. Furthermore, it remains unknown whether ambient humidity or temperature alone contribute to the initiation of the behavioural responses during exercise in the heat. Therefore, the first part of this thesis investigated the effects of endogenous and exogenous female ovarian hormones on behavioural and autonomic responses, in both dry and humid heat environments matched according to the heat stress index, WBGT (**Chapter Five** and **Six**). The results from **Chapter Five** clearly show that behavioural and autonomic responses were less affected by menstrual phase, but were affected by the environmental conditions. In particular, trained women reduced their power output in order to nullify the autonomic strain from a humid heat environment. **Chapter Six** then extended this observation to (trained) women taking combined hormonal contraception, compared to eumenorrheic women in **Chapter Five**. The results from **Chapter Six** indicate that greater autonomic strain was observed in women with hormonal contraception, compared to eumenorrheic women, in both dry and humid heat, whilst the behavioural response was similar between those two groups. Furthermore, the behavioural response was different between dry and humid heat, with power output being lower in the humid heat environment compared to dry heat. The second part of this thesis investigated the effects of ambient temperature *per se* on the interaction of thermoregulatory, cardiovascular and perceptual responses to exercise (**Chapter Seven**), as well as assessing different exercise modalities (variable-intensity versus fixed-intensity exercise) and their effects on thermoregulation when the duration and average power output were matched (**Chapter Eight**). The results from **Chapter Seven** indicate that thermoregulatory and cardiovascular responses were not affected by ambient temperature but that perception was, when vapour pressure was matched between two different thermal profiles. The results from **Chapter Eight** indicate that self-pacing (behaviour) did not modulate thermoregulatory strain, when both self-paced and fixed-intensity were matched at the same exercise intensity and duration. In conclusion, this thesis extends the knowledge-base on behavioural

thermoregulation in trained women and also provides evidence that behavioural and autonomic thermoregulation is influenced more by vapour pressure than ambient temperature of the environment in men. Furthermore, the findings of this thesis confirm that behavioural thermoregulation is effective in modulating physiological strain only when there is a reduction in metabolic heat production.

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

A

ANOVA	Analysis of variance
A-VCO_2	Arteriovenous carbon dioxide content difference
AVP	Arginine vasopressin
Ach	Acetylcholine
ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
au	arbitrary unit

B

BSA	Body surface area
BMI	Body mass index
BP	Blood pressure

C

$\dot{C}$	Convective heat lost
°C	Degree centigrade
$\dot{C}_{res}$	Respiratory conductive heat lost
CO_2	Carbon dioxide
CNS	Central nervous system
Ca^{2+}	Calcium
Cl^{-1}	Chloride
CVR	Cutaneous vascular resistance
CVC	Cutaneous vascular conductance

D

DBP	Diastolic blood pressure
DRY	Dry heat

E

$\dot{E}$	Evaporative heat lost
E_{max}	Maximal evaporative cooling capacity of the environment
E_{req}	Required evaporative cooling for heat balance

EF	Early follicular
eNOS	endothelial nitric oxide synthase
F	
FSH	Follicle stimulating hormone
FBF	Forearm blood flow
FVR	Forearm vascular resistance
G	
g	Gram
GnRH	Gonadotropin–releasing hormone
H	
h	Hour
h_c	Convective heat transfer
HR	Heart rate
HSI	Heat strain index
HUM	Humid heat
HSP	Heat shock protein
K	
Kg	Kilogram
K^+	Potassium
KJ	Kilojoule
Kpa	Kilopascal
L	
L	Litre
LF	Linear factor
LR	Lewis relation
LSR	Local sweat rate
M	
m	Metre
$\dot{M}$	Metabolic heat production
MAP	Mean arterial pressure
Min	Minute
ML	Mid luteal phase

mmHg	Millimeters of mercury
mmol	Millimole
N	
Na^+	Sodium
$nmoll^{-1}$	Nanomole per litre
NO	Nitric oxide
NS	Nervous system
O	
O_2	Oxygen
OCP	Oral contraception pill
OP	Oral contraception
P	
P_A	Ambient water vapour pressure
P_{Sk}	Saturated water vapour pressure at the skin
$P_{ET}CO_2$	Partial pressure of end- tidal CO_2
PL	Pleasant
PO	Power output
Q	
$\dot{Q}$	Cardiac output
*q*F	Quasi-follicular
*q*L	Quasi-luteal
R	
$\dot{R}$	Rate of heat exchange via radiation
RER	Respiratory exchange ratio
RPE	Rate of perceived exertion
rpm	Revolutions per minute
S	
S	Second
$\dot{S}$	Heat Storage
SD	Standard deviation
SBP	Systolic blood pressure
SEE	Standard error of mean
SKBF	Skin blood flow
T	

T_A	Ambient temperature
$\bar{T}_b$	Mean body temperature
TAN	Total adenine nucleotide pool
T_{core}	Core temperature
TD	Thermal discomfort
T_{rec}	Rectal temperature
TS	Thermal sensation
$\bar{T}_{sk}$	Mean skin temperature
TRP	Transient receptor potential
V	
v	Air velocity
VOP	Venous occlusion plethysmography
$\dot{V}CO_2$	Rate of carbon dioxide elimination
$\dot{V}O_2$	Rate of oxygen uptake
$\dot{V}O_2max$	Maximal oxygen uptake
W	
W	Watt
WBGT	Wet-bulb globe temperature
$\dot{W}$	Rate of heat exchange from external work
Y	
Y	Year

LIST OF PUBLICATIONS

Chapter Five

Lei TH, Stannard SR, Perry BG, Schlader ZJ, Cotter JD, Toby Mündel (2017). Influence of menstrual phase and arid *vs.* humid heat stress on autonomic and behavioural thermoregulation during exercise in trained but unacclimated women. J Physiol 595.9(2017): 2823-2837.

Chapter Five

Lei TH & Mündel T (2018). Humid heat stress affects trained female athletes more than does their menstrual phase. Temperature, DOI:10.1080/23328940.2018.1436394.

Chapter Six

Lei TH, Cotter JD, Schlader ZJ, Stannard SR, Perry BG, Barnes MJ , Mündel T (in revision). On exercise thermoregulation in females: interaction of endogenous and exogenous ovarian hormones. J Physiol.

LIST OF FIGURES

LIST OF TABLES

Chapter One

1.0: Introduction

Heat waves during warmer periods are more intense and frequent than before, with no countries around the world immune (Meehl and Tebaldi, 2004). This has resulted in greater mortality rates across the globe in different thermal profiles, from hot-tropical all the way to hot-dry environments (Huang *et al.*, 2010, Conti *et al.*, 2005, Garssen *et al.*, 2005). This effect is unprecedented, and therefore an understanding of how the human body responds to different types of heat stress has now become an important issue for many health practitioners across the globe. In particular, this issue is extremely important for endurance athletes, as many of the famous endurance events are usually held during the warmer months in either tropical or hot dry environments, where both male and female athletes can experience severe heat stress.

Severe heat stress not only reduces exercise performance (Galloway and Maughan, 1997) but, most importantly, it can be a life-threatening situation if athletes do not know how to protect themselves from this situation. Clinical studies reveal that performing prolonged endurance exercise in the heat elevates physiological strain, due in part to the rise of core temperature and heat stroke is more likely when core temperature exceeds the 40°C threshold. If this situation persists, it then causes multiple organ failure (Capacchione and Muldoon, 2009), due to the induction of a systemic inflammatory response syndrome (Leon and Helwig, 2010), which then increases the chance of mortality, if no immediate medical attention is given. Therefore, to prevent the occurrence of this pathological condition, it is necessary to constrain core temperature. Core temperature can be modified by means of physiological and behavioural modifications. The former has a limited capacity to regulate body temperature, whereas the latter has an unlimited capacity (Schlader *et al.*, 2011d, Parsons, 2014). Physiological modification includes vasodilation and an increase in sweating, in order to promote greater convective and evaporative heat loss. The behavioural response during exercise refers to reducing metabolic heat production by reducing exercise intensity to maintain thermal comfort and core temperature (Flouris and Schlader, 2015). This is regarded as self-pacing. A successful self-pacing strategy is not only crucial to constrain core temperature, it may also correspond to success in competition. Due to this reason, self-pacing

in the heat has been one of the major foci of research in recent years.

However, the research on self-pacing in heat is still at an early stage, as current studies have not addressed the following questions: 1) Is the behavioural response different across the natural menstrual or across oral contraceptive cycles, and in different thermal profiles?; and, 2) Is the behavioural response in the heat driven solely by ambient temperature or does ambient humidity play a role, and to what extent can different exercise modalities (fixed- vs. variable-intensity) modify this response? These questions are important to both male and female athletes and their support personnel as they will be able to further optimise their performance without jeopardising their health in differing heat environments.

This is particularly true when dealing with humid heat stress, as engaging in endurance exercise in this environment may induce greater physiological strain compared to dry heat, due to the restriction of evaporative cooling (Che Muhamed *et al.*, 2016a). However, less empirical studies are available to describe the physiological differences between dry and humid heat and few that have investigated whether athletes could utilise a self-pacing strategy to reduce thermoregulatory strain in such a stressful environment. Furthermore, few studies have compared self-pacing and physiological differences between dry and humid heat in female athletes, as it is methodologically difficult to partition their menstrual phases or oral contraceptive (OCP) pill usage.

But this is important not least because future endurance events are going to be held in thermally-stressful environments differing in their profiles, and with an ever-increasing number of female participants, many of whom will be using OCPs.

Whether the reduction in (self-paced) exercise intensity in the heat is driven solely by ambient temperature, remains questionable. This is given by the fact that current consensus articles are based on findings from previous studies (Périard *et al.*, 2011, Ely *et al.*, 2010), where two extreme environments were utilised (hot vs. cold) with simultaneously different temperature and humidity levels for their performance trials. As a consequence, it is difficult to partition whether it is temperature or humidity that causes the reduced exercise performance.

From the observation above, it can be concluded that there is a dearth of literature on behavioural thermoregulation for both sexes during exercise in both dry and humid heat. These issues are important for the health, welfare and personal experiences of athletes and the general public. Therefore, to systematically answer these important questions, this thesis

firstly reviews the relevant literature on temperature regulation in both dry and humid heat for both sexes (**Chapter Two**), and from this the aims and hypotheses are derived accordingly and stated **(Chapter Three). Chapter Four** reviews the merits and limitations of current techniques and protocols and provides justification for the specific measures used throughout the experimental chapters. The first two experimental chapters (**Chapters Five** and **Six**) describe how the fluctuations of endogenous and exogenous female reproductive hormones affect both autonomic and behavioural thermoregulatory responses to exercise in dry and humid heat. **Chapter Seven** extends this research to men and establishes the relationship between ambient temperature, thermoregulatory, cardiovascular and perceptual responses. **Chapter Eight** investigates whether self-pacing is an effective method to reduce thermoregulatory strain in warmer and cooler heat environments matched for vapour pressure. **Chapter Nine** provides a general discussion for conclusion, discussion of limitations and a direction for future research.

Chapter Two

2.0: Review of Literature

The purpose of this chapter is to broadly, and succinctly, introduce the current literature that shapes the concepts that will be developed in this thesis. Further detail, especially specific to each study, can be found in the appropriate Introductions to the experimental chapters (**Chapters Five-Eight**).

2.1: Environmental changes and human behaviour

Humans live in a world with changeable weather, yet can survive only within a very narrow range of core temperatures, from ~20-32°C to ~42°C depending on their circumstances. Although our bodies can operate in only a certain range of temperatures, humans have been shown to have strong resilience against adverse environments, such as the summit of Mount Everest, deserts, tropical rainforests and the Arctic. This occurs because we can behaviourally adjust to environments which we perceive as stressful or dangerous, and thereby maintain thermal homeostasis, termed behaviour modification. Typical behavioural responses to extreme environments include the use of clothing, seeking shelter or building shelter, and adjustments to physical activity. These behavioural modifications greatly enhance our survival rates in extreme environments.

Recently, the area of behavioural thermoregulation in the heat has received increased attention. Global warming intensifies the magnitude as well as the frequency of heat waves across the globe (Meehl and Tebaldi, 2004). Heat waves are characterised as periods of prolonged high ambient temperatures, which are accompanied by high humidity levels (Robinson, 2001), and have resulted in many deaths in New Zealand and across the globe (Hales *et al.*, 2000, Tong *et al.*, 2010, Anderson and Bell, 2011, D'Ippoliti *et al.*, 2010). Such abnormally high ambient temperature and humidity during the summer months in the Northern hemisphere have profound health effects on New Zealand athletes, as they have to travel from a cold weather climate to a hot tropical climate. Therefore, knowing how our bodies respond and how performance changes in these environments is valuable for up-coming sporting events, such as the 2020 Tokyo Summer Olympics. Unfortunately, current studies in behavioural thermoregulation are limited in this regard, as they tend to concentrate on dry heat conditions, as well as avoiding female participants, due to difficulties in controlling for the menstrual

cycle and oral contraceptive pill usage. However, this lack of studies and knowledge on dry and humid heat environments may increase the risk of heat illness in athletes such as those from New Zealand. In particular, female athletes may have a greater incidence of heat related illnesses in humid heat as they have lower thermosensitivity than men, due to a lower evaporative cooling capacity (Gagnon and Kenny, 2011).

As an Asian research scientist from New Zealand, I feel I have the obligation and responsibility to provide correct information to New Zealand athletes (both males and females) on how to behave properly to avoid performance decrements and heat illness symptoms, in both dry and humid heat conditions. Therefore, to provide the correct information in this area, this chapter will first provide a basic overview of temperature regulation, including how humans can behaviourally thermoregulate to avoid fatigue in the heat. Thereafter, this chapter will review the physiological and behavioural differences between dry and humid heat for both sexes. In particular, special focus is placed on reviewing the physiological and behavioural differences between exogenous and endogenous female reproductive hormones. Lastly, this chapter summarises the research findings from previous studies and sets out the research questions that were then investigated further in the experimental chapters.

2.2: Human temperature regulation

Human temperature regulation is a complex interaction between the biophysics of heat transfer and our physiological control systems. The biophysics part describes the avenues of heat exchange, from the core to the skin and from the skin to the environment. The physiological systems detect stimuli and initiate feedback control to restore thermal homeostasis. These stimuli include metabolic heat production from the thermic effect of food and muscular contraction. This knowledge is important for potentially identifying physiological differences between dry and humid heat for both sexes. Therefore, this section will first review how heat is generated and released from our body, and how our body can utilise feedback control to prevent us from over-heating.

2.2.1: Laws of thermodynamics

The laws of thermal dynamics provide the basic mechanism on how heat is formed and dissipates into the environment.

2.2.1.1: First law of thermal dynamics

The first law of thermodynamics describes the concept of energy conservation. It can be derived from the following equation:

$$\Delta E = Q - W \quad (1)$$

Where ΔE is the change in internal energy, Q (joules) is heat and W (joules) is the external work done by the system.

As shown by the equation above, internal energy is increased by adding external heat into the system. This explains why the core temperature increases when the body is exposed to hot environments. Furthermore, this law indicates the heat exchange pathways between the body and its environment, at rest and during exercise (Gagge and Gonzalez, 2011). However, this first law does not identify heat and work interactions, and therefore another law is required.

2.2.1.2: Second law of thermal dynamics

The second law of thermodynamics states that the entropy in an isolated system never decreases, because an isolated system spontaneously evolves towards thermal equilibrium—the maximal entropy (Holliday & Resnick, 2013). Entropy is defined by the following equation:

$$\Delta S = S_f - S_i = \int_i^f dQ/T \quad (2)$$

where S_f is the final entropy, S_i is the initial entropy, dQ is the energy transfer as heat to the system, and T is the system temperature.

The second law of thermodynamics includes the concepts of heat transfer and temperature (Rabi *et al.*, 2012). The entropy inside the body is increased when the temperature of the environment exceeds the mean skin temperature. As a result, an elevated core body temperature is observed. In order to maintain the desired body temperature, heat needs to be dissipated. The dissipation of heat reduces the rise of core temperature and thus prevents the human body from overheating.

2.2.2: Biophysics of heat transfer

The heat balance equation is derived from the following equation (Kenny, 1998):

$$\dot{S} = \dot{M} \pm \dot{W}_k \pm \dot{R} \pm \dot{C} \pm \dot{K} - \dot{E} \quad (3)$$

Where $\dot{S}$ ($W.m^{-2}$) is heat storage, $\dot{M}$ is metabolic production, $\dot{W}_k$ is the external work performed by the individual, $\dot{R}$ is radiative heat exchange, which refers to the transfer of energy via an electromagnetic wave between the environment and the human body, and C and K represent convection and conduction. Finally, $\dot{E}$ represents evaporative heat exchange.

This equation illustrates the pathway of heat exchange at rest and during exercise, which enables the researcher to calculate changes in core temperature at rest and during strenuous exercise. At rest, radiation, convection and evaporation are normally the three major pathways for heat dissipation. However, during exercise, evaporation is the major pathway as radiation and convection are unable to increase much from resting value (Fig. 1).

The biophysics of heat exchange are different between exercise in dry heat and exercise in humid heat. In dry heat, a greater amount of heat loss is achieved through evaporation, as water molecules vaporise on the skin's surface. In humid heat, the dissipation of heat is less efficient, as evaporation is inhibited by the high absolute humidity (Cheung *et al.*, 2000, Kenney, 1985, Sawka *et al.*, 2011). A high absolute humidity reduces the vapour gradient between the skin and the environment, therefore limiting the rate of evaporation. This suggests that exercise in humid heat is potentially more dangerous than exercise in dry heat (Fig. 2).

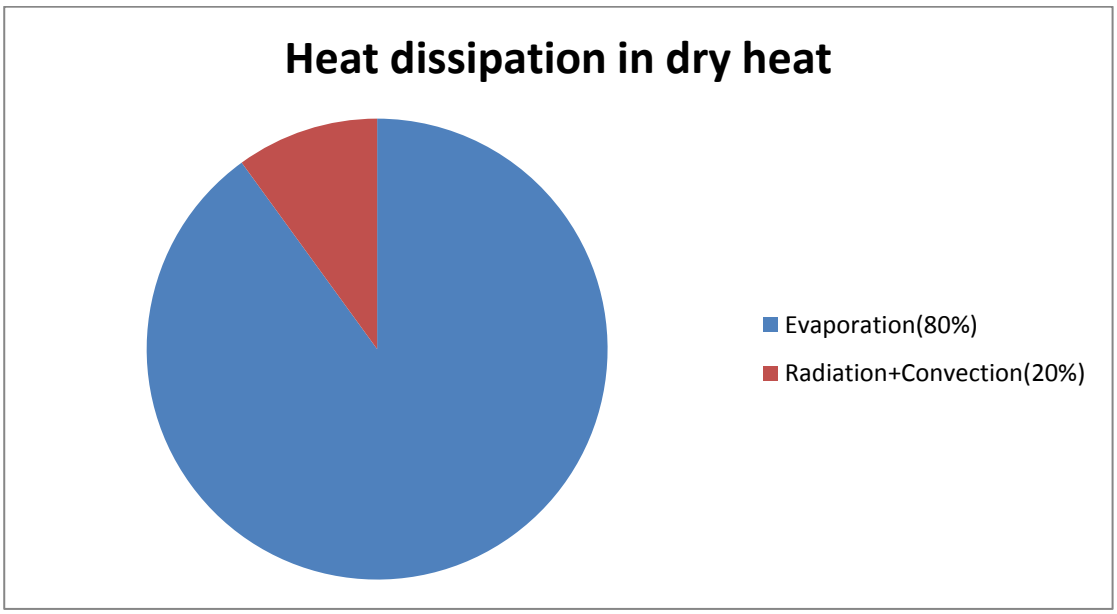

Figure 1: Heat dissipation during exercise in a dry heat environment (Armstrong et al., 1996)

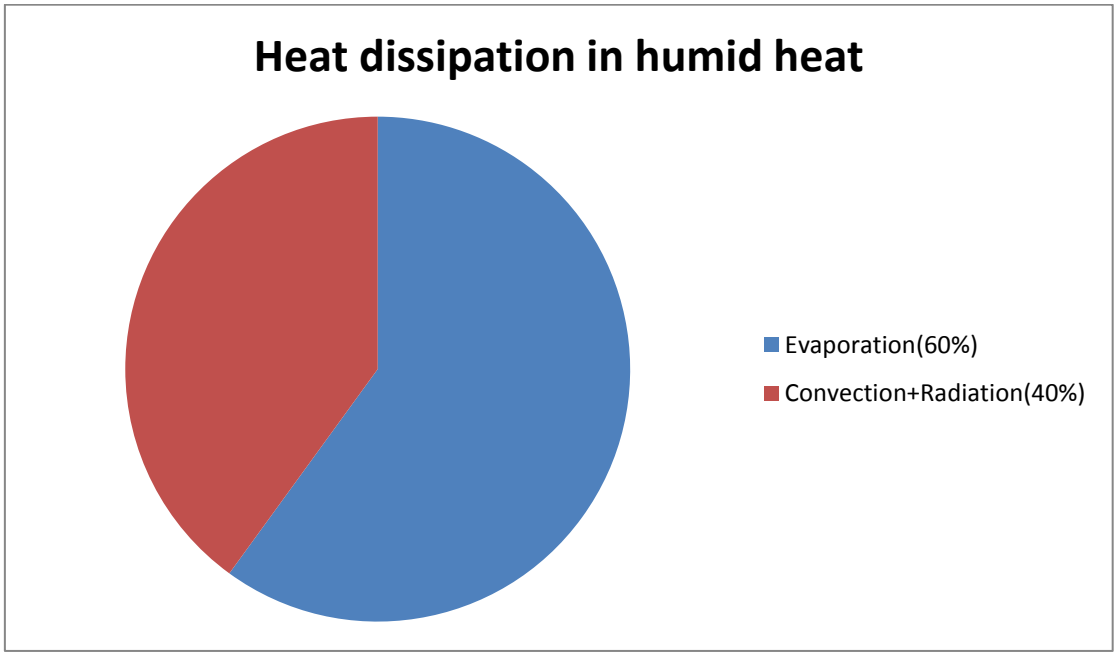

Figure 2: Heat dissipation in a humid heat environment (Armstrong *et al.*, 1996)

2.2.2.1 The compartment model

The Pierce Two-Node Model (Fig. 3) describes the concept of human temperature regulation. It is divided into core and skin compartments. The core compartment serves as both a heat reservoir and a cold reservoir. The heat reservoir represents the existing thermal energy content and the ability to generate heat through metabolic production during exercise or initiate shivering responses when it is cold (Gagge and Gonzalez, 2011). The cold reservoir represents heat sink capacity and the ability to initiate a sweating response to prevent the body from overheating. The skin compartment is the final barrier between the human body and the environment. It is the site for heat dissipation, as well as heat conservation. During cold exposure, cutaneous vasoconstriction results in an increase in skin thickness, thus preventing heat loss. On the contrary, vasodilator activity decreases skin thickness and permits greater heat loss from the body (Gagge and Gonzalez, 2011).

This model indicates the direction of heat transfer, from the core to the tissues, and then from the subcutaneous fat to the skin (Stolwijk, 1980) (Fig. 4). Most importantly, the control of the whole body's sweating drive is based on the theory of the Pierce Two-Node Model, which gives a clear indication of an individual's sweating capacity, in addition to their acclimation

status: (e.g., unacclimatised: 186–195 $g.m^2.h^{-1}.°C^{-1}$; acclimatised: 250–290 $g.m^2.h^{-1}.°C^{-1}$) (Gagge and Gonzalez, 2011). In humid heat, a reduction in sweat rate is coupled with hidromeiosis, whereas the sweat rate during an acute dry heat exposure remains stable (Nielsen *et al.*, 1993). In addition, this model also highlights why in humid heat T_{sk} is usually higher than dry heat as this can promote greater dry heat transfer (convective and radiative heat lost) as well as overcome the effect of vapour pressure to promote greater evaporative heat lost to the environment. However, this also results in greater cardiovascular strain (Refer to Section **2.6.2**) This provides an indication of the differences between the exposures to humid versus dry heat.

Figure 3: Two node (core to shell) model of thermoregulation. Adapted from Gagge and Gonzalez (2011)

2.2.3: Active and passive systems of heat transfer

The human body is a system that detects fluctuations in the internal environment and subsequently initiates an appropriate feedback control mechanism, in order to restore the internal environment (Fiala *et al.*, 1999, Gagge and Gonzalez, 2011, Huizenga *et al.*, 2001, Stolwijk, 1980). This feedback control mechanism can be quantified using mathematical models. In addition, a good mathematical model can work cohesively with the physiological system, to explain the physiological response. To date, most models are centered on passive and active systems (Havenith, 2001).

The passive system (Open loop) is defined as a material that is responsible only for heat transfer to or from the surrounding area. Heat transfer (H) for the passive system is illustrated by the equation below:

$$\Delta H = mcdT/dt \quad (4)$$

where m is the mass of the object or tissue; c: is the specific heat of the object; and dT/dt represents changes in the object or core temperature with respect to time.

This equation gives an indication of heat storage rates at rest and during exercise (via the passive system). At rest, positive heat gain is achieved when ambient temperatures exceed the surface temperature. On the contrary, heat loss occurs when the skin temperature is greater than the environmental temperature. However, when calculating positive heat gain during exercise, metabolic heat production must be considered, in addition to the net heat flux from the ambient environment. The structure of the passive system includes all anatomical structures, such as the body surface area and muscle tissues. Furthermore, this system behaves like an open loop control, with the absence of an effector mechanism (Werner, 1980).

The passive system indicates the direction of heat transfer between the core to the skin and from the skin to the environment. This provides information about maximal evaporative cooling, which includes the effect of clothing, on heat dissipation. Maximal evaporative cooling is defined as the maximal heat loss achieved by the combination of the environment and clothing. Humid heat is categorised as uncompensable, because the energy required for evaporation greatly exceeds the maximal evaporative cooling rate. As a result, a higher core temperature is observed. This explains why exercising in hot a humid condition is potentially more stressful than exercising in dry heat conditions.

The active system (Close loop) includes sensors that detect changes in the initial value, a central controller and an effector mechanisms. This system behaves like a closed-control loop and it is composed of thermal receptors, ascending and descending pathways and the effector mechanisms. The active system initiates a feedback control and a physiological control mechanism, in order to maintain thermal homeostasis. Collectively, both active and passive systems behave like a closed-control loop (Fig. 5), in order to maintain the core body temperature between approximately 37°C and 37.5°C (Werner, 1980).

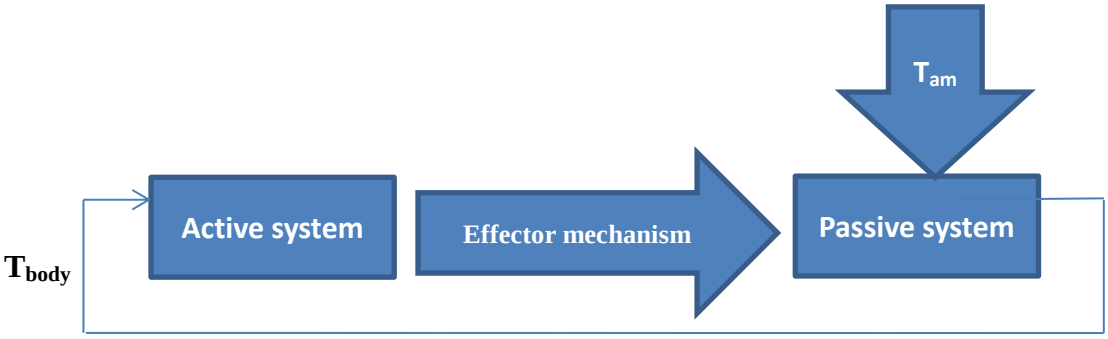

Figure 4: Close-control loop from the active and passive systems (Werner, 2005)

2.2.4: Autonomic regulation

The autonomic system regulates body temperature via thermal sensors, which are composed of an afferent pathway and an efferent pathway to the effector. These components work cohesively to regulate body temperature. Most importantly, the autonomic system provides weighted signals from the environment and allows for appropriate behavioural adaptations. In particular, these signals are derived from the heavily-weighted core temperature and the light-weighted mean skin temperature (Fig. 6). When the difference between the weighted signal and the effector specific value exists, a load error signal is generated. This signal then travels via afferent pathways to the preoptic anterior hypothalamus (POAH) for integration to

generate an effector response; this is carried by an efferent pathway to its effector for an appropriate feedback response.

Figure 5: Core temperature is stable within the range of temperatures bound by the core temperature for shivering and sweating, defined as the interthreshold zone, retrieved from (Mekjavic and Eiken, 2006)

2.2.4.1: Thermosensors

The role of thermosensors is to detect external stimuli and transmit signals to the POAH to initiate heat dissipation or heat retention (Romanovsky, 2007, Vriens *et al.*, 2014). Thermosensors are classified into warm and cold sensitive, and they are responsible for either a heat loss response or heat retention. Thermosensors in the central nervous system are named central thermosensors, and those on the periphery are named the peripheral thermosensors (Romanovsky, 2007).

The role of central thermosensors is to detect changes in temperature and also initiate thermoregulatory responses (Romanovsky, 2007). Central thermosensors are located centrally, in areas such as the spinal cord, the brain stem and the preoptic area. Central thermosensors consist of mainly warm-sensitive sensors and very few cold sensitive sensors. This is because the brain temperature operates at an extremely small range within the thermoneutural zone

and elevating the temperature beyond this zone results in the denature of regulatory proteins, thus resulting in permanent brain damage (Romanovsky, 2007). Although the central thermosensors are primarily comprised of warm sensitive neurons, the cold sensitive neurons also have a role to play in the central nervous system. The cold sensitive neurons inhibit synaptic inputs from the neighbouring warm sensors.

Peripheral thermosensors are predominately cold sensors. These are located beneath the epidermis and their action potential are conveyed by thin myelinated Aδ fibers. Although an abundance of cold sensors are observed in the skin, few warm-sensitive sensors also exist in the deeper dermis of the peripheral area. The signals from warm sensors are transmitted by unmyelinated C fibers to the POAHs for heat loss responses (Morrison and Nakamura, 2011, Romanovsky, 2007).

Transient receptor potential (TRP) ion channels are located in the Peripheral thermosensors and the activation of these channels is temperature-dependent (Fig. 7) (Morrison and Nakamura, 2011, Romanovsky, 2007). These TRP channels are regarded as a molecular thermostat, as they provide thermal sensations from the environment (Vriens *et al.*, 2014, Caterina, 2007). The transient receptor potential superfamily consists of 30 different channels and it is divided into TRPC, TRV, TRPM, TRPML, TRPP and TRPA. However, for the sake of simplicity, only the channels related to thermoregulation are considered in this review, namely TRV3 and TRV4. The TRV3 and TRV4 channels are warm and cold sensitive, with activation thresholds of 33–39 °C and 25–34 °C, respectively (Morrison and Nakamura, 2011). Once the ambient temperature exceeds the activation thresholds of TRV3 and TRV4, temperature ion gated channels are forced opened. This results in the disturbance of the membrane potential and subsequently results in the generation of an action potential (Vriens *et al.*, 2014).

Figure 6: Schematic representation of the dependence of activity on cold-activated (Blue) and heat-activated (Red) thermo TRP channels in heterologous systems, adapted from (Romanovsky, 2007)

2.2.4.2: Thermoafferent pathways

An action potential is generated by the activation of TRP3 and TRP4, due to temperature differences. Subsequently, the action potential is carried by unmyelinated C fibres to the dorsal root ganglion and then to the dorsal horn of the spinal cord. In particular, Lamina I neurons, from the dorsal horn, receive thermo signals from the periphery and carry this information to the insular cortex, with a relay in the posterolateral thalamus, and finally arrive at the POAH (Morrison and Nakamura, 2011, Romanovsky, 2007, Vriens *et al.*, 2014).

2.2.4.3: The effector responses

The main function of the thermal effectors is the regulation of body temperature by initiation of sudomotor and vasomotor drive. Together, these actions prevent overheating during exercise in the heat. The effector responses vary depending on sex, acclimation status (pre-acclimated vs post acclimated) and female reproductive hormones (Sections **2.7** and **2.8**).

2.2.4.3.1: The sudomotor response

The eccrine sweat gland is the dominant site for sweat production at rest and during exercise (Shibasaki *et al.*, 2006). The secretion of sweat from the eccrine sweat gland is extremely important for heat dissipation, as it indirectly contributes to the evaporative cooling process. Sweating by itself cannot ensure evaporative cooling; it is dependent on the gradient between the skin and ambient vapour pressures (absolute humidity) (Sawka *et al.*, 2007). This is, therefore, one of the reasons why humid heat is potentially more stressful than dry heat (**Section 2.6**). The pathway of eccrine sweating and its mechanisms are described in the following section.

Since temperature regulation is a sub-category of the autonomic nervous system, feedback to the eccrine gland uses pre-ganglionic and post-ganglionic neurons to convey information to the effector. For the pre-ganglionic pathway, signals from the POAH pass through the tegmentum of the pons and the medullary raphe regions, to the intermediolateral cell column of the spinal cord. Signals subsequently leave the spinal cord and travel to the white ramus communicans and then to the synapse in the sympathetic ganglia (Shibasaki *et al.*, 2006). For the post-ganglionic pathway, the efferent signal is carried by nonmyelinated C fibers to the gray ramus communicans and then to the sweat gland (Shibasaki *et al.*, 2006).

Acetylcholine (Ach) is released from the cholinergic sudomotor nerve when the efferent thermal signal arrives at the sweat gland. Subsequently, when Ach binds to the muscarinic receptor, the intracellular Ca^{2+} concentration is increased. Subsequently, this increases the permeability of K^{+} and Cl^{-} channels, which then initiates the release of an isotonic precursor fluid from the secretory cells (Shibasaki *et al.*, 2006). As the fluid travels up to the skin surface, isotonic electrolytes are reabsorbed from the epidermis and thus change the sweat content to a hypotonic solution, thereby also leaving the remaining body fluid increasingly hypertonic (Shibasaki *et al.*, 2006, Vetrugno *et al.*, 2003).

2.2.4.3.2: Cutaneous blood flow

The cutaneous vessels have a tremendous ability to accommodate such high perfusion rates during passive heating. For example, skin blood flow during exercise in the heat can increase from 300 mL/min up to 8.1 L/min, which accounts for almost 50% of cardiac output (Rowell, 1974b). This, therefore, highlights the importance of skin blood flow in relation to heat stress. The main functions of cutaneous blood flow during exercise in the heat are to promote

convective heat lost and heat up the skin in order to facilitate evaporative cooling. This is achieved by transporting heated blood from the core to the skin surface, via convection, thus reducing the thermal gradient between the core and skin. As the thermal gradient continues to decrease, the skin temperature begins to rise, allowing radiation and evaporation to take place.

The neurological control of cutaneous blood flow is site specific. Different areas of the skin have different nerve innervations (Johnson and Proppe, 2011). For example, glabrous skin (palms of the hands, soles of the feet, ears, lips, and nose) is under the control of the adrenergic vasoconstrictor system, whereas non-glabrous skin is under the control of both the adrenergic vasoconstrictor system and the sympathetic vasodilator system (Johnson and Proppe, 2011).

The mechanism for cutaneous vasodilation in the glabrous skin is the removal of sympathetic vasoconstrictor activity. This mechanism is supported by a few classical studies (Arnott and Macfie, 1948, Fox and Edholm, 1963, Grant and Holling, 1938, Gaskell, 1956), which observed that anesthetization of vasoconstrictor nerves in the finger (Arnott and Macfie, 1948) or hand (Gaskell, 1956) caused increased cutaneous blood flow, similar to whole body heating. However, since non-glabrous skin covers most of the body surface area, this is usually the site for which active cutaneous vasodilation is described.

The mechanism of cutaneous vasodilation in non-glabrous skin is predominately regulated by the sympathetic vasodilatory system, however, this mechanism remains debatable as there is no single factor to describe the response. In fact, this system is now believed to be concomitantly regulated by the sympathetic cholinergic nerve, downstream of its endothelial function in the cutaneous vessels and co-transmitters (Johnson and Proppe, 2011). In particular, endothelial function is now believed to account for 40 percent of the initiation of active cutaneous vasodilation in non-glabrous skin (Shastry *et al.*, 2000, Johnson, 2010).

Another highlighted mechanism is the release of Ach and co-transmitters from the sympathetic cholinergic nerve (Kellogg *et al.*, 1995). The theory as to why Ach contributes to cutaneous vasodilation is that Ach can simultaneously bind to muscarinic and cutaneous vessels, to mediate cutaneous vasodilation. In addition, Ach can mediate nitric oxide-dependent pathways that initiates cutaneous vasodilation (Shibasaki *et al.*, 2002). However, the role of Ach in cutaneous vasodilation still remains vague, since cutaneous blood flow is not affected when an Ach inhibitor is applied to a treated skin area. This therefore highlights the role of co-transmitters on cutaneous vasodilation (Kellogg *et al.*, 1995).

However, the main challenge to date is that the search for co-transmitters seems endless, as there are many co-transmitters (e.g., substance P, vasoactive intestinal peptide, and calcitonin gene related peptide). Therefore, it is extremely difficult to isolate a single element or co-transmitter that is responsible for cutaneous vasodilation.

2.2.4.4: Reciprocal inhibition

The concept of reciprocal inhibition may not be entirely correct. Briefly, reciprocal inhibition describes warm and cold-sensitive neurons, which mutually inhibit each other following a temperature stimulus. For example, the activation of warm-sensitive neurons simultaneously inhibits the response of the cold-sensitive neurons (Mekjavic and Eiken, 2006). However, recent studies have shown that both cold-defence and warm-defence autonomic responses are initiated by the alternation of warm-sensitive neuron activity. This means that increasing warm-sensitive neuron activity initiates heat-loss, whereas decreasing activity initiates a cold-defence response (Romanovsky, 2007). However, these results have been generated from animal studies and therefore the exact interaction of warm- and cold-sensitive neurons remains debatable.

2.2.4.5: Regulated variables

The main purpose for the thermal effector responses is to modify core and skin temperatures. For example, active cutaneous vasodilation and sweating facilitate evaporative cooling and thus cool the skin and reduce the rise in core temperature. There are many factors that can influence core and skin temperatures, and they are described in the following section.

2.2.4.5.1: Core temperature

The core temperature provides information on total heat storage in the body at rest, or during exercise. It is defined as the summation of all tissue temperatures at a certain depth of the body, without being influenced by circulatory adjustments or temperatures at surface tissues (Commission, 2001). Core temperature responses during rest or exercise in the heat are influenced by circadian rhythms (Refinetti and Menaker, 1992), ambient temperature (Galloway and Maughan, 1997), absolute humidity (Che Muhamed *et al.*, 2016b), drug use (Crandall *et al.*, 2002), reproductive hormones (Kolka and Stephenson, 1997a), exercise intensity and behavioural responses (Schlader *et al.*, 2011a). According to the definition above, there is no true site for measuring the core temperature, because temperature varies among

different sites of the body (Sawka *et al.*, 2011). However, there are several sites available for measuring the core temperature, such as via rectal and oesophageal methods (Moran and Mendal, 2002).

2.2.4.5.2: Skin temperature

Skin temperature is regulated by the ambient temperature as well as the absolute humidity. It is generally used for: (1) the determination of cutaneous blood flow, (2) initiating behavioural responses, (3) determining the thermal input to the POAH (Sawka *et al.*, 2011), (4) generating thermal perceptions.

2.3: Extents of heat stress

The core temperature response to exercise depends on whether the temperature of the environment is a compensable ($E_{req} = E_{max}$) or uncompensable heat stress ($E_{req} > E_{max}$). Compensable heat stress ($E_{req} = E_{max}$) permits attainment of the thermal steady-state. This means that heat dissipation is at least equal to heat production and therefore, there is no further increase in the core temperature (Kraning and Gonzalez, 1991b). On the contrary, uncompensable heat stress ($E_{req} > E_{max}$) represents heat production that exceeds maximal heat dissipation power. As a result, the core temperature increases continuously (Fig. 8) and thus it increases the likelihood of heat-related illnesses (Armstrong *et al.*, 1996, Casa, 1999). Interestingly, compensable heat stress can easily switch to uncompensable heat stress, if the rate of evaporation from the skin's surface is limited (Kraning and Gonzalez, 1991b). This shows that physiological responses in humid heat may be different to those during dry heat exposures.

Figure 7: Comparison of rectal temperatures during intermittent and continuous exercise undertaken in compensable and uncompensable heat stresses, adapted from (Kraning and Gonzalez, 1991a).

2.4: Human behavioural thermoregulation

The previous section (**2.2.4.1.2.1**) highlighted how the discharge of the TRPs, V3 and V4, from the peripheral thermo-sensors carries action potentials to the insular cortex to generate "feelings" and how the POAH conveys this information to the sweat gland and cutaneous vessels, to initiate eccrine sweating and cutaneous vasodilation. This section will describe how the sensation of heat can dictate behavioural responses, at rest and during exercise. Furthermore, this section will highlight why temperature regulation is achieved through both autonomic and behavioural means.

2.4.1: Rest

At rest, the term 'behavioural response' refers to the decision to move from an unpleasant environment to a pleasant environment (Schlader *et al.*, 2013, Cabanac, 2011) or by other behaviour means such as warming or cooling the body through an external thermal source (Flouris and Cheung, 2009) or by building up the microenvironment (Cabanac, 2011). The decision to avoid noxious environments is important to date as it has directly implication to the daily life during the summer months. This response is driven by thermal discomfort, from the thermal-sensory information in the TRP V3 and V4 channels; this thermo-sensory information then travels up to the POAH to generate discomfort and then initiates the move to a pleasant environment. Behavioural responses at rest are preceded by changes in vasomotor tone, from the glabrous and non-glabrous skin (Schlader *et al.*, 2016a, Schlader *et al.*, 2016b). These findings were derived from the studies of Schlader *et al.* (2009), (Schlader *et al.*, 2016b, Schlader *et al.*, 2016a) using the shuttle box approach, where the participant can shuttle between a hot and a cold room. The main finding from these studies was that physiological strain was attenuated once a behavioural response was initiated (Schlader *et al.*, 2013).

The change in vasomotor tone prior to the change in core temperature may be linked to the activation of TRP ion channels, albeit the mechanism behind this still requires further investigation. In particular, a study from Wong and Fieger (2012) indicated that TRP V1 contributed to ~25% of the cutaneous vasodilation, and that the activation of TRP V1 may interact with nitric oxide. However, whether the TRPs, V3 and V4, are simultaneously involved in the generation of thermal perception and the initiation of cutaneous vasodilation, still remains unknown and warrants further investigation. This is deemed necessary as successful discovery of the roles of the TRPs, V3 and V4, may strengthen our knowledge on

why thermal behaviour at rest is preceded by a change in vasomotor tone.

2.4.2: Exercise

During exercise in the heat, the term 'behavioural response' refers to the voluntary reduction of exercise intensity in order to attenuate a rise of core temperature (Tatterson *et al.*, 2000, Tucker *et al.*, 2004, Schlader *et al.*, 2011d). To justify that the reduction in exercise intensity is in fact part of thermoregulatory behaviour, one study (Schlader *et al.*, 2011a) investigated the differences between fixed and self-paced exercise on thermoregulatory responses in uncompensable heat stress, in two different exercise modalities, and concluded that when given the opportunity to exert a behavioural response, the time to complete the fixed amount of work was longer in self-paced exercise than in fixed-intensity exercise. As a result, there was a lower rise in core temperature during self-paced exercise, due to reductions in metabolic heat production and power output. This, therefore, supported the claim that the reduction of performance in the heat was indeed driven by our behaviour. More importantly, this study successfully illustrated that the exerciser can self-select their workload to reduce thermal strain in the heat.

The initiation of behavioural responses in the heat is due to a change in mean skin temperature rather than core temperature. This is supported by the study by Schlader *et al.* (2011c) where skin temperature was manipulated between hot (35.2°C) and cool (29.4°C), using a water-perfused suit; this study concluded that a lower skin temperature at the commencement of exercise rendered better power outputs as compared to a high skin temperature. This is because a higher skin temperature at the beginning of exercise increased thermal discomfort and therefore generated a protective response, in order to avoid overheating in the later trial. This then corresponded to the reduction of power output.

Behavioural responses during exercise in the heat are also influenced by absolute humidity (see **section 2.6.6**), aerobic fitness (Cheung and McLellan, 1998), body composition (Selkirk and McLellan, 2001), hydration (Sawka *et al.*, 2001) and reproductive hormones (Janse *et al.*, (2012) (see **section 2.8.7**). Although hypo-hydration may potentially contribute to the initiation of behavioural responses, this is not the focus of this thesis. The precise physiological mechanism of how the severity of dehydration affects the behavioural response in the heat is highlighted by two excellent review articles (Sawka *et al.*, 2001, Sawka *et al.*, 2012), where they conclude that hypohydration in conjunction with hyperthermia reduces

aerobic performance in the heat. Therefore, this paragraph only highlights the role of aerobic fitness and body composition on behaviour responses in the heat. Highly trained athletes with low percentage of body fat have greater effector responses (lower T_c, greater sweat rate), which is similar to the effect of heat acclimation; this therefore enables them to have a greater time to exhaustion as compared to an untrained cohort (Cheung and McLellan, 1998). This was well documented by the study of Selkirk and McLellan (2001) as they observed that higher aerobic fitness in conjunction with low percentage of body fat had a lower thermoregulatory response and longer time to exhaustion during exercise in the heat. Interestingly, little is known about the effects of different aerobic fitness levels and body composition on self-selected workloads in the heat. This therefore warrants further investigation, as it could provide information on whether body fat serves as a factor to reduce productivity in the heat.

2.5: Acute exposures to dry heat

An acute exposure to dry heat imposes a greater stress on physiological control systems, relative to a temperate environment (Sawka *et al.*, 2007). This includes the cardiovascular system, the musculo-skeletal system and the respiratory system. It is necessary to have a clear understanding of acute physiological responses to dry heat, in order to enhance both sporting performance and working capacity.

2.5.1: Core and skin temperature responses

Exercising at a moderate intensity or above in a dry heat environment elevates both core and skin temperatures, compared to those in temperate environments. (Galloway and Maughan, 1997, Lind, 1963, Périard *et al.*, 2011). This is because the rate of heat production greatly exceeds heat dissipation and thus results in a greater rise in core temperature. As core temperature continues to rise, it transports the heated blood, via conduction and convection, towards the skin and thus a higher skin temperature is observed.

2.5.2: Cardiovascular drift

Acute dry heat exposures increase cardiovascular strain compared to temperate environments (Cheung and Sleivert, 2004). This is because most of the blood supply is redistributed to the cutaneous region and skeletal muscles for the greater purpose of maintaining heat dissipation and metabolism of the working muscle (Sawka *et al.*, 2011). Notably, maximal skin blood flow can reach a value of eight litres per minute during acute heat stress (Johnson and Proppe,

2011). This redistribution of blood supply towards the cutaneous regions reduces venous return and subsequently results in the reduction of stroke volume (Rowell, 1974a) (Fig. 9). As a result, heart rate is elevated, in order to maintain a reasonable level of cardiac output. This is called cardiovascular strain (Rowell, 1974a).

Figure 8: Cardiovascular responses during sustained moderate intensity (70% of VO_{2max}) exercise in temperate and hot conditions, retrieved from Sawka et al (2011).

2.5.3: The relationship between skeletal muscle blood flow and skin blood flow

During dynamic exercise in a dry heat environment, muscle blood flow receives a greater blood supply than cutaneous blood flow. Numerous studies show that blood flow to the muscle remains unchanged in the euhydrate state, whereas skin blood flow either plateaus or is reduced, after exceeding a core temperature of 38 °C (Fig. 10) (González-Alonso *et al.*, 2008, Sawka *et al.*, 2011). This is achieved by increasing vasoconstrictive activity in the cutaneous vessels (González-Alonso *et al.*, 2008) and thus redirecting the remainder of the blood to the skeletal muscles for metabolic purposes. Such redistribution of the blood supply results in a higher core temperature and induces premature fatigue (González-Alonso *et al.*, 2008).

Muscle blood flow could be reduced in a dehydrated state; however, this does not compromise whole body $\dot{V}O_2$ and skeletal $\dot{V}O_2$ as greater O_2 extraction was observed in the dehydrated compared to euhydrated trial (González-Alonso *et al.*, 1998). Therefore, non-extreme reductions in skeletal muscle blood flow apparently do not contribute towards fatigue when exercising for a prolonged duration in the heat. However, current studies have yet to resolve whether the increased of O_2 extraction could possibly compensate for the severe decrease in muscle blood flow during exercise and therefore warrants further investigation.

Figure 9: Schematic comparison of the thermoregulatory control of skin blood flow at rest (passive heating) and during dynamic exercise, retrieved from González-Alonso *et al.* (2008).

2.5.4: Blood flow to the visceral organs

Exercising in dry heat results in vasoconstriction to the visceral organs (splanchnic, GI tract, renal and), in order to maintain adequate blood supply to both the cutaneous region and the skeletal muscles (Musch *et al.*, 1987). Current research findings from animal trials and human trials indicate that the average reduction of splanchnic blood flow is between 36% to 80% (Rehrer *et al.*, 2001), for renal blood flow it is between 27–36% and the reduction of brain blood flow is between 36–38 % (Johnson and Proppe, 2011, Rowell, 1974b). However, such modulations of blood flow for prolonged periods result in premature fatigue and exercise-induced endotoxemia. This is because the reduction of blood delivery to the GI tract increases its permeability and this jeopardises the integrity of the GI tract. As a consequence, leakage of lipopolysaccharides from the intestine wall increases heat storage and subsequently may lead to heat stroke (Lambert, 2004).

2.5.5: Sudomotor adjustment

Eccrine sweating is significantly elevated in the heat compared to in a temperate environment (Galloway and Maughan, 1997, Shapiro *et al.*, 1980a). More importantly, the rate of sweating increases with a rise in ambient temperature (Galloway and Maughan, 1997). The purpose of additional sweating in the heat is to facilitate greater evaporative cooling at the skin surface. However, as a consequence, this results in the reduction of total blood volume, if fluid supply is not available. Subsequently, greater cardiovascular strain is observed, due to the reduction of both cardiac output and stroke volume (González-Alonso *et al.*, 1997).

2.5.6: Metabolic adjustments

Skeletal muscle metabolism in the heat has minor or nearly no influence on endurance performance (Febbraio, 2000, Febbraio, 2001). As compared to an ambient environment, the current literature unanimously agrees that substrate utilisation in dry heat shifts towards carbohydrate metabolism, with less fat metabolism (Febbraio, 2000, Febbraio, 2001). The potential mechanisms for increasing carbohydrate metabolism are muscle temperature (the Q_{10} effect), reduced delivery of skeletal muscle blood flow, alternation of the neuromuscular recruiting pattern, an increase in (nor)adrenaline secretion and a reduction in the total adenine nucleotide pool (TAN = ATP + ADP + AMP). However, studies have revealed that glucose concentrations inside both the plasma and the muscle are elevated at the point of fatigue in dry heat, compared to in a temperate environment (hot conditions: 300 $mmol.kg^{-1}$ dry weight

vs temperate conditions: 150 mmol.kg^{-1} dry weight) (Febbraio, 2000, Febbraio, 2001). This unique mechanism may be due to an increasing sympatho-adrenal response that triggers the secretion of (nor) adrenaline, which subsequently results in hepatic glucose production. Therefore, glycogenolysis significantly exceeds glucose utilisation. As a result, glucose concentration inside the muscle and plasma are elevated and so it can be said that exercise in dry heat results in relative hyperglycaemia (Febbraio, 2000, Febbraio, 2001). Therefore, the shift towards an increase in carbohydrate metabolism does not reduce endurance performance in a dry heat environment.

A current limitation for all studies involving metabolic adjustments in dry heat is a lack of sex-specificity. In a temperate environment, women are found to have higher rates of fat utilisation than men and thus this gives them greater potential in ultra-endurance events. However, no current studies have investigated the effect of the menstrual phase on muscle metabolism in a dry heat environment and, therefore, this specific area warrants further investigation.

2.5.7: Ventilation adjustments

Hyperventilation occurs when the human body is exposed to dry heat conditions and this unique breathing pattern is called thermal hyperpnea (Fig. 11) (Nybo *et al.*, 2002, White, 2006). It is characterised as an increase in minute ventilation ($\dot{V}E$), simultaneously decreasing the arterial partial pressure of carbon dioxide (hypocapnia). As a result, global cerebral blood flow is reduced (Nielsen and Nybo, 2003) and this may decrease physical performance in a dry heat environment, due to central fatigue. In addition, hyperthermia-induced hyperventilation has been shown to alter the sensitivity of cutaneous vessel dilation, which gives the possibility that hyperthermia-induced hyperventilation may alter cardiovascular stability in a dry heat environment (Hayashi *et al.*, 2009). In particular, cutaneous blood flow at the forearm is reduced in a hypocapnic state (Fujii *et al.*, 2012). Interestingly, blood flow at the forehead is not reduced in a hypocapnic state. This then begs the question of whether the reduction in cutaneous blood flow was redirected to the brain in order to maintain adequate cerebral perfusion. However, no previous studies have described this relationship and this therefore warrants further investigation.

Figure 10: Effects of limiting the rise in rectal temperature, in humans, on pulmonary ventilation and arterial CO_2 tension, during prolong submaximal work at 65% maximal oxygen uptake, retrieved from White (2006).

2.5.8: Exercise performance in dry heat

Endurance performance is attenuated in dry heat environments compared to temperate environments (Galloway and Maughan, 1997, Périard *et al.*, 2011, Schlader *et al.*, 2011d, Ely *et al.*, 2010). This is supported by both time to exhaustion (González-Alonso *et al.*, 1999, Galloway and Maughan, 1997) and self-selected pace exercise (Schlader *et al.*, 2011d, Périard *et al.*, 2011). The reduction in endurance performance in the heat involves the critical core temperature hypothesis and the anticipatory regulation theory, rather than the critical temperature hypothesis alone (Schlader *et al.*, 2011e). Previously, the critical core temperature theory was regarded as the main contributor to fatigue in the heat, i.e., exhaustion occurs at a core temperature of around 40°C (González-Alonso *et al.*, 1999). However, this can only be applied to a laboratory setting, since elite runners endure core temperatures greater than 40°C during actual endurance events, without any medical complications (Maron *et al.*, 1977). In addition, during self-paced trials, a reduction in power output happens prior to reaching the critical core temperature of 40°C (Tucker *et al.*, 2004, Périard *et al.*, 2011, Schlader *et al.*, 2011d). This shows the need for the anticipatory regulation theory. In brief, this theory proposes that fatigue occurs because of protective actions to avoid physiological breakdown (Marino, 2004), that is, to avoid heat-related illnesses or any damage to the body. However, the anticipatory regulation theory varies depending on exercise mode—time to exhaustion versus self-paced exercise. For example, the development of fatigue during a time to exhaustion protocol is affected by critical temperature, as this affects arousal, as indicated by altered brain wave activity (the α/β wave ratio) (Nielsen *et al.*, 2001), which then results in the termination of exercise before further damage can be done. This is explained by the inability of the brain to sustain voluntary muscle contractions during severe hyperthermia (Nybo and Nielsen, 2001a). More interestingly, even with progressive cooling immediately after the attainment of the critical temperature, force generation is not recovered compared to baseline (Morrison *et al.*, 2004). However, during self-paced exercise, the perception of thermal consequence starts right at the beginning of exercise and therefore allows the exerciser to adjust their exercise intensity in order to prevent premature fatigue in the heat (Schlader *et al.*, 2011a). As a result, a rise in core temperature is usually limited during moderate duration, self-paced exercise. This highlights the importance of smart pacing during endurance events in the heat, as this can preserve the capacity for an end-spurt at the end of the competition.

2.6: Acute humid heat exposure

Acute humid heat exposure is much more stressful than dry heat exposure, due to restricted evaporative cooling and the much greater reliance on evaporative cooling in the heat (Avellini *et al.*, 1979). This elevates physiological strain and then impairs endurance performance compared to dry heat. This section will first review the biophysical differences between dry and humid heat and then describe how these differences elevate physiological strain and thereby impair our performance in these environments.

2.6.1: Core temperature differences

Evaporative cooling in humid heat is greatly inhibited due to the saturation of vapour pressure from the environment (Che Muhamed *et al.*, 2016a). This reduces the evaporative gradient between the skin and the environment. Subsequently, this alters the ratio between required evaporative heat loss (E_{req}) and maximal evaporative cooling (E_{max}) in a humid heat environment. E_{req} represents the metabolic heat from the body that is required for evaporative cooling. E_{max} represents the maximal evaporative capacity from the combination of clothing and environment. When E_{max} is reduced due to a high vapour pressure, this metabolic heat cannot be dissipated and thus higher core and skin temperatures are observed (Che Muhamed *et al.*, 2016a). As a result, this induces greater cardiovascular strain compared to that experienced in dry heat environments (**2.6.2**).

2.6.2: Cardiovascular adjustments

Exercise in humid heat increases cardiovascular strain (Che Muhamed *et al.*, 2016a) and therefore reduces physical performance compared to exercise in dry heat (Maughan *et al.*, 2012, Che Muhamed *et al.*, 2016a). This is because evaporative cooling is inhibited by high vapour pressures ($E_{req} > E_{max}$) and in order to overcome such energy differences, it is necessary to increase skin temperature by elevating the core to skin conductance. This means that greater delivery of skin blood flow is necessary, in order to achieve a higher skin temperature. This greater cutaneous pooling effect may reduce venous return and thus result in the reduction of stroke volume. As a result, it is anticipated that cardiovascular drift may be more pronounced in a humid heat than in a dry heat environment. This is supported by Che Muhamed *et al.* (2016a), which they investigated the effect of different levels of humidity on cardiovascular strain and confirmed that a greater circulatory strain was observed as the humidity level increased. As greater cardiovascular strain is evident in humid heat, this poses

the question of whether exercise in humid heat environment may induce greater cardiovascular dysfunction. Unfortunately, due to ethical reasons, no human studies are available in this area, but this mechanism is supported by an animal study (Wang *et al.*, 2014). Specifically, this animal study demonstrated that acute humid heat exposure increases oxidative stress and ultimately results in cardiomyocyte apoptosis. This mechanism may be because humid heat exposure increases the secretion of angiotensin II and inhibits antioxidant gene expression, which then up-regulates reactive oxygen species (ROS) production (Wang *et al.*, 2014). The production of ROS has a direct role in cellular apoptosis. This indicates that the differences between humid and dry heat may be different at a molecular level. However, whether the results from this animal model can be applied to humans still remains unknown and thus warrants further investigation.

2.6.3: Sudomotor differences

Exercise in humid heat is usually associated with drippage of sweat due to the restricted evaporative cooling from the environment (Candas *et al.*, 1980). As a consequence, this causes a reduction in sweat rate due to blockage of the eccrine sweat gland (Ogawa *et al.*, 1984). This situation is called hidromeiosis. However, there are virtually no studies to describe whether the induction of hidromeiosis is much shorter in humid heat compared to dry heat. Therefore on-going investigation in this area is required.

2.6.4: Metabolic challenges

In accordance with the Q_{10} theory, carbohydrate oxidation rates may be higher in humid heat since the greater rise in core temperature in humid heat may also result in higher muscle temperatures. However, many studies have shown that increased carbohydrate metabolism in the heat is not associated with any performance decrement (Febbraio, 2000). In fact, the thermoregulatory challenge is the main contributor to fatigue in the heat.

2.6.5: Ventilation and relationship between cerebral, cutaneous and muscle blood flow

Hyperventilation is usually present in humid heat in airway obstructive patients (Strauss *et al.*, 1978). However, to date, limited studies have investigated whether hyperventilation is more severe in humid heat compared to dry heat. Furthermore, no studies are available to describe whether cerebral blood flow is affected by humid heat due to hyperventilation and whether

there is competition between cutaneous blood flow and muscle blood flow against cerebral blood flow during exercise in humid heat. This, therefore, requires further investigation to clarify its mechanism.

2.6.6: Performance differences

Aerobic performance is greatly impaired in humid heat compared to dry heat (Maughan *et al.*, 2012, Che Muhamed *et al.*, 2016a). This is because the greater rise in core temperature results in greater cardiovascular strain, which then impairs exercise capacity. However, interestingly, previous studies have not investigated the differences in behavioural responses between dry and humid heat. In fact, previous studies have focused solely on time to exhaustion rather than self-selected pace exercise. Using self-paced exercise is more performance specific than fixed-intensity, as this can reflect whether pacing is different between dry and humid heat environments.

Despite the aforementioned issues, behavioural responses in hot and temperate environments still remain unclear. For example, many studies have suggested that endurance performance is reduced in the heat due to temperature differences (Ely *et al.*, 2010, Périard *et al.*, 2011, Schlader *et al.*, 2011d, Tucker *et al.*, 2004), but this supposition does not consider that hot and temperate conditions differ in terms of ambient temperature as well as humidity levels, with hot environments being more hot and humid whereas the temperate environment cooler and drier. It is logical that performance will be impaired in a hot environment compared to in a cool dry environment due to restricted evaporative cooling. Therefore, to determine whether ambient temperature plays a role in our behavioural responses in the heat, it is necessary to control for (ie.,match) absolute humidity; however, no data are available in this area and this therefore requires further investigation.

2.6.7: Equalised heat strain from dry heat and humid heat environments

Performing endurance events in humid heat environments places the human body under greater stress than a dry heat environment. Therefore, in order to compare the physiological differences between dry heat and humid heat, equalised heat strain between dry heat and humid heat is necessary. The Wet Bulb Globe Temperature (WBGT) is currently the most used method during exercise to equalise heat strain from different environments because it takes into account dry air, humid air, wind speed and radiation. Previous studies (Havenith *et al.*, 1998, Smolander *et al.*, 1987) have used WBGT to equalise the heat strain between dry

and humid environments. However, some researchers have suggested that the WBGT underestimates physiological strain in the humid heat setting (Brocherie and Millet, 2015), because it neglects to take into account absolute humidity, clothing, sex differences and exercise intensity. In fact, performing physical exercise in high humidity is much more stressful than in dry heat, even when heat strain is equalised (Budd, 2008). Furthermore, Nielsen et al. (1999) also disagree with using WBGT for equalising stress levels between humid and dry heat because WBGT does not consider individual variability in heat tolerance. Instead, they propose using the same rate of rising core temperature as an index to equalise stress levels between humid and dry heat. Since only Nielsen et al. (1999) have used the rate of core temperature rise to equalise heat stress between humid and dry heat environments, more research is needed.

2.7: Female reproductive physiology

2.7.1: The ovarian cycle

The ovarian cycle describes the monthly maturation of the ovum. This cycle is divided into two consecutive phases: the follicular phase and the luteal phase (Silberstein and Merriam, 2000).

The follicular phase involves follicle development (Fig. 12), which lasts approximately 14 days. During this phase, hormonal actions stimulate follicle growth and produce oestrogen. This important hormonal interaction begins with the secretion of gonadotropin–releasing hormone (GnRH) from the anterior pituitary, which triggers the secretions of follicle stimulating hormone (FSH) and luteinizing hormone (LH). FSH stimulates the development of the primordial follicle into the vesicular follicle, which sets up ovulation (Silberstein and Merriam, 2000). LH combines with the thecal cells to produce androgens that diffuses into the basement membrane and converts into oestrogens. Once oestrogen is secreted, it initiates a negative feedback action to inhibit the secretion of FSH and LH from the anterior pituitary. Although the initial oestrogen secretion inhibits the secretion of FSH and LH, continuous accumulation of oestrogens in the plasma deactivates negative feedback and activates positive feedback. This results in a ‘burst-like’ release of FSH: and (to a lesser extent) LH into the plasma, which then triggers ovulation by rupturing the follicle (Farage *et al.*, 2009, Silberstein and Merriam, 2000).

The luteal phase is also regarded as the post-ovulatory phase (Fig 12). It transforms the

ruptured follicles into a new endocrine gland called the corpus luteum. Once fertilisation occurs, the corpus luteum maintains adequate secretion of oestrogen and LH until the placenta is formed. In the absence of fertilisation, the degeneration of the corpus luteum results in the cessation of hormonal output and thus it restarts the whole cycle again (Farage *et al.*, 2009, Silberstein and Merriam, 2000).

2.7.2: The menstrual cycle

The menstrual cycle describes the cyclic change of the uterine wall, as it responds to fluctuation of the ovarian hormones (Charkoudian and Stachenfeld, 2014). A major change in female physiology is needed, in order to prepare for pregnancy. The menstrual cycle characterized by three consecutive phases: menstrual phase, proliferative phase and secretory phase.

The menstrual phase (days 1–5) is characterized by continuous shedding and bleeding of the endometrium from the uterine wall. At the beginning of the menstrual phase, the ovarian hormones are at their lowest concentration and most of the endometrium detaches from the uterine wall, despite being the deepest part of the endometrium. Subsequently, the detached tissue and blood pass through the vagina by menstrual flow. This is accompanied by bleeding for three to five days. At the end of day five, the ovarian hormones start to rise and thus leads to the beginning of the proliferative phase (Farage *et al.*, 2009, Silberstein and Merriam, 2000).

The proliferative phase is best described as the rebuilding of the endometrium as it responds to oestrogen levels in the plasma. This cycle usually starts at day six and finishes at day fourteen. The elevated oestrogen level in the plasma results in the formation of a new functional layer and it also converts the thick cervical mucus into thin crystalline mucus. As those layers thicken, its glands enlarge and spiral arteries increase in greater numbers. As a result, the endothelium wall is now thick and well vascularised (Farage *et al.*, 2009, Silberstein and Merriam, 2000). At the end of the last day, ovulation occurs and thus commences the beginning of the secretory phase.

The secretory phase prepares for implantation of the fertilized egg. This usually starts at day fifteen and ends at twenty-eight days into the menstrual cycle. During this phase, the effects of progesterone cause the spiral arteries to elaborate and to enlarge the gland. The enlargement of the gland causes the secretion of glycogen into the uterine cavity, which

prepares it for implantation. However, if fertilization does not occur, progesterone levels decline rapidly and thus the endometrium is deprived of hormonal support. Subsequently, the spiral arteries go into spasm. This results in the cessation of oxygen supply to the endometrium and results in apoptosis of the endothelial cells. As a result, the whole menstrual cycle starts all over again.

Figure 11: Hormonal fluctuations during the menstrual cycle and their relation to the ovarian cycle, retrieved from THE CONVERSATION.com.

2.7.3: Hormone contraception

The ovarian cycle can be manipulated using hormonal contraception. The primary purpose for hormonal contraception is to provide birth control or reduce the effect of menorrhagia. This is achieved by supressing the production of oestrogen and progesterone endogenously and thereby preventing ovulation (Fotherby, 1996). Hormonal contraception comes in various forms, but oral contraceptives are the most popular method for hormonal contraception as they do not involve any invasive procedures. In particular, combined oestrogen and progesterone pills are more common than the progesterone only pill, as they can reduce the adverse effects of progesterone (e.g. the rise of T_{core} and delaying the onset of thermo-effector responses) (Burrows and Peters, 2007). The combined pill differs in terms of its form, as according to dosage: monophasic, biphasic or triphasic. The monophasic pill provides women with a fixed dosage of oestrogen and progesterone over the 21-day cycle. On the other hand, the biphasic pill has a fixed amount of oestrogen, but two dosages of progesterone, on days 7–10 and on days 11–14. The triphasic pill contains three dosages of either oestrogen or progesterone that are elevated throughout the 21-day cycle (Burrows and Peters, 2007). The monophasic and triphasic pills are the most common pills for athletes as well as for the general public. In particular, almost 40% percent of women nowadays are using oral contraceptives and this has also become more prevalent in the athletic population (Bennell *et al.*, 1999) as most female athletes use it for birth control or to avoid negative effects of their menstrual cycle (Rechichi *et al.*, 2009).

Despite the advantages of birth control and the manipulation of the menstrual cycle, the side effects of the combined pill have been linked to alterations in thermoregulation (**Section 2.9**), an increased risk of venous thrombosis (Lidegaard *et al.*, 2009) and possibly effects on athletic performance in the heat.

2.8: The menstrual cycle and acute heat exposure

The fluctuation in female reproductive hormones has a significant influence on thermoregulatory responses in the heat. This section will describe the combined and separate effects of oestrogen and progesterone on thermoregulatory responses in the heat, at different phases of the menstrual cycle.

2.8.1: Core temperature adjustments

Core temperature is significantly elevated by approximately 0.3 to 0.5°C in the mid-luteal phase compared to the early follicular phase, at rest and throughout fixed-intensity exercise (Marshall, 1963, de Jonge, 2003). Notably, the rise in core temperature is due to the increase in progesterone concentration inside the plasma (Kolka and Stephenson, 1997a). This may be explained by the fact that progesterone can penetrate through the blood–brain barrier and then exert a direct action on sex steroid receptors, which then decreases the firing rate of warm sensitive neurons from the POAH (Nakayama *et al.*, 1975).

Although the rise in core temperature is evident in the heat during the luteal phase of the menstrual cycle, the rise in core temperature may be greater in humid heat environments, due to restricted evaporative cooling. Therefore, physiological strain may be greater in humid heat during the luteal phase of the menstrual cycle. However, no studies have compared dry and humid heat exposures for athletic women during different phases of the menstrual cycle, as previous investigations have focussed on military outcomes (participants and protocols). (Frye and Kamon, 1983, Morimoto *et al.*, 1967a, Shapiro *et al.*, 1980a); this therefore requires further investigation.

The elevation of resting core temperature in the luteal phase of the menstrual cycle compromises the vasomotor and sudomotor responses during exercise in the heat.

2.8.2: Cutaneous adjustments

The threshold for acute cutaneous vasodilation in the luteal phase occurs at a higher mean body temperature than the early follicular phase. This is because progesterone acts as an central inhibitor for the central vasodilatory system (Charkoudian and Johnson, 1997). Furthermore, progesterone is believed to decrease endothelial function (Williams *et al.*, 2001) by downregulating the expression of nitric oxide synthase (Miller *et al.*, 1996), which subsequently contributes to the restriction of cutaneous vasodilation. Oestrogen, on the other hand, has the opposite effect from progesterone—it facilitates cutaneous vasodilation (Charkoudian and Johnson, 2000, Brooks-Asplund *et al.*, 2000) and upregulates nitric oxide synthase (Rosselli *et al.*, 1995). Furthermore, when oestrogen combines with progesterone, oestrogen can override the side effects of progesterone in young females during acute heat exposure (Stachenfeld *et al.*, 2000). However, there are some studies that have suggested that progesterone still has a dominant effect when combined with oestrogen (Charkoudian and

Johnson, 1999b, Brooks-Asplund *et al.*, 2000), notably in postmenopausal women (Brooks-Asplund *et al.*, 2000).

The delayed threshold for cutaneous vasodilation in the luteal phase is still debatable due to two major reasons. First, previous studies have focused on sedentary populations, whilst neglecting to investigate highly-trained populations. Highly trained athletes have lower fluctuations in reproductive hormones and a greater ability to vasodilate (Kuwahara *et al.*, 2005a) compared to a sedentary cohort. Therefore, the delay in cutaneous vasodilation may not be present in a highly-trained population. Secondly, most of those studies (Charkoudian and Johnson, 1999b) were conducted using a passive heating model, whilst neglecting the exercise approach. Although a study from Stephenson and Kolka (1985) indicated that the delayed onset of cutaneous vasodilation was still present during exercise in the heat for eumenorrheic women, this study population was derived from a non-athletic population and they investigated only in dry heat setting as well as having no statistical analysis due to a small sample size (N = 4). As humid heat is generally regarded as more stressful than dry heat due to the restriction of evaporative cooling (Che Muhamed *et al.*, 2016a), the delayed onset for cutaneous vasodilation may not be present as greater delivery of blood flow towards the cutaneous region is needed in order to facilitate greater dry heat loss. However, whether this theory is still valid during exercise in humid heat for highly-trained eumenorrheic women remains unknown and thus warrants further investigation.

2.8.3: Sudomotor adjustments

The threshold of sweating is shifted towards a higher mean body temperature in the luteal phase compared to the early follicular phase (Stephenson and Kolka, 1985, Kolka and Stephenson, 1989). This reduces the evaporative heat loss capacity in dry heat and results in greater heat storage during exercise. Although the threshold of sweating is shifted towards a higher mean body temperature, the sensitivity (slope) is not different between follicular and luteal phases (Kolka and Stephenson, 1989). Furthermore, sweat gland activation remains the same between follicular and luteal phases (Sargent and Weinman, 1966). Therefore, it is suspected that progesterone may act as an inhibitor in the CNS, but this still requires more studies in order for a firm conclusion to be drawn. Also, as mentioned previously, the delayed threshold for sweating may not be evident in highly-trained female athletes, as they have less fluctuation in female reproductive hormones and have greater effector responses than an untrained population. This requires further investigation.

2.8.4: Ventilation adjustments

Minute ventilation is increased in the luteal phase of the menstrual cycle, due to high concentrations of progesterone (de Jonge, 2003, Marsh and Jenkins, 2002). A clinical study has revealed that progesterone increases the phrenic nerve activity—a nerve that innervates the diaphragm—and thus causes hyperventilation to occur (Bayliss and Millhorn, 1992). This subsequently reduces the $P_{ET}CO_2$ during exercise in the heat (Hayashi *et al.*, 2012); but whether this alters cerebral blood flow remains unknown. This requires further investigation to explain whether the luteal phase of the menstrual cycle is associated with greater cerebralvascular effects and potentially greater risks of heat syncope and central fatigue in the heat.

2.8.5: Metabolic adjustments

Substrate utilisation does not vary across the menstrual cycle. However, women are different from men in terms of substrate utilisation (Tarnopolsky, 2000). Studies have shown that oestrogen promotes glycogen uptake and shifts substrate metabolism towards free fatty acid metabolism (Tarnopolsky, 2000, Braun and Horton, 2001). This may enhance womens' abilities for ultra-endurance exercise, compared to men.

To date, no studies have investigated whether the fluctuation of female reproductive hormones has an effect on substrate utilisation in the heat. However, previous studies have indicated that substrate utilisation in the heat has a minimal effect on endurance performance (Farage *et al.*, 2009, Febbraio, 2000, Febbraio, 2001). Therefore, even if there is an alteration on substrate utilisation in heat due to reproductive hormones, its influence on endurance performance in heat is likely to be limited. However, it is still necessary to understand the effect of the fluctuation of female reproductive hormones on substrate utilisation in the heat (Oosthuyse *et al.*, 2010) . In-depth knowledge of this particular area would enable researchers to develop supplementation strategies before and after competition, thereby improving physical performance.

2.8.6: Blood volume adjustments and haematological alternations

Resting blood volume and plasma osmolality are lower during the luteal phase of the menstrual cycle compared to in the early follicular phase (Stachenfeld *et al.*, 2001). A lower resting plasma osmolality triggers early occurrence of the thirst sensation and an early release

of AVP, during passive heating. This is attributed to the effect of oestrogen (Calzone *et al.*, 2001) as oestrogen can upregulate the activity of AVP-synthesizing neurons in the hypothalamus (Akaishi and Sakuma, 1990). The mechanism behind the lower blood volume during the luteal phase still remains debatable, as most studies have only included two time points (early follicular and mid-luteal phase). Also, no difference, in terms of blood volume, has found between the follicular and luteal phases, during passive heating and during exercise in the heat (Sims *et al.*, 2007, Sims *et al.*, 2008, Stachenfeld *et al.*, 1999). The changes in blood volume also relate to the blood lost during menstruation and so the reduced of blood volume may not be associated with reproductive hormones per se (de Jonge, 2003).

From the information above, it is clear that reproductive hormones may alter osmoregulation and therefore monitoring hydration status prior to the commencement of heat stress trial is essential in order to reduce the effect of dehydration.

2.8.7: The menstrual cycle and exercise performance in dry heat

Some studies have shown that the luteal phase of the menstrual cycle is associated with reduced endurance performance in either dry or humid heat conditions, compared to in the early follicular phase (Janse *et al.*, 2012, Tenaglia *et al.*, 1999) . Changes in performance-related variables include a reduced time to exhaustion; a higher resting core temperature; a higher heart rate; and an increased $\dot{V}E$ (Janse *et al.*, 2012). However, whether the luteal phase is associated with a reduction in endurance performance in the heat still remains debatable, due to three major reasons. First, previous studies (Janse *et al.*, 2012, Tenaglia *et al.*, 1999) include only sedentary or moderately physically-active females; therefore, their interpretations may not be applied to a highly-trained population. Notably, highly-trained athletes have greater effector responses, similar to the effect of heat acclimation (Kuwahara *et al.*, 2005a), which may nullify physiological strain and performance decrements during the luteal phase. Second, protocols used in previous studies were not performance specific, as they used time to exhaustion rather than time-trial approaches. Using a time-trial approach is usually more performance specific, as it is more similar to real-life competition. Lastly, existing studies do not clearly articulate which type of heat stress (dry or humid) is most likely to reduce endurance performance. When the previously-described limitations are considered, it can be said that the knowledge of the menstrual cycle and performance in the heat is incomplete and thus further research is needed.

2.9: The OCP and exercise in the heat

2.9.1: Core temperature

Core temperature is significantly elevated for women who are using the hormonal contraceptive pill, relative to the follicular phase (Kenny *et al.*, 2008) or the placebo week of the hormonal contraceptive period (Martin and Buono, 1997). The synthetic component of progestin is believed to elevate resting core temperature, similar to the endogenous effect of progesterone (Tenaglia *et al.*, 1999, Rogers and Baker, 1996). However, current research is still limited in explaining the synthetic effects of progestin on temperature regulation and how this is different from the endogenous production of progesterone. For example, it is unknown whether they both have the ability to inhibit the firing rate of the warm sensitive neurons. Furthermore, whether temperature regulation is different between the high hormone phase of the OCP and the luteal phase still remains unknown. Those issues require further investigation.

The elevation of resting core temperature from the synthetic component of progestin subsequently affects the vasomotor and sudomotor responses during exercise in the heat.

2.9.2: Cutaneous vasodilation

The threshold for active cutaneous vasodilation is shifted towards a higher internal temperature during the active pill week compared to the placebo week of oral contraception (Charkoudian and Johnson, 1997, Charkoudian and Johnson, 1999b, Charkoudian and Johnson, 2000). This delayed onset threshold of cutaneous vasodilation is due to the synthetic component of progestin (Stachenfeld *et al.*, 2000), which exerts a direct effect on the hypothalamic thermoregulatory centre on temperature regulation, similar to the effect of progesterone. However, the exact mechanism for the effects of synthetic progestin on the thermoregulatory set point still remains unknown, as this involves interactions between the CNS and inflammatory markers (Charkoudian and Johnson, 2000).

Despite knowing that cutaneous vasodilation is shifted towards a higher internal temperature during the active pill phase, no previous comparisons between the effects of exogenous versus endogenous female reproductive hormone on cutaneous vasodilation are present in the literature. This is because previous studies have used OCP to manipulate the course of the nature menstrual cycle and to identify which hormone is responsible for cutaneous

vasodilation (Charkoudian and Johnson, 1997, Charkoudian and Johnson, 1999b). However, there is a need to identify the differences between synthetic hormones and endogenous female reproductive hormones on cutaneous vasodilation, as this could help to explain why using OCP usage is associated with a greater risk of venous thrombosis (Jick *et al.*, 1995). In particular, synthetic female reproductive hormones usually have a stronger potency than endogenous female reproductive hormones and may therefore induce greater thermoregulatory strain during exercise in the heat.

2.9.3: Sudomotor adjustments

The threshold for sweating is shifted towards a higher mean body temperature during the active pill period compared to the placebo week (Grucza *et al.*, 1993, Rogers and Baker, 1996). However, this only occurs without the presence of oestrogen. When progestin combines with oestrogen, it nullifies the delayed effect of cutaneous vasodilation (Stachenfeld *et al.*, 2000). Research by Stachenfeld *et al.* (2000) suggested that the synthetic component could modify the effect of progestin on eccrine sweating, but no differences were found between the combined OCP group and the eumenorrheic group. The biological mechanism of how oestrogen modulates the effect of progestin on sudomotor function still remains unknown and thus requires further investigation.

2.9.4: Blood volume adjustments

Total blood volume is not different between exogenous and endogenous female reproductive hormones (Stachenfeld *et al.*, 1999). However, the follicular phase of the menstrual cycle involves higher plasma osmolality than that of the combined oral contraceptive pill. In terms of electrolyte handling between exogenous and endogenous female reproductive hormones, the luteal phase of the menstrual cycle involves greater sodium retention and plasma renin activity compared to the OCP and the follicular phase (Stachenfeld *et al.*, 1999). Overall, although sodium retention and plasma renin activity are greater during the luteal phase compared to the OCP, this does not correspond to the reduction in plasma volume observed in an acute heat exposure. Furthermore, some studies have shown that when adequate electrolyte drink is consumed, no changes in aldosterone or AVP occur (Sims *et al.*, 2007, Sims *et al.*, 2008). This indicates that so long as adequate hydration is provided, there is no difference in body fluid balance between exogenous and endogenous female reproductive hormones.

2.9.5: Performance differences

There are very few studies available to describe whether endurance performance in both dry and humid heat are different during the active pill weeks versus the placebo week of the OCP cycle in the heat. Furthermore, whether this is different from a highly trained eumenorrheic population still remains unknown. Previously, the focus of physical performance between OCP cycle was in occupational workers (Tenaglia *et al.*, 1999), and the only finding from athletes was in relation to female team sport athletes (Sunderland and Nevill (2003). However, once again, this study investigated a dry heat setting only. Therefore, future studies should investigate whether endurance performance and autonomic responses are different between OCP and eumenorrheic populations amongst highly trained female athletes in both dry and humid heat environments so that sports practitioners and coaches can focus on different strategies according to their hormone level as well as in different environmental profiles.

2.10: Summary of the literature

The main purpose of this literature review was to provide a broad overview of the topic areas that will be discussed in more detail and investigated during this thesis. Three novel concerns have been identified. First, there is a lack of supporting evidence that behavioural responses in the heat are driven by ambient temperature. This is because previous investigations, and differing environments, are not equal in terms of their humidity (vapour pressure). Secondly, the physiological and behavioural differences between dry and humid heat have been under-researched in the eumenorrheic population, especially athletic women. Finally, no studies are available to describe the effects of endogenous and exogenous female reproductive hormones on autonomic and behavioural thermoregulation in dry and humid heat exposures. These issues form the general aims (**Chapter Three**), general methodology (**Chapter Four**) and experimental chapters **(Chapters Five- Eight)** of this thesis.

Chapter Three

3.0: Research Aims and Hypotheses

The purpose of this thesis is to: 1) clarify the role of endogenous and exogenous female reproductive hormones on autonomic and behavioural responses between exercising in dry and humid heat stress; 2) investigate how ambient temperature, *per se*, can influence thermoregulatory, cardiovascular and perceptual responses; 3) investigate the efficacy of exercise modality (fixed- and variable-intensity) on thermoregulatory strain, in differing thermal profiles but matched for vapour pressure. In order to achieve these goals, it was important to first identify the differences between eumenorrheic women and women taking combined hormonal contraception on autonomic and behavioural thermoregulation in dry and humid heat (General Aim I). There should be evidence to indicate that the behavioural and/or autonomic responses are significantly affected by the differing environments in these two cohorts, this information could then be used to construct General Aim II, which endeavoured to investigate the role of ambient temperature per se on thermoregulatory, cardiovascular and perceptual responses, and compare different exercise modalities in both warmer and cooler environments matched for vapour pressure. This was based on the premise in General Aim I, which indicates that absolute humidity is the main regulator of behavioural and autonomic responses in heat. Therefore, in order to investigate solely the effect of temperature on autonomic and behavioural responses, it was necessary to fix the absolute humidity between different heat environments. Collectively, the findings of General Aims I and II improve our understanding of thermoregulatory responses in different heat environments, for both men and women.

3.1: Aims

The first objective for **General Aim I** was to elucidate the influence of fluctuating reproductive hormones on autonomic and behavioural responses in acute dry and humid heat exposure **(Chapter Five)**. In particular, this study addressed the unresolved issue of whether the menstrual cycle, *per se*, is associated with a reduction of exercise performance in different types of heat stress environments (dry or humid). Ultimately, this could have an impact on heat acclimation strategies, for example, in regard to different thermal profiles during different phases of the menstrual cycle. Also, the findings from **General Aim I** could

potentially provide a pragmatic pacing strategy on how to avoid heat related-illness in any type of heat stress environment.

Following this investigation on the effect of the menstrual cycle in acute dry and humid exposure, **General Aim I** then compared the effect of endogenous *versus* exogenous female reproductive hormones on behavioural and autonomic differences in dry and humid heat exposure (**Chapter Six**). This occurs because the strong potency of synthetic hormones has long been assumed to induce greater thermoregulatory strain, compared to eumenorrheic women; and yet limited studies were available to provide sufficient evidence to support this hypothesis. In addition, the findings from **General Aim I** could ultimately provide valid information for drug companies, for example, in regards to altering their prescription for hormonal contraception, in order to reduce its side effect on temperature regulation.

Following the evidence from **General Aim I,** which indicates that a higher level of ambient humidity can induce greater physiological strain and reduce endurance performance compared to dry heat, the studies from **General Aim II** then attempted to explore the role of ambient temperature, *per se*, on thermoregulatory, cardiovascular and perceptual responses (**Chapter Seven**), and to ascertain whether different exercise modalities could potentially alter thermoregulatory responses (**Chapter Eight**) in both warmer and cooler conditions matched for vapour pressure. The resulting impact could be as follows: 1) to justify the claim that a successful pacing strategy can attenuate thermal strain in a hotter environment and thereby reduce the chance of obtaining a heat related illness; 2) to provide a guideline on how to select an appropriate pacing strategy according to different thermal profiles; and 3) the results from **General Aim II** could potentially provide a better approach to equalise heat strain between thermal environments, and, therefore, it may be beneficial to sports organisations, communities or military personnel, when deciding to suspend or terminate training in different thermal profiles (dry *versus* humid).

General Aim I and **General Aim II** can be divided into more specific objectives described as follows:

General Aim I

1.) To investigate the behavioural and autonomic differences between menstrual phases under dry and humid heat in eumenorrheic women (**Chapter Five**).

2.) To investigate the effect of exogenous *versus* endogenous female reproductive hormones on autonomic and behavioural differences in dry and humid heat (**Chapter Six**).

General Aim II

1.) To investigate whether ambient temperature alone has an influence on thermoregulatory, cardiovascular and perceptual responses in male athletes (**Chapter Seven**).

2.) To investigate whether different exercise modalities (fixed-intensity *versus* variable) induce different thermoregulatory strain in different thermal profiles in male athletes (**Chapter Eight**).

3.2: Hypotheses

1.) The menstrual cycle will not affect female athletes autonomic and behavioural responses in both dry and humid heat (**Chapter Five**).

2.) Thermoregulatory strain will be nullified by thermoregulatory behaviour in eumenorrheoic women (not taking OCP) and in women taking hormonal contraception **(Chapters Five** and **Six)**.

3.) Self-selected pace will be lower in humid heat, but it will not be different between eumenorrheoic women (not taking OCP) and women taking combined hormonal contraception **(Chapters Five** and **Six)**.

4.) Autonomic strain will be greater in women taking combined hormonal contraception than eumenorrheoic women **(Chapters Five** and **Six)**.

5.) The thermoregulatory and cardiovascular responses are not different between environments matched for absolute humidity, despite different perceptual response **(Chapter Seven)**.

6.) Thermoregulatory strain will not differ between fixed and self-selected pace, when metabolic heat production is equivalent **(Chapters** and **Eight)**.

Chapter Four

4.0: General Methodology

The purpose of this chapter is to provide an overview of the experimental procedures common to all experimental chapters in this thesis, and to provide justification and critique for why specific measurements were selected. Further detail unique to any experimental studies is provided in subsequent chapters (**Chapters Five-Eight**).

4.1: Participants

A total of twenty (20) moderate to highly trained female endurance athletes and fourteen (14) moderate to highly trained male endurance athletes were recruited for this thesis. The participants' characteristics are summarized in Table 1. All studies from this thesis were approved by the Massey University Ethical Committee: Southern A (See **APPENDIX 1** and **2**), according to the Declaration of Helsinki.

Table 1: Participants' anthropometric characteristics. Data are expressed as mean ± SD, The bioelectrical impedance machine was not available after the completion of subject number eight.

Variables	**Combined OCP (N=10)**	**Eumenorrheic (N=10)**	**Men (N=14)**
Age (yr)	26 ± 5.7	34 ± 9.0	31.5 ± 12.1
Height (cm)	167.3 ± 5.7	164.5 ± 4.7	177.8 ± 5.6
Weight (kg)	66.7 ± 9.1	62.1 ± 3.6	76.4 ± 8.5
BMI (kg/m^2)	23.7 ± 1.9	23 ± 1.8	24.1 ±2 .2
% Body fat	23.9 ± 5.3	24 ± 4.7	13.5 ± 4.4 **(N=8)**
Body Surface Area (m^2)	1.76 ± 0.13	1.67 ± 0.06	1.93 ± 0.12
$\dot{V}O_{2max}$ (ml/kg/min)	57.6 ± 8	56.7 ± 6.9	59 ± 9.2
PO (W)	286.2 ± 28.8	261 ± 29	392.7 ± 53
PO (W/kg)	4.2 ± 0.4	4.2 ± 0.7	5.2 ± 0.9

4.2: Experimental protocol

The experimental protocol began with resting measures, followed by a warm-up period. Immediately after the warm-up period, a 30minute time-trial was completed. The warm-up period for **Chapters Five** and **Six** was two 6minute incremental stages, with power outputs of 125W and 150W, respectively, whilst the warm-up period for **Chapters Six** and **Seven** was 6 minutes at 125W. The reason for including two incremental stages for **Chapters Five and Six** was to observe physiological responses at a fixed intensity and to compare the physiological responses between dry and humid heat.

4.3: Seasonal control

To minimise the effect of heat acclimatisation, all experiments were conducted during the autumn to spring period, where the ambient temperature rarely exceeded 22°C (5°C - 22 °C, ~70%RH). In addition, no participant had spent time in warmer climates or training environments within the month preceding testing.

4.4: Pre-experimental controls

Two days prior to any experimental trial, participants abstained from alcohol and exercise and only consumed caffeine as per their habitual use (as abstinence would in itself confound from withdrawal effects). Additionally, participants were provided with a standardised dinner of two Watties Snack Meals (Heinz Watties, Hastings, New Zealand), providing 1363 (247) kJ consisting of 53 (6) g carbohydrate, 12 (4) g protein and 8 (0.3) g fat the night preceding the trial and were asked to consume the same light meal (consisting of toast or cereal) between 2 and 4 h prior to visiting the laboratory for the trial. This dietary and exercise control minimised variation in pre-trial metabolic state. Fluid was encouraged and an euhydrated state was further ensured by instructing the participants to drink 500 ml of water 2 h prior to each trial. Hydration status was confirmed by urine specific gravity of a value being less than 1.01.

To avoid the effect of circadian rhythms on core temperature fluctuations, all heat trials were conducted at the same time of day and were separated by at least two days to avoid the development of fatigue. Lastly, all heat stress trials were randomised in order.

4.5: Measurements

4.5.1: Anthropometric

Height and weight were measured with a stadiometer (Seca, Bonn, Germany, accurate to 0.1 cm) and an electronic scale (Jandever, Taiwan, accurate to 0.01kg), from which body surface area was calculated. The electronic scale was calibrated using standard weights. Percent of body fat was measured using bioelectrical impedance (InBody 230, Korea).

4.5.2: Respiratory measurements

Expired respiratory gases were collected and analysed for oxygen consumption ($\dot{V}O_2$) and carbon dioxide elimination ($\dot{V}CO_2$), ventilation ($\dot{V}E$) and respiratory exchange ratio (RER) using an online, breath-by-breath system (VacuMed Vista Turbofit, Ventura, CA, USA) with a gas sampling period of 30 s. This system was calibrated before each trial using a zero and *β*-standard gas concentrations (15% oxygen and 5.02% carbon dioxide). The volume was calibrated using a 3 litre calibration syringe (VacuMed 3l, USA).

4.5.3: Submaximal and maximal oxygen consumption tests

The submaximal cycling exercise protocol varied between men and women, whilst the protocol for maximal oxygen uptake ($\dot{V}O_{2max}$) was the same for both sexes. All submaximal and $\dot{V}O_{2max}$ exercise was conducted on an electronically-braked cycle ergometer (Lode, Excalibur, Groningen, Netherlands) with participant-specific set up for the seat, handle bars and pedals in a standard laboratory environment (18–22°C) with a fan-generated airflow of 19 $km.h^{-1}$. The submaximal protocol for women consisted of four consecutive 6-minute incremental stages of cycling exercise, at 100W, 125W, 150W and 175W, whereas, for men, it was 100W, 150W, 200W and 250W. $\dot{V}O_2$ was measured during the last two minutes of each stage. Following ten minutes rest from the submaximal exercise, the $\dot{V}O_{2max}$ test was conducted. The test started at 100W for both sexes and increased 25W per minute until volitional exhaustion. The total duration of the $\dot{V}O_{2max}$ was less then 14 minutes for both sexes. The linear relationship between oxygen consumption and power output was used to extrapolate the workload of 75% $\dot{V}O_{2max}$ during all of the self-paced trials in the heat (**Chapter Five, Six and Seven**), according to this equation: $W = LF.(RPM)^2$ (Jeukendrup *et al.*, 1996), where W = workload, LF = linear factor and RPM = cadence.

4.5.4: Body temperature measurement

Rectal temperature was used as an index of core temperature. Each participant was instructed to self-insert a calibrated rectal thermistor (Covidien Mon-a-Therm, USA; accurate to 0.1°C) 12 cm beyond their anal sphincter. Skin temperature was measured using four calibrated skin thermistors (Grant Instrument Ltd, Cambridge, UK; accurate to 0.2°C) at the chest, forearm, hamstring and calf region. Each skin thermistor was secured using surgical tape (3M, Healthcare, USA). Weighted skin temperature was calculated using the following equation: $\bar{T}_{sk} = 0.3(T_{Chest} + T_{Forearm}) + 0.2(T_{thigh} + T_{Calf})$ (Ramanathan, 1964). Both rectal and skin thermistors were connected to squirrel data loggers (Measurement Advantage, USA) and analysed and displayed by data acquisition software (TracerDAQ, Measurement Advantage, USA) at a sampling rate of one second. To account for the relative influences of T_{core} and T_{sk} on the activation of heat loss responses (Hertzman *et al.,* 1952), mean body temperature ($\bar{T}_b$) was calculated as: $0.8 \, . \, T_{rec} + 0.2 \, T_{sk}$ (Hardy and Stolwijk, 1966) (Stolwijk & Hardy, 1966).

4.5.4.1: Validity of the measurements

Rectal temperature to measure core temperature is only one of the many indices that can be used to reference core temperature, however as one of the primary dependent variables of this thesis, it is therefore worthwhile providing a justification of why rectal temperature was used.

The pulmonary artery is considered to be the gold-standard of core temperature measurement, since this artery brings blood from the core to the surrounding tissues (Moran and Mendal, 2002). It is administered by inserting a catheter into the pulmonary artery. However, since this method is highly invasive and requires expensive apparatus and medical expertise, it is not a practical method for measuring core temperature during exercise.

Oesophageal temperature has similar accuracy to the rectal temperature. It responds rapidly to small changes in blood temperature and therefore, it is regarded as the best site for measuring core temperature (Sawka *et al.*, 2011). However, this site is not practical in many settings, due to difficulties inserting the probe, irritation to the nasal passage and subject discomfort (Moran and Mendal, 2002).

Taking all information together, rectal temperature remains a better method than the aforementioned methods simply because it is more acceptable in nature. Furthermore, rectal temperature and oesophageal temperature achieve similar validity and therefore, both are

reliable when representing core temperature at rest or during exercise (ICC: 0.90; r^2= 0.81) (Mündel et al., 2014).

4.5.5: Thermodynamics calculations

Heat stress compensability was estimated using the heat strain index (HSI), with >1.0 indicating uncompensable heat stress (Cheung *et al.*, 2000). HSI was calculated as the ratio of the required evaporative cooling for heat balance (E_{req}; in W.m^{-2}) and the maximal evaporative capacity of the environment (E_{max}; in W.m^{-2}) (Belding and Hatch, 1955). E_{req} was calculated by the following equation:

$$E_{req} = M - W \pm (C + R) \pm (C_{res} - E_{res})$$

where M is the rate of metabolic heat production (W.m^{-2}), calculated as follows (Kenney, 1998):

$$\dot{M} = (352(0.23RER + 0.77)\,\dot{V}O_2)/BSA$$

W is the rate of energy lost as external work (W.m^{-2}). $C + R$ is the rate of heat transfer from convection (C; W.m^{-2}) and radiation (R; W.m^{-2}), calculated as the sum of:

$C = hc(T_{Sk} - T_A)$ and $R = 4.7(T_A - T_{Sk})$ (Kenney, 1998),

where hc is the convective heat transfer coefficient (W.m^{-2} °C) (Kerslake, 1972) and T_A is the ambient temperature (°C). $C_{res} + E_{res}$ is the rate of respiratory conductive (C_{res}) and evaporative (E_{res}) heat transfer, and was calculated as follows (Kenney, 1998):

$$C_{res} + E_{res} = (0.0012 \cdot M \cdot (34 - T_A)) + (0.0023 \cdot M \cdot (44 - P_A)) \quad (11)$$

where P_A is ambient vapour pressure (kPa). E_{max} was calculated as

$$E_{max} = LR * hc * (P_{Sk} - P_A)$$

where LR is the Lewis Relation (16.5°C kPa) and P_{Sk} is the saturated vapour pressure at the skin (in kPa). In addition, the rate of evaporative heat loss (E; in W.m^{-2}) was estimated according to the following equation (Kerslake, 1972):

$$\dot{E} = (P_{Sk} - P_A) \cdot \sqrt{v} \cdot 124$$

where v is air velocity (0.5 ms^{-1}). The rate of body heat storage (S, in W.m^{-2}) at each recording interval was calculated as follows:

$$\dot{S} = \dot{M} - \dot{W}_k + \dot{E} + \dot{C} + \dot{R} + \dot{C}_{res} + \dot{E}_{res}$$

4.5.6: Cardiovascular measurements

4.5.6.1: Heart rate

Heart rate was measured using a heart rate monitor (Polar Vantage XL, Polar Electro, Kempele, Finland) by detection of the R–R interval; heart rate was displayed on a heart rate monitor watch (Polar Vantage XL, Polar Electro, Kempele, Finland).

4.5.6.2: Blood pressure measurement

Blood pressure (BP) was measured using a stethoscope (Littmann, 3M, USA) and a sphygmomanometer (ALPK2, Japan) over the right brachial artery at heart level, in duplicate and by the same experienced operator. Mean arterial pressure (MAP) was calculated as diastolic blood pressure + 1/3 pulse pressure.

4.5.6.3: Cardiac output and stroke volume

Cardiac output ($\dot{Q}$) was obtained using indirect Fick's Equation: $\dot{V}CO_2 = \dot{Q}$ /A-VCO_2. $\dot{V}CO_2$ was measured by the metabolic cart as described previously (**Section 4.5.2**). A–VCO_2 represents the arteriovenous carbon dioxide concentration differences and was estimated using venous and arterial PCO_2 according to McHardy (1967). $ETCO_2$ was measured as a surrogate of arterial PCO_2 prior to the rebreathing procedure, whilst venous $ETCO_2$ was obtained after the rebreathing procedure, using Defares (1958) rebreathing method and corrected according to Paterson and Cunningham (1976). For the rebreathing procedure, the participant was asked to rebreathe from a 4 L anaesthesia bag (Kruuse, Demark) filled with a gas mixture containing 35% O_2 and 4% CO_2. In order to prevent hypoxemia, the bag was filled with a high concentration of O_2 and at least three times the participant's tidal volume, as obtained from the previous section (**Section 4.5.2**). When the baseline $ETCO_2$ values were stable, a three-way stopcock connected to the anaesthesia bag was opened and the subject rebreathed from the anaesthesia bag until the capnograph tracing plateaued. All of the end tidal gases were analysed by a gas analyser (AD Instrument, Australia) and acquired by the data acquisition system (PowerLab, AD Instrument, Australia) and displayed using Labchart Software (PowerLab, AD Instrument, Australia) in real time, as well as in an offline analysis. Stroke volume was obtained by dividing cardiac output by heart rate.

4.5.6.3.1: Golden standard for cardiac output measurement and validity for CO_2 rebreathing

The thermodilution method is regarded as the golden standard for cardiac output measurement. It is achieved by inserting a Swan–Ganz catheter into the pulmonary artery. The Swan–Ganz catheter has at least two lumens with a thermistor at the distal end of the catheter, to detect changes in blood temperature. Once the catheter is inside the pulmonary artery, a 5% ice saline is injected through the proximal port and cardiac output is calculated as the area under the curve of the change in blood temperature, according to the modified Stewart–Hamilton Equation (Reuter *et al.*, 2010). However, this procedure is highly invasive and can result in severe complications, like pneumothorax, dysrhythmias and perforation of the heart (Lavdaniti, 2008) and therefore it is not a suitable method for this thesis.

The CO_2 rebreathing method by Defares (1958) is a reliable method to use in this study as it has high reliability in measuring thermodilution (Pearson Correlation: r = 0.81) (Beekman *et al.*, 1984). Furthermore, it has been used in heat stress exercise (Schlader *et al.*, 2011a) as well as in severely ill patients (Franciosa, 1977). Collectively, this provides a good justification on why the CO_2 rebreathing method was selected for this thesis.

4.5.7: Blood flow measurement

Forearm blood flow can be measured by either venous occlusion plethysmography or by laser Doppler flowmetry. Each method has its advantages and its limitations. The purposes of this section are: 1) To describe the measurement procedure for each instrument, as both instruments were used for data collection of this thesis; 2) to provide a justification on why venous occlusion was selected for **Chapters Five** and **Six**, whilst laser Doppler flowmetry was selected for **Chapters Seven** and **Eight.**

4.5.7.1: Venous occlusion plethysmography

Venous occlusion plethysmography (VOP) was used to measure cutaneous perfusion in **Chapters Five** and **Six**. The basis of this technique is that, when the venous drainage of the forearm is transiently occluded, the increase of volume expansion from the occluded forearm is directly related to the arterial inflow and thus provides an indication of cutaneous perfusion. To detect the degree of forearm volume expansion, a calibrated mercury-filled strain gauge (Hokanson, USA) was secured on the widest part of the forearm with the surgical tape (3M,

USA) and was attached to a custom-built amplifier, with its voltage output being analysed by the data acquisition system (PowerLab, AD Instrument, Australia). This procedure began by suspending the forearm at the heart level and quickly inflating a pressure cuff from the upper arm to 50 mmHg, to occlude forearm venous blood flow. Subsequently, volume expansion from the forearm was detected by the mercury strain gauge and an electrical signal was generated. The electrical signal was processed by the data acquisition software (PowerLab, AD Instrument, Australia) with a low pass filter of 50 Hz. Once the signal plateaued, the pressure cuff was deflated and removed from the participant.

VOP is a reliable method to measure cutaneous perfusion as it gives an actual measurement unit instead of an arbitrary unit. However, VOP does not permit real time display and so it does not support experiments involving continuous measurement of cutaneous perfusion during exercise in the heat. However, since the purpose for **Chapters Five** and **Six** was to quantify the actual cutaneous perfusion between dry and humid heat at separate time points in different menstrual phases, the use of OCP was appropriate. Forearm vascular resistance was calculated as forearm blood flow/MAP.

4.5.7.2: Laser Doppler flowmetry

Cutaneous perfusion was measured by laser Doppler flowmetry (Moor Instrument, UK) in **Chapters Seven** and **Eight**. The reason for selecting laser Doppler flowmetry was because the experimental design for **Chapters Seven** and **Eight** required a continuous trace of the cutaneous perfusion throughout the exercise in dry and humid heat. This could not have been achieved without using laser Doppler, as VOP does not support this feature. The principles of laser Doppler flowmetry are based on the Doppler shift in frequency from a moving erythrocyte towards the laser light. In particular, a higher Doppler frequency from the erythrocyte indicates a higher perfusion rate of the underlying skin surface. To achieve this Doppler shift, two calibrated laser Doppler probes were affixed to the widest part of the forearm and at the back, by an adhesive ring (3M, USA), taking care to avoid the vein. Then, a laser light (785nm ± 10) was emitted from the laser diode of the monitor and transmitted via the optic probe heat into the skin tissue. The laser light scattered across the skin tissue and the erythrocyte. The collision of laser light and the movement of the erythrocyte resulted in a Doppler shift in frequency; this shift in frequency was detected by the photo receiver from the optic cable and underwent analogue and digital signal processing to produce the flux of the

red blood cell. The flux of the red blood cell was then used as an index of cutaneous perfusion. The net flux of the erythrocyte was calculated according to the following equation:

$\mathbf{k_1}\int_{\boldsymbol{\omega 1}}^{\boldsymbol{\omega 2}} \boldsymbol{\omega}\mathbf{P}(\boldsymbol{\omega}).\frac{\mathbf{d}\,\boldsymbol{\omega}}{\boldsymbol{DC^2}} - \boldsymbol{Noise}$ where K_1 is the scaling constants used for calibration, $\boldsymbol{\omega 1}$ and $\boldsymbol{\omega 2}$ are lower and upper bandwidth limits, $\mathbf{P}(\boldsymbol{\omega})$ represents the optical power density at the Doppler frequency shift $\boldsymbol{\omega}$, DC is the light intensity and noise represents the dark and short noise components.

However, laser Doppler cannot give an actual value of flow rate as it is based on the Doppler shift of the erythrocyte and its output can only be applied within the area of investigation. That is, it cannot give an actual value for the entire segmental blood flow as the value varies at different forearm sites (Johnson *et al.*, 1984). Therefore, it is suggested that laser Doppler and venous occlusion plethysmography are combined together when it comes to the investigation of segmental blood flow.

4.5.7.3: Agreement between venous occlusion plethysmography and laser Doppler flowmetry

Laser Doppler and venous occlusion plethysmography share the same validity as each other and therefore both are accurate methods for detecting cutaneous perfusion (Johnson *et al.*, 1984, Høyer *et al.*, 2013). However, the selection of either laser Doppler or venous occlusion plethysmography is solely dependent on the research questions, as mentioned in the previous section (**Section: 4.5.7.2)**.

4.5.8: Sudomotor measurement

To assess sudomotor responses, all experimental chapters in this thesis include both measures of whole body sweat rate and local sweat rate (LSR). This is because whole body sweat rate gives an index of the total sweat production during the exercise, whilst local sweat rate represents the local effect of sweating. By including both measurements together, it gives a clear picture of how sweating is affected by endogenous and exogenous female reproductive hormones and whether it is different between fixed and self-paced exercise, in both dry and humid heat environments.

4.5.8.1: Whole body sweat rate

Whole body sweat rate (WBSR) was calculated using nude weight change before and after the heat stress and corrected for total fluid consumption (as no urine production occurred). Specifically, it was calculated according to this equation:

Whole body sweat rate (L/hour) = (Pre-nude weight (kg) – Post-nude weight (kg) + fluid intake (kg))/Time (hour)

where pre-nude weight is the body weight before the trial and post-nude weight is the body weight after the trial. Time refers to the total time that is spent in the heat chamber. The total time is the same (1 Hour) for all Experimental chapters.

4.5.8.2: Local sweat rate

Local sweat rate (LSR) was measured using a ventilated capsule (Graichen *et al.*, 1982). The capsule (3.5 cm^2) was secured to the neck dorsally and ventilated with dry air at 0.4 litres min^{-1}. The effluent gas was sensed by capacitance sensors that consisted of a temperature (Honeywell Ltd, New Zealand) and humidity sensor (National Semiconductor, Santa Clara, CA, USA). Together, these two sensors transformed the change in humidity and temperature inside the sealed capsule into a voltage output, which was acquired by the data acquisition system (PowerLab, AD Instrument, Australia) to enable local sweat rate calculation. Prior to the experiment, the humidity sensor was calibrated using a two-point calibration, using dry gas (0% humidity) as the low point and fully saturated air (100% humidity) as the high point. Likewise, the temperature sensor used room air (20°C) as the low point and a heated temperature of 38 °C as the high point. The neck was used because all limbs were used for other measures and it was not exposed directly to the fan.

4.5.9: Perceptual measures

Borg's rating of perceived exertion (RPE) was measured using the 15-grade scale, from six to 20 (Borg, 1970), whilst thermal sensation (TS) and discomfort (TD) were measured using seven and four point scales, as described by Gagge *et al.* (1967). Skin wetness and pleasant were measured using the 13 grade scale from -3 to +3 according to (Filingeri *et al.*, 2015, Attia, 1984).

All the perceptual forms are outlined in the appendix (See **APPENDIX 3-7**).

4.6: Statistical analysis

All statistical analyses were completed using SPSS software for windows (IBM SPSS Statistics 20, NY, USA). Descriptive values are reported as means and standard deviation (SD) unless stated otherwise. Homogeneity of variance was examined using Levene's test and the normality of the data was examined using the Kolmogorov–Smirnov Test. Generally, all data were analysed with ANOVA, but were different in terms of their models, as different experimental chapters focus on different research questions. Therefore, specific ANOVA procedures are outlined in each experimental chapter. In order to examine thermoeffector responses, mean body temperature (Tb) was plotted against either local sweat rate or vasomotor response and was analysed using a simple linear regression [$y = y0+a.x$]. The onset threshold was defined as the *y*-intercept ($y0$) of the regression line, with values at baseline, while thermosensitivity was defined as the slope. Statistical significant level was set at $p \leq 0.05$.

Chapter Five

5.0: Influence of menstrual phase and thermal profile on autonomic and behavioural thermoregulation during exercise in trained women

Abstract

This chapter studied thermoregulatory responses of ten well-trained [$\dot{V}O_{2max}$, 57 (7) mL.min^{-1}.kg^{-1}] eumenorrheic women exercising in dry and humid heat, across their menstrual cycle. They completed four trials, each of resting and cycling at fixed intensities (125 and150 W), to assess autonomic regulation, then self-paced intensity (30 min work trial), to assess behavioural regulation. Trials were in early-follicular (EF) and mid-luteal (ML) phases in dry (DRY) and humid (HUM) heat matched for wet bulb globe temperature (WBGT, 27°C). During rest and fixed-intensity exercise, rectal temperature was~ 0.2°C higher in ML than EF (p < 0.01) independent of environment (p = 0.66). Mean skin temperature did not differ between menstrual phases ($p \geq 0.13$) but was higher in DRY than HUM (p < 0.01). Local sweat rate and/or forearm blood flow differed as a function of menstrual phase and environment (interaction: $p \leq 0.01$). Exercise performance did not differ between phases [EF: 257 (37), ML: 255 (43) kJ, p = 0.62], but was 7 (9)% higher in DRY than HUM [263 (39), 248 (40) kJ; P < 0.01] in conjunction with equivalent autonomic regulation and thermal strain but higher evaporative cooling [16(6) W m^2; p < 0.01]. In well-trained women exercising in the heat: (1) menstrual phase did not affect performance, (2) humidity impaired performance due to reduced evaporative cooling despite matched WBGT and (3) behavioural responses nullified thermodynamic and autonomic differences associated with menstrual phase and dry *vs.* humid heat.

5.1: Introduction

In eumenorrheic women the approximate monthly rhythm of the reproductive cycle is divided into follicular and luteal phases based on the function of the uterus and ovary, and corresponding fluctuations in hormonal concentrations (Stephenson and Kolka, 1993b). Progestogens and oestrogens, the steroidal ovarian hormones, influence several non-reproductive organs and systems including thermoregulation (Charkoudian and Stachenfeld, 2014).

Oestrogens generally promote heat dissipation and lower body temperature whereas progestogens have the opposite effect (Charkoudian and Stachenfeld, 2014). Core body temperature (T_{core}) is regulated approximately 0.3–0.5°C higher during the luteal phase (Harvey and Crockett, 1932, Stephenson and Kolka, 1993a). The notion of a shift in thermoregulatory set-point is supported by an elevated T_{core} at rest and during passive and active heat stress, and by an increased T_{core} threshold for thermoregulatory effector responses such as sweating and cutaneous vasodilatation (Stachenfeld *et al.*, 2000, Kuwahara *et al.*, 2005a, Kuwahara *et al.*, 2005b). This shift in set-point and threshold results in higher T_{core} during the luteal phase particularly when women exercise with environmental heat stress (Avellini *et al.*, 1979, Carpenter and Nunneley, 1988, Kolka and Stephenson, 1997b, Tenaglia *et al.*, 1999, Janse *et al.*, 2012), leading several authors to suggest that women should avoid competition or face a disadvantage when performing exercise with environmental heat stress during their luteal phase (Stephenson and Kolka, 1993b, de Jonge, 2003, Charkoudian and Joyner, 2004, Janse *et al.*, 2012). Yet there is a need for norms and recommendations specific to these differing exercise responses (Charkoudian and Joyner, 2004), especially as there remains an under-representation of women in sport and exercise research (Costello *et al.*, 2014) and that > 40% of women believe that their menstrual cycle has a negative impact on training and performance (Bruinvels *et al.*, 2016). Only five published investigations appear to have tested the notion of a reduced exercise heat-stress tolerance or performance during the luteal phase of the menstrual cycle. Two earlier investigations, which employed a fixed-intensity (constant power) approach, identified that exercise heat stress tolerance was reduced by ~11 and ~16% during the mid-luteal compared to early follicular phase (Avellini *et al.*, 1980, Tenaglia *et al.*, 1999), whereas Kolka and Stephenson (1997) found no difference between early and late follicular or mid-luteal phases. More recently, Sunderland and Nevill

(2003) demonstrated that high-intensity intermittent running performance in the heat remained unaltered between the mid-follicular and mid-luteal phases, whereas Janse *et al.* (2012) reported a ~6% reduction in endurance time in the mid-luteal phase during an incremental test to exhaustion in the heat.

From a thermoregulatory standpoint, the limited and conflicting results above cannot be directly applied to a well-trained, competitive woman for several reasons. First, training status markedly alters effector responses, with trained women demonstrating enhanced sweating and cutaneous vasodilatation compared to untrained women, and aerobic training *per se* improving these effector responses (Roberts *et al.*, 1977, Drinkwater, 1984, Kuwahara *et al.*, 2005a, Kuwahara *et al.*, 2005b). Trained and competitive female endurance athletes typically display a maximal aerobic uptake ($\dot{V}O_{2max}$) of > 3 l min^{-1} or > 55 ml kg^{-1} min^{-1}(Drinkwater, 1984), so it can be reasoned that in only two (Avellini *et al.*, 1980, Sunderland and Nevill, 2003) of the above-mentioned investigations would participants fit these criteria. Second and relatedly, trained women have reduced reproductive hormone concentrations and fluctuation between menstrual phases and associated smaller difference in the bi-phasic T_{core} (Dale *et al.*, 1979, Bullen *et al.*, 1984, Kuwahara *et al.*, 2005a, Kuwahara *et al.*, 2005b). Indeed, Kuwahara *et al.* (2005a), Kuwahara *et al.* (2005b) have observed less phase-related differences in effector and T_{core} responses for trained than untrained women. Third, all previous investigations have used a bout of fixed-intensity (constant power), sub-maximal exercise to exhaustion as their mode of investigation. These protocols have poor face-validity, but more importantly they deprive us of our most effective, powerful and nearly limitless(Benzinger, 1969b, Parsons, 2014) form of thermoregulation: behaviour.

During (face-valid) exercise heat stress when able to self-pace (variable intensity), it has been demonstrated that men 'behave' by reducing exercise intensity, and therefore metabolic heat production, which modifies heat exchange and allows for an improved compensability of the thermal environment principally via a reduction in required evaporation, that ultimately results in a reduced thermoregulatory strain (Schlader *et al.*, 2011a, Schlader *et al.*, 2011c). There is, however, evidence from passive heat stress models indicating that such thermoregulatory behaviour is altered by the menstrual cycle, with reports that the threshold for an affective/behavioural response is shifted during the luteal phase (Cunningham, 1971, Scarperi and Bleichert, 1983, Shoemaker and Refinetti, 1996); this remains untested during exercise.

Forthcoming (at the time of writing) large international events (2016 Summer Olympics in Rio de Janeiro, 2018 Commonwealth Games on the Gold Coast, 2018 Asian Games in Jakarta, 2019 IAAF World Championships in Doha) will expose athletes to high levels of environmental heat stress and the number of women participating at this elite level is ever increasing, with the latest editions of the above-named international events reporting 39–44% of competitors as women. However, these environments differ in their ambient thermal profile, from warm-humid to dry-hot, with the latter usually permitting greater (full) evaporation of sweat whilst the former does not and thus high rates of evaporative cooling are not possible. Previous investigations have determined that when exposed to approximate environmental heat but humid *versus* dry in nature, women sweat less but more efficiently upon exercising when exposed to humid heat as demonstrated by similar T_{core} responses(Morimoto *et al.*, 1967b, Frye and Kamon, 1983) although no effect of the menstrual phase was apparent (Shapiro *et al.*, 1980a). However, the same limitations as discussed above apply to these studies (training status and fixed-intensity exercise) and these potential differences in thermoregulatory control across menstrual phase may interact with differences in the thermal environment (i.e. dry *versus* evaporative heat transfer), which would warrant examining in highly trained women (i.e. by virtue of higher heat loss requirement). Furthermore, to our knowledge no published investigation has compared how women perform when exposed to equivalent dry and humid heat during exercise.

From the above, it can be concluded that research into how well-trained women respond to environmental heat stress across the menstrual cycle is markedly sparse. This study sought to characterise and compare the behavioural and autonomic thermoregulatory responses of well-trained, eumenorrheic women to exercise when exposed to equivalent dry and humid heat stress during the early follicular and mid-luteal phase of their menstrual cycle.

5.2: Methods

5.2.1: Ethical approval

This study was approved by the Massey University Human Ethics committee: Southern A (14/99).

5.2.2: Participants

Ten eumenorrhoeic (Menstrual cycle length: 28-32 days cycle), aerobically well-trained and competitive women cyclists and triathletes volunteered for this study. Participants'characteristics were outlined in **Table 2** (**Section 4.1**).

5.2.3: Experimental overview

Season control was mentioned in **Section 4.3**. All participants attended the laboratory on six occasions: (1) preliminary submaximal and maximal tests, (2) experimental familiarization and (3–6) experimental trials. The four experimental trials were a full crossover of menstrual phase (early follicular and mid-luteal) and environment [dry and humid, at matched wet bulb globe temperature (WBGT)]. All trials were counterbalanced except that the same order of dry or humid environment was retained for each menstrual phase within participants. Experimental trials were conducted at the same time of day (± 1 h), and following > 24 h of dietary and exercise control. Each trial consisted of 12 min fixed-intensity cycling followed immediately by a 30 min self-paced cycling performance trial. All exercise was on an electronically braked cycle ergometer (Lode Excalibur, Groningen, The Netherlands) with participant-specific set up for the seat, handle bars and pedals.

5.2.4: Preliminary testing and familiarisation

Submaximal and maximal capacity tests were undertaken in the follicular phase to minimise potential physiological effects of the menstrual cycle on $\dot{V}O_{2max}$ performance. The procedure and protocol for preliminary testing was outlined in **Section 4.53**.

At least 24 h following preliminary testing, familiarization trial was undertaken to ensure participants were accustomed to the experimental procedures and to minimise learning effects. These trials replicated entirely the experimental trials outline below.

5.2.5: Dietary and Exercise Control

Dietary and Exercise control were considered and were described in **Section 4.4**.

5.2.6: Menstrual cycle and type of heat stress

Participants were tested during the early follicular (EF) and mid-luteal (ML) phases, to maximise differences in oestrogen and progesterone concentrations and permit comparison with results of previous studies (Avellini *et al.*, 1980, Kolka and Stephenson, 1997b, Tenaglia *et al.*, 1999, Sunderland and Nevill, 2003, Janse *et al.*, 2012). Testing occurred on days 3 (1) and 6 (1) (EF), and 18 (2) and 21 (3) (ML) following start of menses, with 12 (2) days separating the second EF and first ML trials. Hatcher (1990) suggested that a progesterone level of > 9.5 nmol l^{-1} is good evidence that ovulation has occurred. Using this criterion, three participants were excluded from all data analysis whilst another had lower [progesterone] in her first ML trial (4.1 nmol l^{-1}). This participant's results were examined and found to be consistent in magnitude and direction with those of other participants. Furthermore, all statistical analyses were completed with and without this participant and omitting her had no impact on the magnitude or direction of statistical results other than reducing observed power. Therefore, her data was included in the final analyses for $n = 10$.

In accordance with previous studies investigating the influence of humid (HUM) *versus* dry (DRY) environmental heat in women (Morimoto *et al.*, 1967b, Shapiro *et al.*, 1980b, Frye and Kamon, 1983) heat stress was indexed using WBGT because, while limited, it is the most widely used empirical index (Brotherhood, 2008, Budd, 2008). This decision-making was guided by what typical or possible extreme conditions athletes would encounter at the 2016 Summer Olympics and 2018 Commonwealth Games (humid) compared to the 2019 IAAF World Championships (dry), so a WBGT equivalent to 27°C was chosen to elicit our HUM [29 (1)°C, 81 (3) % relative humidity] and DRY [34 (0.2)°C, 41 (3) % relative humidity] environments. Absolute humidity in these two environments was 3.4 (0.1) and 2.2 (0.3) kPa, respectively. Within each menstrual phase, exposure to DRY and HUM environments was separated by 3 (1) days.

5.2.7: Experimental procedure

These four sessions were conducted in the same environmental chamber with the 19 km.h^{-1} airflow mentioned above; however, the fan was turned off for each ~2 min data collection period (of each 6 min stage/interval) to minimise interference of airflow on measurement. On arrival to the laboratory hydration status was confirmed by urine specific gravity as mentioned in **Section 4.4,** nude weight was recorded, and then they self-inserted a rectal thermistor. A blood sample was obtained from the antecubital vein, following which participants entered the environmental chamber wearing only cycling shorts and top, shoes and socks. Participants rested seated on the ergometer for 20 min during which they were instrumented and baseline measurements were recorded. Participants then completed 6 min cycling at each of 125W and 150W, to allow sufficient warm-up and fixed-intensity responses to be recorded. Physiological measurements taken during the final 2 min of each intensity included expired gas, heart rate (HR), blood pressure (BP), forearm blood flow (FBF), and cardiac output ($\dot{Q}$), whilst rectal (T_{rec}) and skin ($\bar{T}_{sk}$) temperatures as well as local sweat rate (LSR) were measured continuously. Immediately on completion of the 150 W bout, the ergometer was set to linear mode as it was mentioned in the previous section (**Section 4.5.3**), where participants were instructed to perform as much work as possible over 30 min. During this 30min self-paced period, work completed (kJ), HR, and expired gas were recorded every 6 min, whilst T_{rec}, $\bar{T}_{sk}$ and LSR were measured continuously. Total work completed was used as our performance criterion, whereas the time profile of power output was used as our behavioural. Water was recorded and supplied ad libitum throughout the entire heat stress exercise.

5.2.8: Measurements

The details of procedures for anthropometric, respiratory, cardiovascular, body temperature, sudomotor, and biophysics measures were clearly outlined in **Chapter four (**Anthropometric: **Section 4.5.1**; Respiratory: **Section: 4.5.2**; cutaneous blood flow: **Section 4.5.7.1**; Cardiovascular: **Section 4.5.6**; Body temperature: **Section: 4.5.4**; Sudomotor: **Section 4.5.8**; Biophysics: **Section 4.5.5**).

5.2.8.1: Hormonal analysis

Blood was collected by venipuncture into a vacutainer (Becton-Dickinson, Oxford, UK) containing clot activator. Following inversion and clotting, the whole blood was centrifuged at 4°C and 805 *g* for 12 min and aliquots of serum were transferred into Eppendorf tubes

(Genuine Axygen Quality, USA) and stored at −80 °C until further analysis. Serum samples were analysed using enzyme-linked immune assays for 17β-oestradiol (Demeditec Diagnostics, Kiel, Germany) and progesterone (IBL International, Hamburg, Germany) with a sensitivity of 22.7 pmol l^{-1} and 0.14 nmol l^{-1}, respectively, and an intra-assay variation of 4 and 6%, respectively.

5.2.9: Statistical analysis

Basic descriptive statistic and the normality of the data were outlined in **section 4.5.10**. The data for this experimental chapter were analysed by three-way (menstrual phase × heat stress × time) ANOVA for repeated measures. Resting and fixed-intensity exercise data were analysed separately from self-paced exercise data. Sphericity was assessed and where the assumption of sphericity could not be assumed, adjustments to the degrees of freedom were made ($\varepsilon > 0.75$ = Huynh-Feldt; $\varepsilon < 0.75$ = Greenhouse-Geisser). Where main or interaction effects occurred, *post hoc* pairwise analyses were performed using a paired samples *t*-test (Bonferroni correction where relevant), with statistical significance set at $P \leq 0.05$. To examine how menstrual phase and type of heat stress affected the thermal control of the effector responses (LSR and FBF), the linear regression method was used as described clearly in **Section 4.5.10** and compared using two-way (menstrual phase × heat stress) ANOVA. Given its putative role in the shift in thermoregulatory set-point and threshold for effector responses between menstrual phases, we sought to further determine whether [progesterone] was associated with or predicted the key variables of exercise performance and resting T_{rec}, FBF and LSR. First, we described the form and strength of bivariate association using Pearson's correlation coefficient for both the absolute values and change (Δ) between menstrual phases. Next, we used hierarchical multiple regression to estimate the effect of [progesterone] on these key variables while controlling for potential confounding from [oestrogen].

5.3: Results

5.3.1: Ovarian Hormone concentrations

Progesterone [EF: 1.7 (1.2) *vs* ML: 53.5 (51.8) nmol l^{-1}] and 17β-oestradiol [EF: 185 (166) *vs* ML: 364 (259) pmol l^{-1}] concentrations were significantly higher in the luteal phase (both $p < 0.001$) but not different for each environment ($p = 0.43$ and $p = 0.24$, respectively).

5.3.2: Exercise performance and behaviour

Work capacity was similar between menstrual phases [EF: 257 (37) *vs* ML: 255 (43) kJ, $p = 0.62$] but was 7 (9) % higher in DRY than in HUM [263 (39) *vs* 248(40) kJ; $p = 0.001$] (Fig. 14). Accordingly, mean power output was unaffected by menstrual phase ($p = 0.87$) but 5 (8) % higher in DRY than in HUM [146 (22) *vs* 139 (22) W; $p < 0.01$]. When viewing behaviour as the self-paced exercise profile, behaviour differed between environments as a function of menstrual phase (environment × phase × time: $p = 0.03$). Specifically, participants reduced workload more rapidly in HUM, which was more pronounced in EF than in ML.

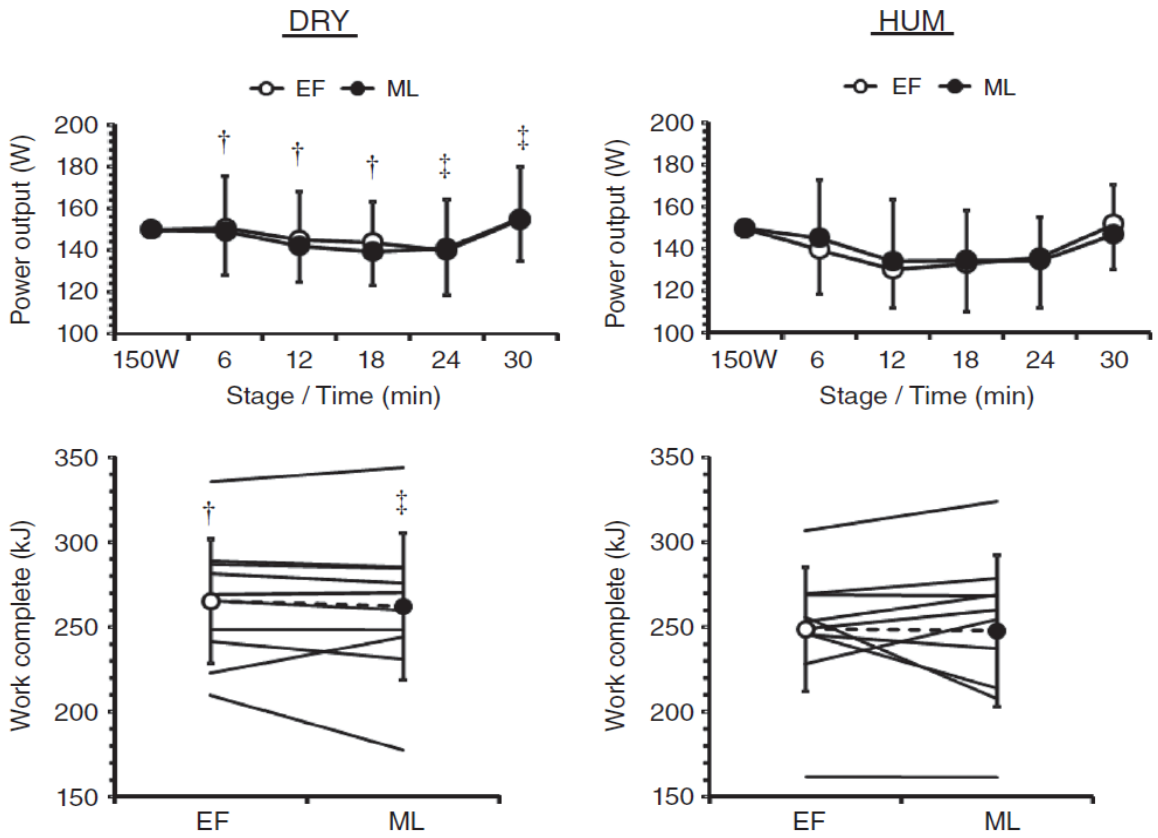

Figure 12: Mean (SD) power output (n=10) and individual and mean (SD) work capacity (n=10) during exercise in dry (DRY) and humid (HUM) heat during the early follicular (EF) and mid-luteal (ML) phase.

†Significant difference between corresponding EF-HUM value, ‡ Significant difference between corresponding ML-HUM value.

5.3.3: Thermoregulatory measures

5.3.3.1: Body temperature

T_{rec} when resting was 0.21 (0.14)°C higher in ML than in EF ($p < 0.01$) and remained higher by 0.16 (0.10)°C during fixed-intensity exercise ($p < 0.01$) (Fig. 15). The rise in T_{rec} throughout this exercise ($p < 0.01$) was not dependent on menstrual phase or environment (interaction: $p = 0.66$). During self-paced exercise T_{rec} differed between environments as a function of menstrual phase (environment × phase × time: $p = 0.02$). Specifically, the between-phase differences seen at rest [0.13 (0.14)°C] and fixed-intensity exercise [0.45 (0.24)°C] during DRY were not evident during self-paced exercise [−0.04 (0.21)°C], whilst the resting [0.29 (0.29)°C] and fixed-intensity [0.25 (0.14)°C] differences persisted throughout self-paced exercise [0.23 (0.28)°C] during HUM. The rise in T_{rec} was smaller during ML than EF [1.23 (0.27) *vs*. 1.40 (0.18)°C, $p = 0.05$] but similar between environments [DRY: 1.34 (0.26) *vs*.HUM:1.28 (0.24)°C, $p = 0.55$].

Resting $\bar{T}_{sk}$ was similar between menstrual phases ($p = 0.13$) but was 1.3 (0.5)°C higher during DRY than HUM ($p < 0.01$) (Fig. 15). During fixed-intensity exercise $\bar{T}_{sk}$ was similar between menstrual phases ($p = 0.26$) but differed between environments as a function of work-rate (environment × work-rate: $p = 0.01$) such that the difference between environments was halved to 0.7 (0.4)°C. During self-paced exercise $\bar{T}_{sk}$ differed between environments as a function of menstrual phase (environment × phase × time: $p = 0.04$). Specifically, end-exercise $\bar{T}_{sk}$ values were attained in the following order: EF-DRY > ML-DRY > ML-HUM > EF-HUM.

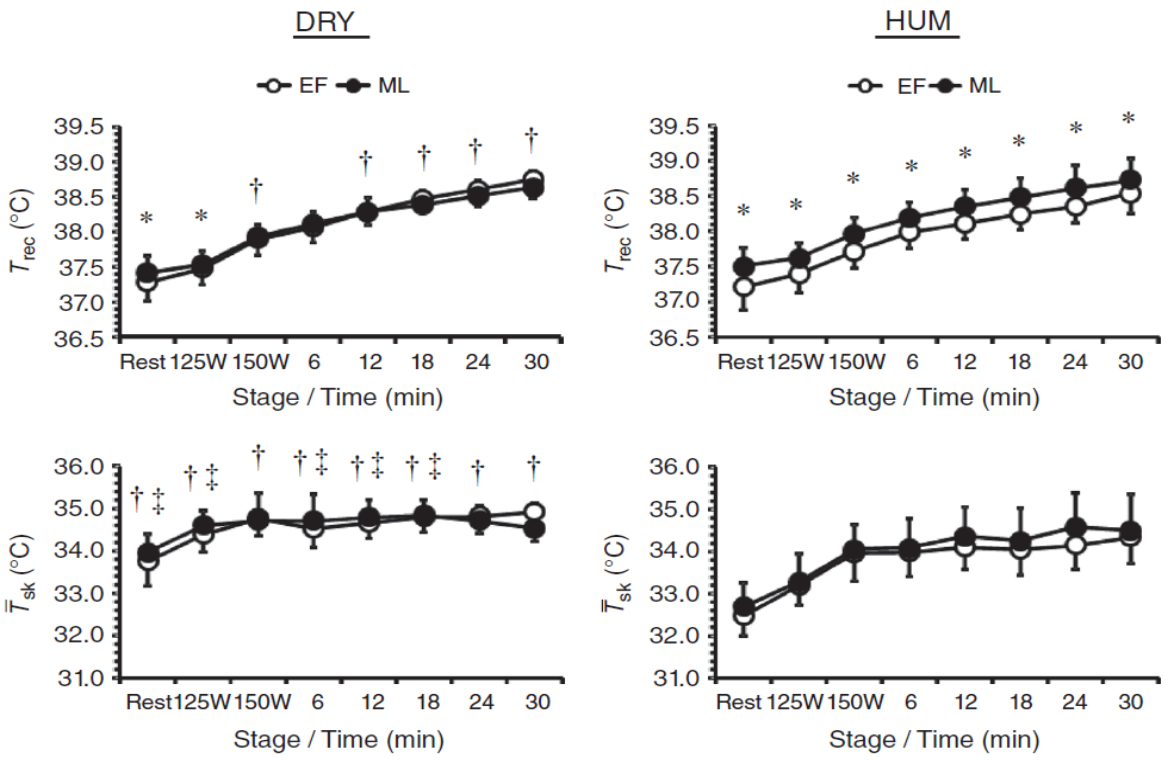

Figure 13: Mean (SD) rectal temperature (T_{rec}, n = 10) and weighted mean skin temperature $T_{\bar{sk}}$, n =10) during exercise in dry (DRY) and humid (HUM) heat during the early follicular (EF) and mid-luteal (ML) phase.

∗Significant difference between EF-ML within environment, †significant difference between corresponding EF-HUM value, ‡significant difference between corresponding ML-HUM value.

5.3.3.2: Cardiovascular and thermoeffectors

Resting $\dot{Q}$, SV and MAP were similar between menstrual phases and environments (all p > 0.38) whereas resting FVR differed between environments as a function of menstrual phase (environment × phase: p = 0.02) being higher in ML-DRY than in EF-DRY and ML-HUM (Table 3 and Fig. 16). During fixed-intensity exercise $\dot{Q}$ and SV were similar between menstrual phases (both p > 0 .74) and environments (both p > 0.54) but increased above resting values before plateauing (both p < 0.01). During fixed-intensity exercise MAP differed between menstrual phases as a function of work-rate (phase × time: p = 0.03) whilst FVR differed between environments as a function of menstrual phase (environment × phase: p = 0.01) such that ML-DRY > EF-DRY > ML-HUM > EF-HUM. Resting LSR differed between environments as a function of menstrual phase (environment × phase: p = 0.01) such that EF-DRY was lower than ML-DRY and EF-HUM, whilst resting FBF was similar between menstrual phases (p = 0.15) but was lower in DRY than HUM (p < 0.01; Fig.16). During fixed-intensity exercise LSR was similar between menstrual phases and environments (both p > 0.14) but increased with work-rate (p < 0.01) whilst FBF differed between environments as a function of menstrual phase (environment × menstrual phase × work-rate: p = 0.01). During self-paced exercise LSR was similar between menstrual phases and environments (both p > 0.11) but increased over time (p < 0.01). Neither onset threshold nor thermosensitivity of the effector responses were affected by menstrual phase or environment (all p > 0.26). Water consumption was similar between menstrual phases and environments [806 (422) ml; both p > 0.14] whilst WBSR was similar between menstrual phases and environments [937 (262) g h^{-1}; both p > 0.42], resulting in a 1.6 (0.5) % loss of body mass that was similar between menstrual phases and environments (both p > 0.43).

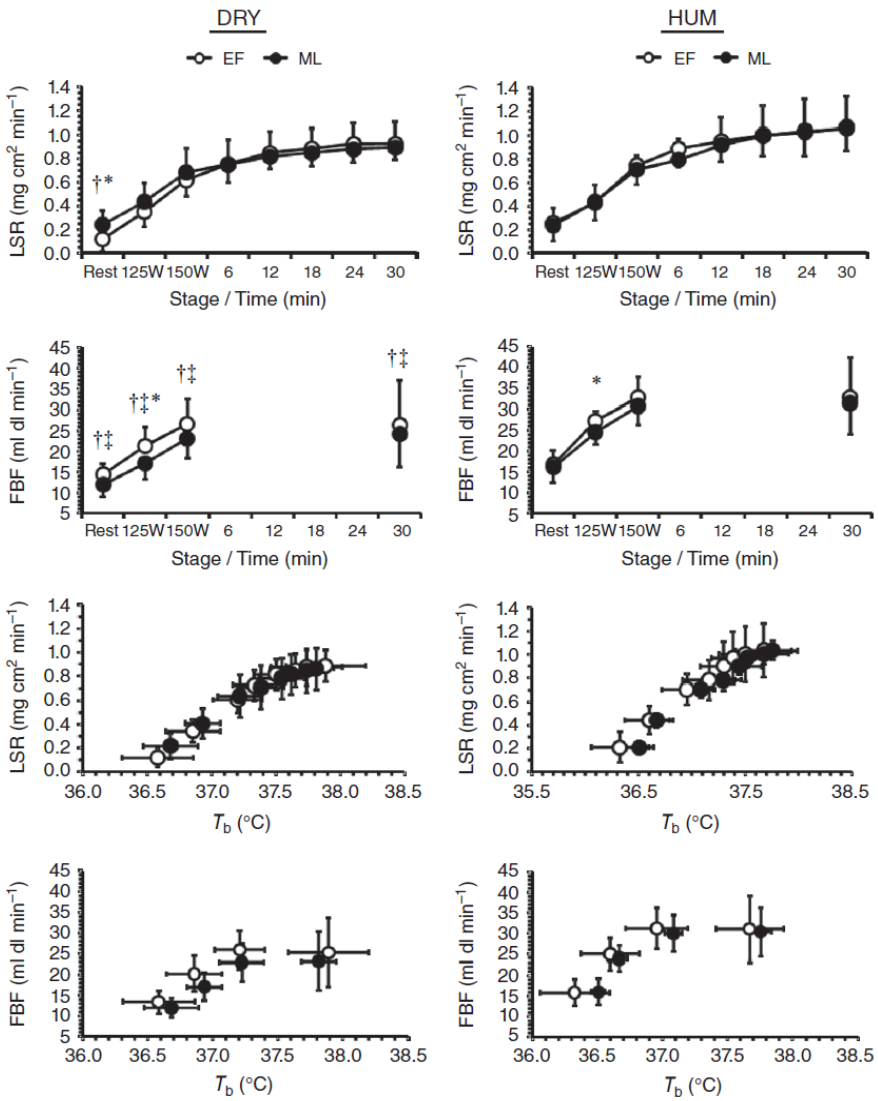

Figure 14: Mean (SD) local sweat rate (LSR, n=9) and forearm blood flow (FBF, n=10) against time and mean body temperature($\bar{T}$b) during exercise in dry (DRY) and humid(HUM) heat during the early follicular (EF) and mid-luteal (ML) phase**.**

∗Significant difference between EF-ML within environment, †significant difference between corresponding EF-HUM value, ‡significant difference between corresponding ML-HUM value.

5.3.3.3: Thermodynamics

Resting M was similar between menstrual phases and environments (both $p > 0.79$) whereas resting E was similar between menstrual phases ($p = 0.41$) but was 22 (10) $W.m^{-2}$ higher in DRY than HUM ($p < 0.01$), with negligible S (Fig. 17). Resting E_{max} was similar between menstrual phases ($p = 0.48$) but was 39 (16) $W.m^{-2}$ higher in DRY than HUM ($p < 0.01$) whereas E_{req} was similar between menstrual phases and environments (both $p > 0.48$), which meant that the HSI was similar between menstrual phases and environments (both $p > 0.44$). During fixed-intensity exercise M and E were similar between menstrual phases (both $p > 0.25$) but differed between environments as a function of work-rate (environment × time: both $p < 0.03$). As a result, S was similar between menstrual phases ($p = 0.58$) but was 35 (18) $W.m^{-2}$ lower in DRY than HUM ($p < 0.01$). During fixed-intensity exercise E_{max}, E_{req} and consequently HSI were similar between menstrual phases (all $p > 0.29$) but differed between environments as a function of work-rate (environment × work-rate: all $p < 0.04$). During self-paced exercise M, E and S were similar between menstrual phases (all $p > 0.67$) with only E being 16 (6) $W.m^{-2}$ higher in DRY than HUM ($p < 0.01$). During self-paced exercise E_{max}, E_{req} and consequently HSI were similar between menstrual phases (all $p > 0.39$) with only E_{max} being 27 (10) $W.m^{-2}$ higher in DRY than HUM ($p < 0.01$).

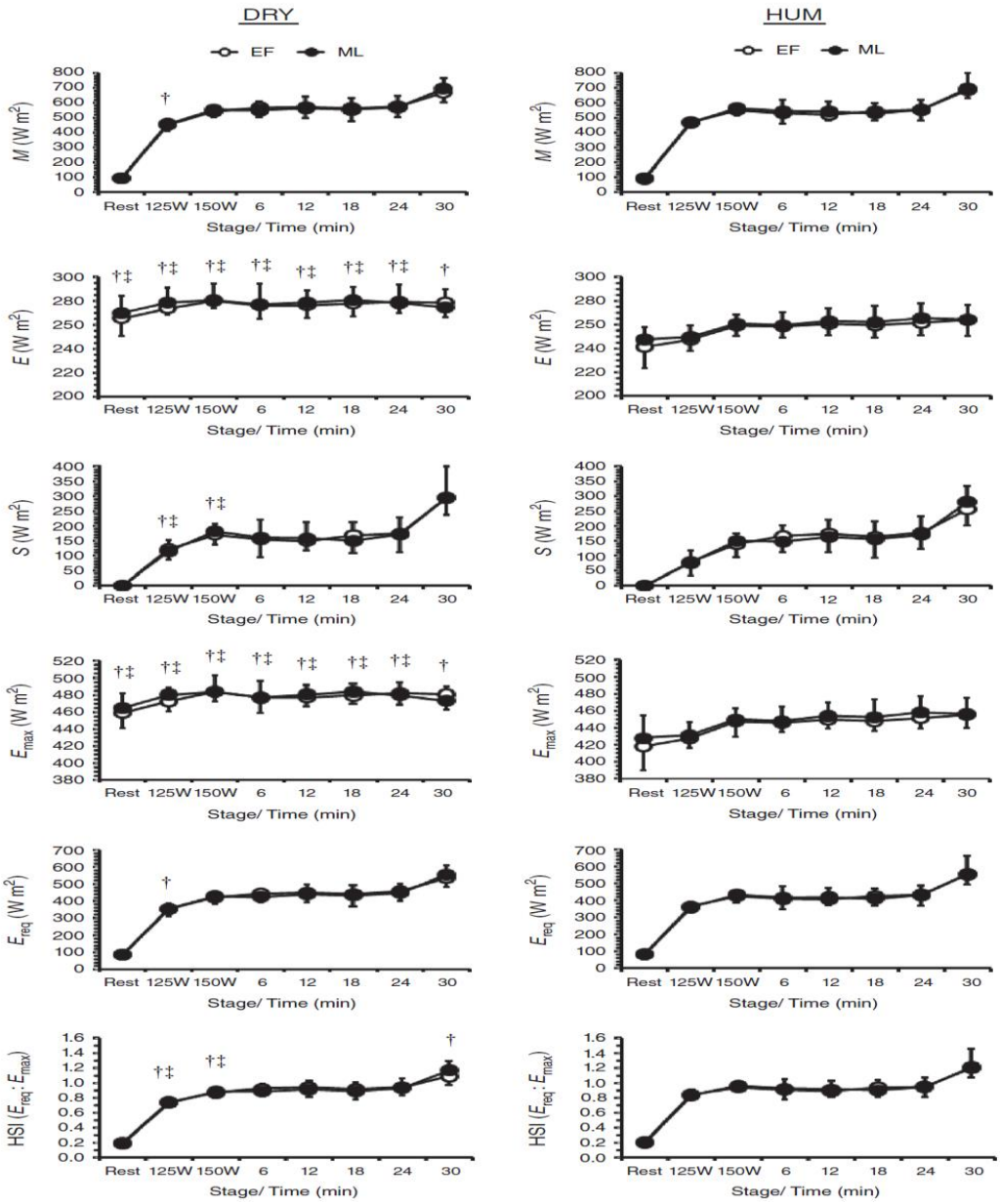

Figure 15: Mean (SD) rate of metabolic heat production (M, n = 9), rate of evaporative heat loss (E, n = 9), rate of heat storage (S, n = 9), maximal evaporative capacity of the environment (Emax, n = 9), required evaporative cooling for heat balance (Ereq, n = 9) and heat strain index (HSI, n = 9) during exercise in dry (DRY) and humid (HUM) heat during the early follicular (EF) and mid-luteal (ML) phase

∗Significant difference between EF-ML within environment, †significant difference between corresponding EF-HUM value, ‡significant difference between corresponding ML-HUM value.

Table 2: Mean arterial pressure (MAP, n=10), cardiac output($\dot{Q}$, n=8), forearm vascular resistance (FVR, n=10) and stroke volume (SV, n=8) at rest and during fixed-intensity exercise in dry(DRY) and humid(HUM) heat during the early follicular(EF) and mid-luteal(ML) Phase

	DRY						**HUM**					
	EF			*ML*			*EF*			*ML*		
	Rest	**125 W**	**150 W**	**Rest**	**125 W**	**150 W**	**Rest**	**125 W**	**150 W**	**Rest**	**125 W**	**150 W**
MAP (mmHg)	83(6)	93(8)*	100(7)	85(5)	95(5)*	100(4)	85(5)	94(6) *	102(6)	84(6)	94(9)*	99(9)
$\dot{Q}$ ($L\ min^{-1}$)	7(3)	22(4)*	21(4)	7(3)	20(4)*	22(3)	7(2)	21(5) *	21(5)	7(2)	20(5)*	23(4)
FVR ($mm\ Hg\ min\ dL^{-1}$)	5.8(1.3)	4.4(1.2)*	3.8(1.2)*	7.6(2.3)†	5.8(1.4)* †	4.4(0.8)*	5.2(1.1) †	3.5(0.4) *†	3.2(0.5)*	5.4(1.3) ‡	3.9(0.7) *‡	3.3(0.6) *‡
SV (mL)	98(32)	160(25)*	136(36)	103(37)	146(30)*	144(22)	97(36)	154(28)*	135(25)	94(30)	145(35)*	143(20)

Values are mean (SD).
∗Significant difference to preceding time-point.
†Significant difference to corresponding EF-DRY time-point.
‡Significant difference to corresponding ML-DRY time-point.

5.3.3.4: Correlation and regression analyses

The [progesterone] correlated with [oestrogen] in absolute terms (r=0.69, p<0.01) and in the extent of rise from EF to ML (r = 0.47, p = 0.04). The [progesterone] moderately predicted resting T_{rec} (r^2 = 0.14, β = 0.37, p = 0.02), while the rise in [progesterone] moderately to strongly predicted the rise in resting LSR (r^2 = 0.31, β = −0.55, p = 0.02), with no more than 4% of remaining variability in T_{rec} or LSR being accounted for by the rise in oestrogen.

5.4: Discussion

Research into how well-trained women respond to either DRY or HUM across the menstrual cycle remains sparse despite it being known that women sweat more efficiently than men, and that ovarian hormones exert multiple physiological effects including on sweating function. Therefore, this investigation characterised and compared the behavioural and autonomic thermoregulatory responses of well-trained, eumenorrheic women to exercise when exposed to equivalent dry and humid heat stress during the early follicular and mid-luteal phases of their menstrual cycle. The novel results are that: (1) self-paced exercise performance (i.e. total work) was not affected by menstrual phase but was impaired by HUM, (2) whilst the autonomic (thermoeffector onset thresholds and thermosensitivities) thermoregulatory responses were similar between menstrual phases and environments, the behavioural response (i.e. exercise pacing) differed between environments as a function of menstrual phase, and (3) the ovarian hormone concentrations and fluctuations between menstrual phases were not attenuated in these well-trained women relative to values previously reported in less-trained women. These results indicate that under the conditions of this investigation, trained women behaviourally thermoregulate to minimise autonomic differences but at the expense of their exercise performance under humid heat stress.

Performance was unaffected by menstrual phase in either environment.

The performance data (Fig. 14) support some earlier findings that menstrual phase does not affect heat-stress tolerance (Kolka and Stephenson, 1997b, Sunderland and Nevill, 2003), but are in contrast to other reports in which tolerance/performance was reduced by ~ 6–16% in ML compared to EF(Avellini *et al.*, 1980, Tenaglia *et al.*, 1999, Janse *et al.*, 2012). Importantly, however, these previous investigations did not allow participants to pace themselves and were performed to exhaustion, thereby limiting their ecological validity for performance requirements.

Thermoregulatory differences across menstrual phases and ambient conditions were nullified by thermoregulatory behaviour.

It has been demonstrated previously in men that reductions in work-rate in the heat are a form of behavioural thermoregulation that serve to improve heat exchange (Schlader *et al.*, 2011d) that cannot be achieved when the work-rate is constant (Schlader *et al.*, 2011a). The current results extend these observations to women. This is perhaps best illustrated by the impact of

changing from one exercise modality to the other (constant *vs.* variable intensity). For instance, at rest or during fixed-intensity exercise (125 or 150 W) the effector (LSR, FBF, FVR; Fig. 16 and Table 2) and thermodynamic (*M, S,* E_{req}; Fig. 17) responses display differences between menstrual phase and/or environments, but these differences disappear when allowed to self-pace. At its most pronounced this exercise behaviour constituted a reduction in power output ~31 W or ~12% of peak aerobic power (Fig. 14). Such a sustained (6min) reduction in work-rate resulted in a ~238 W m^2 lower *M* that required ~219 W m^2 less evaporative cooling for heat balance. It has been shown previously that total heat loss, predominantly from *E*, during exercise in compensable environments, such as those in this study (Fig. 17, HSI), is dependent on E_{req}, and *M* largely determines E_{req} (Gagnon *et al.*, 2013). Thus, it follows that in these participants the behavioural adjustments during exercise minimised autonomic differences (LSR, WBSR). The behavioural adjustments displayed by these participants in the current study (Fig. 14) display 'classic' pacing in the heat, whereby physiological strain is constrained and allows a 'reserve' for an end-spurt that reduces the likelihood of exhaustion and heat illness (Schlader *et al.*, 2011e).

Thermoregulatory behaviour differed between environments as a function of menstrual phase.

Another novel result is that these women reduced workload more rapidly and performed worse in HUM compared to DRY, despite matched WBGTs. This is probably due to low(er) rates of evaporative cooling (Fig. 17) driven by the reduced vapour pressure gradient between skin and environment, which is consistent with previous observations (Morimoto *et al.*, 1967b, Shapiro *et al.*, 1980b, Frye and Kamon, 1983). However, it should be noted that whilst this performance decrement in humid *versus* dry heat has been demonstrated previously in men (Gupta *et al.*, 1984) it was unknown whether this would be replicated in women due to their greater sweating efficiency in this environment (Frye and Kamon, 1983, Morimoto *et al.*, 1967b, Shapiro *et al.*, 1980b). Furthermore, the observation that the initiation of thermal behaviour in HUM occurred earlier in EF than in ML (Fig. 14) supports previous investigations that used passive-heating behavioural models to determine that the threshold for affective/behavioural response is menstrual phase-dependent (Cunningham, 1971, Scarperi and Bleichert, 1983, Shoemaker and Refinetti, 1996). Nevertheless, it should be highlighted that this behaviour during HUM was not sufficient to completely diminish the differences observed for T_{rec} (from rest) in spite of no differences for *S*.

Phase-related differences in T_{core} but not ovarian [hormone] were not attenuated compared to

less trained women.

The finding of an elevated T_{core} at rest and during fixed-intensity exercise during ML compared to EF is consistent with those of others (Avellini *et al.*, 1980, Carpenter and Nunneley, 1988, Kolka and Stephenson, 1997b, Tenaglia *et al.*, 1999, Janse *et al.*, 2012). The results also support previous observations that trained women have a smaller difference in the bi-phasic T_{core} and no phase related difference for the T_{core} threshold for sweating or cutaneous vasodilatation (Kuwahara *et al.*, 2005a, Kuwahara *et al.*, 2005b). Yet, the current participants did not have the reduced ovarian hormone concentrations or between-phase fluctuations that were evident in previous studies (Dale *et al.*, 1979, Bullen *et al.*, 1984, Kuwahara *et al.*, 2005a, Kuwahara *et al.*, 2005b). However, when examining these participant data further by separating into greater (n = 5, 59–70 ml min^{-1} kg^{-1}) and lesser (n = 5, 48–56 ml min^{-1} kg^{-1}) trained, a clear difference in the absolute and relative (Δ) hormone concentrations appeared such that *a posteriori* analysis confirmed $\dot{V}O_{2max}$ correlated with [oestrogen] (r = 0.79, p < 0.01) but not [progesterone] (r = −0.14, P = 0.55).

Considerations

The design of comparing the responses of women between EF and ML used within this study has the specific advantage of being applicable to competitive women experiencing their natural, endogenous hormonal changes. Furthermore, this rationale was based on (1) maximising the differences in [oestrogen] and [progesterone] occurring naturally/endogenously, (2) permitting comparison with and therefore expansion beyond previous results, and (3) the previous research indicating that women self-report training and performance to be impacted negatively by their menstrual cycle. However, whilst this approach ‘captures’ the phases of lowest hormone exposure and peak [progesterone] it does not include for comparison the late-follicular (pre-ovulatory) phase, when [oestrogen] peaks and during which it has been demonstrated that resting T_{core} and the threshold for thermoregulatory effector responses are shifted to a lower T_{core} (Stephenson and Kolka, 1999). However, the same authors did not observe any exercise performance change between this late-follicular, EF and ML phases (Kolka and Stephenson, 1997a). It must also be noted that women are in EF and ML for ~50% of their reproductive lives, and that hormone exposure does not determine effect, i.e. receptor activity (Stachenfeld and Taylor, 2014). Moreover, the ovarian and other reproductive (luteinising and follicle stimulating) hormones exert independent effects, and in combination their effects on the body’s systems are complex and

multi-faceted, and extend beyond thermoregulation. Thus, other experimental designs are required (e.g. hormonal contraception or suppression) to allow causal inferences to be made (Stachenfeld and Taylor, 2014). Many elite athletes often have irregular menstrual cycles or take the oral contraceptive pill (OCP) for reasons of contraception and/or to negate pre-menstrual symptoms and manipulate the menstrual cycle timing for travel, training and competition (Bennell *et al.*, 1999). Previous investigations on OCP-users have reported that the phase-related elevation in T_{core} is maintained during exercise in the heat (Sunderland and Nevill, 2003, Tenaglia *et al.*, 1999). However, this increased resting and exercising T_{core} and the concomitant increase in the T_{core} threshold for sweating differs according to the type of OCP used, i.e. combined synthetic oestrogen + progesterone *versus* progesterone only (Stachenfeld *et al.*, 2000). Nevertheless, to our knowledge, only two published studies have measured exercise performance with heat stress using matched groups (OCP *vs.* eumenorrheic) and the same limitations apply of not having used self-paced protocols or well-trained women (Sunderland and Nevill, 2003, Tenaglia *et al.*, 1999). Therefore, whether well-trained women using OCP differ in their thermo-behavioural and performance responses from matched eumenorrheic or to different thermal stress (dry *vs*. humid) remains unknown. This issue then forms the study rationale in **Chapter Six.**

One (de)limitation includes the lack of an untrained cohort. They were not included because – at least in a sporting context – performance is less imperative, and hence they may rely on behavioural thermoregulation to a relatively greater extent (i.e. reduce power output) and may introduce other confounding effects by exhibiting different perceptual and behavioural tolerance and motivation (McLellan, 2001, Tikuisis *et al.*, 2002). One limitation was not including other physiological measures that are impacted by ovarian (and other reproductive) hormones and could contribute to performance/behaviour or homeostasis during exercise in the heat (especially leg blood flow and arterial oxygenation). The focus was on autonomic and behavioural thermoregulation, and even during more prolonged and severe hyperthermia with marked dehydration, oxygen delivery to the active musculature is preserved despite a reduced blood flow–at least in men (González-Alonso *et al.*, 1998, González-Alonso *et al.*, 2008). Furthermore, using the $P_{ET}CO_2$ data (not shown) as an indication of alveolar ventilation, arterial oxygenation seems unlikely to have been influenced by menstrual phase or environment, as no significant main or interaction effects were evident. Finally, the current design included a period of fixed-intensity exercise and a period of variable-intensity exercise that were unequal in duration, and it is unlikely that a thermoregulatory steady state had been

achieved during the fixed-intensity exercise; therefore, as per the previous observations in men (Schlader *et al.*, 2011a, Schlader *et al.*, 2011d), a longer (> 20 min) and duration-matched protocol would strengthen the current results and form, in part, the rationale for **Chapters Seven** and **Eight**.

Practical Application

When exercising in warm-humid conditions, and during the luteal phase of the menstrual cycle, pre-cooling and per-cooling (during the race) using e.g. ice slurry would be recommended as this has been shown to reduce thermal strain and may potentially result in better endurance performance in this particular thermal environment (Kay *et al.* 1999*).*

Chapter Six

6.0: Influence of oral contraceptive pill and thermal profile on autonomic and behavioural thermoregulation during exercise in trained women

Abstract

This chapter studied thermoregulatory responses of ten well-trained ($\dot{V}O_2$max, 57 (7) mL. min^{-1}. kg^{-1}) women taking a combined, mono-phasic oral contraceptive pill (OCP; ≥ 12 months) during exercise in dry and humid heat, across their active OCP cycle. They completed four trials, each of resting and cycling at fixed intensities (125 and 150 W), to assess autonomic regulation, then self-paced intensity (30-min work trial), to assess behavioural regulation. Trials were in *quasi*-follicular (*q*F) and *quasi*-luteal (*q*L) phases in dry (DRY) and humid (HUM) heat matched for wet bulb globe temperature (WBGT, 27°C). During rest and exercise at 125 W, rectal temperature was 0.15°C higher in *q*L than *q*F (p = 0.05) independent of environment (p = 0.17). The onset threshold and thermosensitivity of local sweat rate and forearm blood flow relative to mean body temperature was unaffected by the OCP cycle (both p > 0.30). Exercise performance did not differ between *quasi*-phases (*q*F: 268 (31), *q*L: 263 (26) kJ, p = 0.31), but was 5 (7)% higher in DRY than HUM (273 (29), 258 (28) kJ; p = 0.03). When compared to their matched eumenorrhoeic athletes in **Chapter Five**, chronic OCP use impaired the thermoeffector onset thresholds and thermosensitivities (all p < 0.01). In well-trained, OCP-using women exercising in the heat: i) a performance-thermoregulatory trade-off occurred that required behavioural adjustment, ii) humidity impaired performance due to reduced evaporative cooling despite matched WBGT, and iii) autonomic but not behavioural thermoregulatory responses were impaired compared to **Chapter Five females**.

6.1: Introduction

The primary ovarian steroidal hormones influence several non-reproductive organs and systems including thermoregulation, whereby oestrogens promote heat dissipation and lower core body temperature (T_{core}) whereas progestogens are thermogenic (Israel and Schneller, 1950, Charkoudian and Stachenfeld, 2014). Studies have investigated the impact of these hormones on temperature regulation during different phases of the menstrual cycle. In eumenorrhoeic women the thermoregulatory balance-point shows T_{core} to be regulated approximately 0.4 °C higher during the post-ovulatory (luteal) phase at rest and during passive and active heat stress, as the rise in progesterone exerts its dominant effect (Harvey and Crockett, 1932, Stephenson and Kolka, 1993a). This is accompanied by an increased T_{core} threshold for thermoregulatory effector responses such as sweating and cutaneous vasodilation (Stachenfeld *et al.*, 2000, Inoue *et al.*, 2005). Therefore, several authors have suggested that when performing exercise under environmental heat stress during their luteal phase, women should avoid competition or face a thermoregulatory *and* performance disadvantage (Stephenson and Kolka, 1993a, de Jonge, 2003, Charkoudian and Joyner, 2004, Janse *et al.*, 2012). However, for a well-trained and competitive female athlete this may not be the case. Firstly, trained females have a greater capacity to deal with a heat load due to their enhanced thermoeffector responses compared to less-trained counterparts (Kuwahara *et al.*, 2005a, Kuwahara *et al.*, 2005b). Secondly, trained women show smaller bi-phasic effects on T_{core} and thermoeffector responses due to reduced ovarian hormone concentrations and fluctuation between their menstrual phases (Kuwahara *et al.*, 2005a, Kuwahara *et al.*, 2005b). Thirdly, most previous investigations have utilised a fixed-intensity exercise protocol that is less face-valid and does not examine the fundamental premise of heat balance: that heat loss needs only to equal heat production (e.g. Gagnon *et al.*, 2013). Therefore, the findings from **Chapter Five** demonstrated that when well-trained females can use behavioural thermoregulation (self-pacing) during exercise heat stress, exercise intensity, and therefore, metabolic heat production is reduced. This eases the required evaporation and decreases thermoregulatory strain to the point where the menstrual phase-related thermodynamic and autonomic differences become nullified. Furthermore, these menstrual phase-related effects were relatively small in well-trained women.

Prevalence of OC use is high (42-83%) among athletically competitive and elite females, three-quarters of whom reportedly use a combined mono-phasic OC pill (OCP, Rechichi *et al.*, 2009). The combined mono-phasic OCP provides a constant dose of synthetic oestrogen and

progestogen for 21 days followed by 7 days of a placebo. Previous investigations on OCP users report that the phase-related elevation in T_{core} and concomitant increase in the T_{core} threshold for effector responses is maintained during active and passive heating, and that this shift can be regarded as a strong and residual effect of the phase of the menstrual cycle (Grucza *et al.*, 1993, Martin and Buono, 1997, Rogers and Baker, 1996, Charkoudian and Johnson, 1997, Tenaglia *et al.*, 1999, Sunderland and Nevill, 2003). However, whilst these investigations generally describe their comparison between a *quasi*-follicular and *quasi*-luteal phase, comparison always occurred when the females were taking active OC compared to their placebo week (withdrawal). This raises several important considerations. Firstly, the variable tissue washout rates mean that the exogenous hormones, or their metabolites, likely remain elevated and able to exert an effect (Israel and Schneller, 1950, Rothchild and Barnes, 1952, Charkoudian and Stachenfeld, 2014). Secondly, towards the end of the placebo week the concentration of endogenous oestrogens increases, and as such this phase should be viewed as a transitory hormonal phase and not, in fact, a controlled "low" hormonal phase (cf. OC use as a "high" hormonal phase, Rechichi *et al*., 2008; Charkoudian and Stachenfeld, 2014). Thirdly, the synthetic progestins found in OCPs differ in some of their basic actions to those of endogenous progesterone, likely influencing physiological systems differently (Charkoudian and Stachenfeld, 2014). Thus, the supposition that an endogenous rhythm of the menstrual cycle is maintained during OC use has not properly been investigated, as to the authors' knowledge no data exist pertaining to variation within the active OCP cycle. This was the principle objective of this experimental chapter. Moreover, data exist to indicate that chronic OCP use may alter women's T_{core} and thermoeffector responses to active and passive heating, as evidenced by greater resting T_{core} and attenuated changes in mean body temperature and gain for local sweat rate (Grucza *et al.*, 1993, Sunderland and Nevill, 2003). Therefore, a secondary objective was to compare the results of the current OCP cohort matched in relevant physical and fitness characteristics with the eumenorrhoeic cohort in **Chapter Five**.

Forthcoming large international events (e.g. 2018 Commonwealth Games on the Gold Coast, 2019 IAAF World Championships in Doha, 2020 Summer Olympic Games in Tokyo) will expose female athletes to high levels of environmental heat stress, and the number of women participating at this elite level is ever increasing, now approximating that of males. However, these environments differ in their ambient thermal profile, with arid environments usually permitting greater (full) evaporation of sweat whilst humid tropical environments do not. In

Chapter Five, eumenorrhoeic athletes' performance was impaired in humid compared to dry ambient heat matched for wet bulb globe temperature (WBGT, 27ºC). To the candidate's knowledge, no previous study has compared thermoregulatory and performance responses in women taking OCP when exposed to humid *versus* dry heat. This was the final objective.

6.2: Method

6.2.1: Ethical approval

This study was approved by the Massey University Human Ethics committee: Southern A (14/99).

6.2.2: Participants

Ten endurance trained female athletes, who had been using oral contraceptive pill were recruited. All OCP participants were taking a mono-phasic combination OCP (≥ 1 y) that provides a constant level of hormones for 21 days followed by a placebo pill for 7 days. Five were taking Ginet® (REX Medical Ltd, New Zealand; containing cyproterone acetate 2 mg and ethinylestradiol 35 µg), four Ava 20 ED® (Teva Pharma Ltd, New Zealand; containing levonorgestrel 0.1 mg and ethinylestradiol 20 µg) and one Norimin® (Pfizer Ltd, New Zealand; containing norethisterone 0.5 mg and ethinylestradiol 35 µg). This group was then matched with the eumenorrheic women in **Chapter Five** according to their percentage of body fat, body surface area, $\dot{V}O_2$max, peak power output, which can be seen in **Table 2** (**Section 4.1**).

6.2.3: Experimental Overview

Data collection was conducted during the autumn to spring period, when the temperature in Palmerston North is rarely above 22 °C and no participants had spent any time in a hot climate for at least one month prior to the trial. All participants reported to the laboratory on six separate occasions: (1) preliminary submaximal and maximal tests; (2) familiarisation session; and (3-6) heat stress trials. All subjects participated in the heat stress trials in a cross-over fashion; (eumenorric- early follicular phase and mid-luteal phase; OCP- first week of the combined hormone tablet and last week of the combined hormone tablet) and both the dry and humid heat environments were matched by WBGT (27°C). All trials were counter-balanced and conducted at the same time of day (±0.5 hours) and followed > 24 h of dietary and exercise control. All heat stress trials were conducted using the cycle ergometer (Lode Excalibur, Groningen, The Netherlands) with specific adjustment of the saddle height and handle bar, according to individual preference.

6.2.4: Preliminary testing and familiarisation

In order to minimise the effect of the female reproductive hormone on substrate utilisation during submaximal and maximal exercise (Casazza *et al.,* 2002, Lebrun *et al.,* 2003), both the

submaximal exercise test and maximal aerobic capacity tests ($\dot{V}O_2$max Test) were conducted during the early follicular phase for the eumenorrheic women and in the no pill/placebo pill period for the combined contraceptive pill women. The procedure and protocol submaximal and maximal exercise ($\dot{V}O_2$max) test was outlined in **Section 4.53**. Furthermore, familiarisation was also mentioned in **Section 4.53.**

6.2.5: Dietary and Exercise control

Dietary and exercise control were considered and were described in **Section 4.4**.

6.2.6: Hormone control and type of heat stress

Participants were tested during the *q*F and *q*L phases to permit comparison with the eumenorrhoeic group. Testing occurred on days 3-5 and 18-20 following start of OCP use, whereas testing of the eumenorrhoeic group occurred on days 3-6 (early follicular) and 18-21 (mid-luteal) following start of menses. This was unavoidable due to the study's hypothesis and therefore design, and as such meant that the current OCP users were being tested on days 10-12 (*quasi* mid-late follicular) and 25-27 (*quasi* mid-late luteal) following start of menses.

The environmental condition in this chapter was exactly the same as in **Chapter Five** as it was mentioned clearly in **Section 5.2.6**.

6.2.7: Experimental procedure

This chapter carried on from **Chapter Five**, as all of the data from **Chapter Five** were used to compare directly against the women taking combined oral contraception. Due to this reason, the experimental procedure was exactly the same as in **Chapter Five** and was mentioned clearly in **Section 5.2.7**.

6.2.8: Measurements

The details of measurement procedures for anthropometric, respiratory, cardiovascular, body temperature, sudomotor, and biophysics measures were clearly outlined in **Chapter four (**anthropometric: **Section 4.5.1**; respiratory: **Section: 4.5.2**; cutaneous blood flow: **Section 4.5.7.1**; cardiovascular: **Section 4.5.6**; body temperature: **Section: 4.5.4**; sudomotor: Section 4.5.8; biophysics: **Section 4.5.5**).

6.2.8.1: Hormonal analysis

Blood was collected by venipuncture into a vacutainer (Becton-Dickinson, Oxford, UK)

containing a clot activator. Following inversion and clotting for at least 30 minutes, the whole blood was centrifuged at 4°C and 805 *g* for 12 minutes and aliquots of serum were transferred into Eppendorf tubes (Genuine Axygen Quality, USA) and stored at −80 °C for later analysis. Serum samples were analysed using enzyme-linked immune assays for 17*β*-oestradiol (Demeditec Diagnostics, Kiel, Germany) and progesterone (IBL International, Hamburg, Germany) with a sensitivity of 22.7 pmol l^{-1} and 0.14 nmol l^{-1}, respectively, and an intra-assay variation of 4 and 6%, respectively.

6.2.9: Statistical analysis

All statistical analyses were performed with SPSS software for windows (IBM SPSS Statistics 20, NY, USA). Descriptive values were obtained and reported as means and standard deviation (SD) unless stated otherwise. Levene's test was used to ensure data did not differ substantially from a normal distribution. Data were analysed using three-way (OCP phase × environment × time) ANOVA for repeated measures. Resting and fixed-intensity exercise data were analysed separately from self-paced exercise data. Sphericity was assessed and where the assumption of sphericity could not be assumed, adjustments to the degrees of freedom were made ($\varepsilon > 0.75$ = Huynh-Feldt; $\varepsilon < 0.75$ = Greenhouse-Geisser). Where main or interaction effects occurred, *post-hoc* pairwise analyses were performed using a paired samples *t*-test (Bonferroni correction where relevant), with statistical significance set at $P \leq 0.05$. To examine how OCP phase and type of heat stress affected the thermal control of the effector responses (LSR and FBF), the visually determined linear portion of each response against T_b was analysed using simple linear regression [y=y0+a*x] and compared using two-way (menstrual phase × heat stress) ANOVA. The onset threshold was defined as the y-intercept (Y0) of the regression line with values at baseline, while the thermosensitivity was defined as the slope (a) of the regression line. To allow comparison between this OCP and previous eumenorrhoeic group (**Chapter 5**), a fourth (between-group) factor was introduced using a mixed-model (group × OCP phase × environment × time) ANOVA, with the group effect reported.

6.3: Results

6.3.1: Hormone concentrations

OCP use maintained constant endogenous concentrations of both progesterone and 17β-oestradiol between *q*F and *q*L (p = 0.93 and p = 0.62, respectively, Table 3), and concentrations were also not different between days of testing within a *quasi*-phase (p = 0.24 and p = 0.22, respectively). Between-group analysis revealed that endogenous concentrations of progesterone ($p < 0.01$) but not 17β-oestradiol (p = 0.07) were significantly lower in the current OCP than previous eumenorrhoeic group in **Chapter Five**.

Table 3: Individual and group progesterone and 17β-oestradiol concentrations during the (quasi-) follicular (F) and luteal (L) phase for the matched eummenorrheic (Chapter 5) and oral contraceptive pill (OCP) groups.

	Progesterone (nmol L^{-1}) $				**17β-Oestradiol (pmol L^{-1})**			
	F		*L*		*F*		*L*	
Participant	**EUM**	**OCP**	**EUM**	**OCP**	**EUM**	**OCP**	**EUM**	**OCP**
1	0.3, 0.3	0.4., 0.4	30, 42	0.5, 0.5	195, 202	79, 75	290, 264	89, 102
2	0.6, 3.8	-	49, 43	-	33, 7	-	330, 301	-
3	2.2, 2.9	0.0, 0.1	219, 168	0.0, 0.1	503, 602	8, 4	1057, 833	3, 0
4	0.3, 0.3	0.2, 0.3	4.1, 11	0.4, 0.5	88, 132	96, 95	176, 723	90, 77
5	2.9, 1.0	0.2, 0.1	39, 21	0.5, 0.2	92, 103	56, 57	162, 165	71, 62
6	2.9, 1.9	0.3, 0.2	18, 39	0.2, 0.3	117, 44	24, 67	198, 198	36, 29
7	1.3, 0.6	0.5, 0.4	61, 60	0.5, 0.8	40, 62	60, 66	139, 261	64, 72
8	1.3, 2.2	0.9, 1.4	19, 61	0.5, 1.1	147, 136	28, 64	242, 169	76, 46
9	3.5, 3.2	0.1, 1.0	35, 59	0.2, 0.2	396, 430	1, 9	426, 716	0, 12
10	1.0, 1.3	0.2, 0.1	27, 65	0.2, 0.2	176, 195	10, 2	272, 363	4, 7
Mean (SD)	**1.7 (1.2)**	**0.4 (0.4)**	**54 (52)**	**0.4 (0.3)**	**185 (166)**	**44 (33)**	**364 (259)**	**47 (36)**

6.3.2: Exercise performance and behaviour

Work capacity was similar between OCP phases (EF: 268 (31) *vs* ML: 263 (26) kJ, $p = 0.31$) but was 5 (7) % higher in DRY than in HUM (273 (29) *vs* 258 (28) kJ; $p = 0.03$, Fig.18). Accordingly, mean power output was unaffected by OCP phase ($p = 0.44$) but 5 (7) % higher in DRY than in HUM (152 (16) *vs* 143 (16) W; $p = 0.03$). When viewing behaviour as the self-paced exercise profile, behaviour was similar between OCP phases ($p = 0.44$) but was 8 (10) W higher in DRY than in HUM ($p = 0.03$) and changed over time ($p < 0.01$). Between-group analysis revealed no differences between the OCP and eumenorrhoeic groups for work capacity ($p = 0.50$) or power output profile ($p = 0.52$).

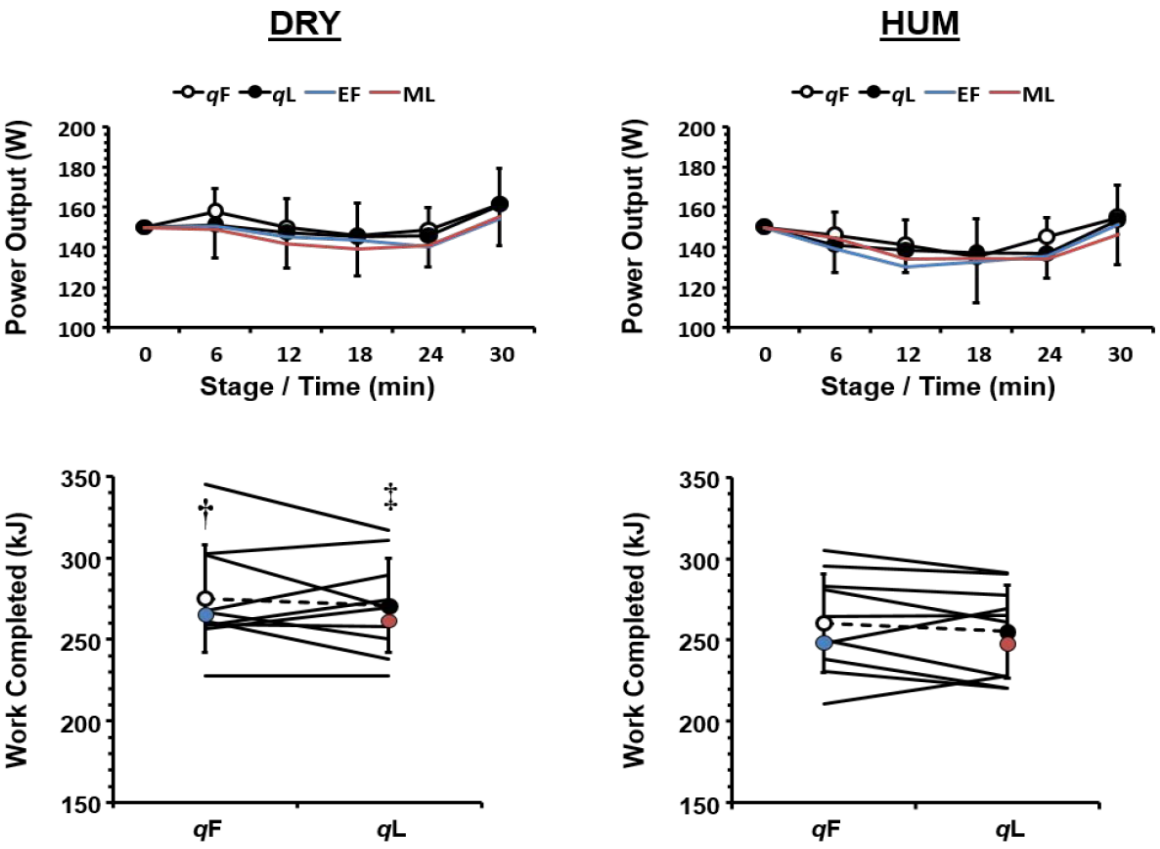

Figure 16: Mean (SD) power output (n = 10) and individual and mean (SD) work capacity (n = 10) during exercise in dry (DRY) and humid (HUM) heat during the quasi-follicular (qF) and quasi-luteal (qL) phase.

† indicates significant difference between corresponding qF-HUM value. ‡ indicates significant difference between corresponding qL-HUM value. Mean early follicular (EF) **and** mid-luteal (ML) values are provided for the previous eumenorrhoeic cohort in Chapter Five.

6.3.3: Thermoregulatory measures

6.3.3.1: Body temperature

The T_{rec} when resting was 0.15 (0.21)°C higher in *q*L than in *q*F (p = 0.05) and was 0.12 (0.12)°C higher during HUM than DRY (p = 0.01, Fig.19). The rise in T_{rec} during fixed-intensity exercise differed between OCP phases as a function of work-rate (OCP phase × time: p = 0.05) but was not dependent on environment (interaction: p = 0.17), whereby the between-phase difference seen at rest was still evident at 125 W (0.12 (0.19) °C) but not 150 W (0.06 (0.21)°C). During self-paced exercise T_{rec} was similar between OCP phases (p = 0.74) and environments (p = 0.54) but continued to increase with time (p < 0.01) until the end of exercise.

Resting $\bar{T}_{sk}$ was similar between OCP phases (p = 0.78) but was 1.2 (0.7)°C higher during DRY than HUM (p < 0.01, Fig.19). During fixed-intensity exercise $\bar{T}_{sk}$ was similar between OCP phases (p = 0.85) but differed between environments as a function of work-rate (environment × work-rate: p < 0.01) such that the difference between work-rates was greater in HUM (0.79 (0.42)°C) than DRY (0.19 (0.52)°C). During self-paced exercise $\bar{T}_{sk}$ differed between environments as a function of time (environment × time: p = 0.01). Specifically, $\bar{T}_{sk}$ values were maintained constant during DRY but continued to increase by ~0.7°C during HUM.

Between-group analysis revealed no differences between the OCP and eumenorrhoeic groups for T_{rec} (all p > 0.47) or $\bar{T}_{sk}$ (all p > 0.58) during any stage of the protocol.

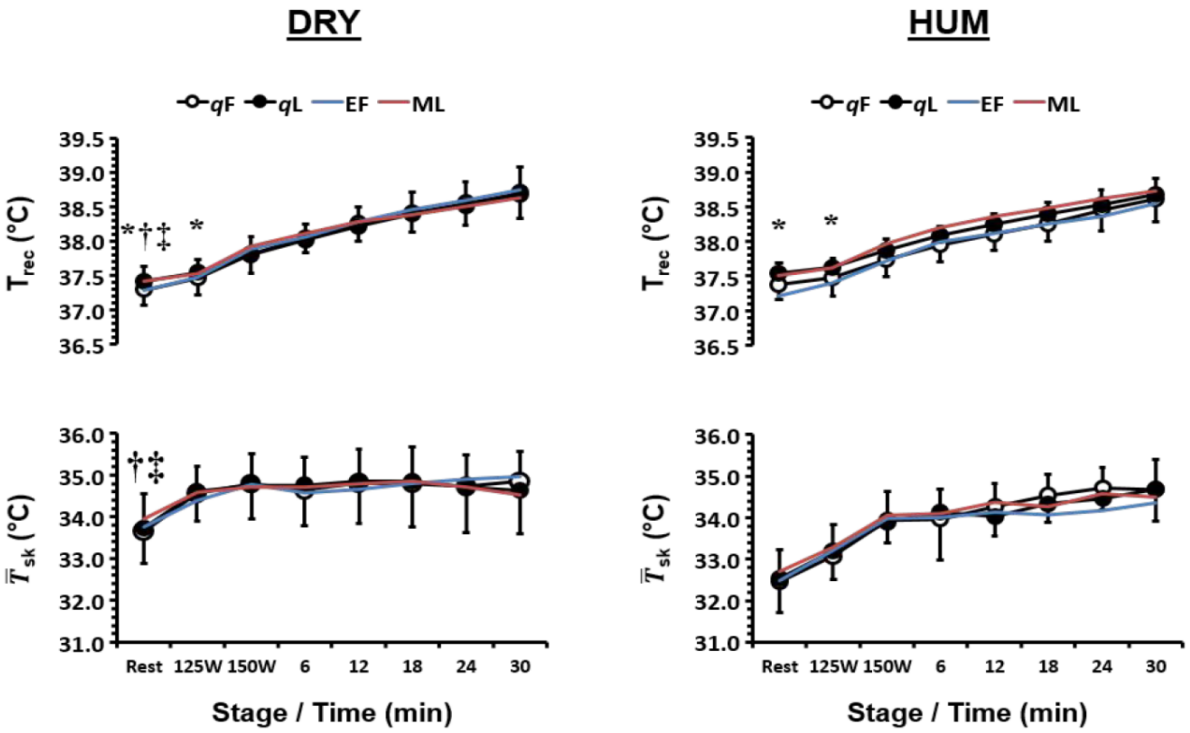

Figure 17: Mean (SD) rectal temperature (T_{rec}, n = 10) and weighted mean skin temperature ($\bar{T}_{sk}$, n = 10) during exercise in dry (DRY) and humid (HUM) heat during the quasi-follicular (*q*F) and quasi-luteal (*q*L) phase.

* indicates significant difference between *q*F-*q*L within environment, † indicates significant difference between corresponding *q*F-HUM value, ‡ indicates significant difference between corresponding *q*L-HUM value. Mean early follicular (EF) and mid-luteal (ML) values are provided for the previous eumenorrhoeic cohort in Chapter Five.

6.3.3.2: Cardiovascular and thermoeffectors

Resting SV, $\dot{Q}$ and MAP were similar between OCP phases and environments (all $p > 0.22$, Table 4). Whereas, resting HR was 2 (2) beats min^{-1} higher in DRY with this more evident in *q*F than *q*L (environment × OCP phase: $p = 0.08$), and resting FVR was 2.9 (2.6) mm Hg min dL^{-1} higher in DRY than HUM ($p = 0.02$), although this may have been more evident in *q*F than *q*L (environment × OCP phase: $p = 0.08$). During fixed-intensity exercise, $\dot{Q}$ and possibly also SV differed between environments as a function of OCP phase and work-rate (environment × phase × time: $p = 0.01$, and $p = 0.09$, respectively) such that values were highest at 150 W during *q*L-HUM. MAP and HR were similar between OCP phases and environments (all $p > 0.16$), but increased with work-rate (both $p < 0.01$). Thus, during fixed-intensity exercise FVR was 1.5 (1.0) mm Hg min dL^{-1} higher in DRY than HUM ($p < 0.01$) and differed between OCP phase as a function of work-rate (OCP phase × time: $p < 0.01$) such that FVR was lower during *q*L than *q*F at 125 W (by 1.6 (2.2) mL dL min mm Hg^{-1}) but not at 150 W (0.2 (1.8) mL dL min mm Hg^{-1}). During self-paced exercise, HR changed over time ($p < 0.01$) independent of OCP phase and environment (both $p > 0.24$) with a characteristic end-spurt higher than all previous time-points by > 10 beats min^{-1}.

Between-group analysis revealed no effect of OCP usage for SV, HR, $\dot{Q}$ and MAP (all $p > 0.23$), however FVR was 2.7 (2.3; $p < 0.01$) and 1.1 (1.1; $p = 0.01$) mm Hg min dL^{-1} higher at rest and during fixed-intensity exercise, respectively, in the current OCP than previous eumenorrhoeic group.

Table 4: Mean arterial pressure (MAP, n = 8), cardiac output ($\dot{Q}$, n = 6), forearm vascular resistance (FVR, n = 8) and stroke volume (SV, n = 6) at rest and during fixed-intensity exercise in dry (DRY) and humid (HUM) heat during the quasi-follicular (qF) and quasi -luteal (qL) phase. Values are mean (SD).

	DRY						**HUM**					
	qF			*qL*			*qF*			*qL*		
	Rest	**125 W**	**150 W**	**Rest**	**125 W**	**150 W**	**Rest**	**125 W**	**150 W**	**Rest**	**125 W**	**150 W**
MAP (mmHg)	90 (6)	98 (5)	103 (7)	84 (5)	95 (7)	100 (7)	87 (7)	98 (6)	103 (6)	84 (6)	95 (8)	100 (9)
$\dot{Q}$ (L min^{-1})	8 (2)	20 (3)	21 (4)	7 (2)	22 (7)	20 (3)	7 (2)	20 (2)	21 (3)	8 (4)	20 (3)	23 (3)$^{\#}$
FVR (mm Hg min dL^{-1})	11.9 (5.8)	7.4 (2.3)	5.6 (1.9)	7.7 (1.5)^{a}	5.3 (1.7)	4.9 (1.5)	7.2 (1.4)^{a}	5.2 (0.9)	3.8 (0.5)	6.6 (1.6)	4.2 (1.8)	4.0 (1.6)
SV (mL)	109 (33)	138 (24)	133 (25)	94 (33)	160 (59)	128 (28)	91 (20)	140 (18)	132 (21)	122 (72)	149 (31)	154 (23)^{bc}

$^{\#}$ Significant difference to preceding time-point; ^{a} Significant difference to corresponding *q*F-DRY time-point; ^{c} Significant difference to corresponding *q*F-HUM time-point.

Resting LSR was similar between OCP phases and environments (both $p > 0.21$, Fig.20), whilst resting FBF differed between environments as a function of OCP phase (environment × OCP phase: $p = 0.05$) such that values were higher for HUM than DRY at both *quasi*-phases and higher in *q*L than *q*F during DRY. During fixed-intensity exercise LSR was 0.07 (0.06) mg cm^{-2} min^{-1} lower in *q*L than in *q*F ($p < 0.01$), 0.08 (0.10) mg cm^{-2} min^{-1}) and lower during DRY than HUM ($p = 0.04$) and increased with work-rate ($p < 0.01$). Whereas, FBF differed between OCP phases as a function of environment (environment × OCP phase: $p = 0.05$) and work-rate (time × OCP phase: $p < 0.01$), such that FBF was higher during DRY in *q*F (by 7 (3) mL dL^{-1} min^{-1}) but not *q*L (by 4 (6) mL dL min^{-1}) and increased from 125 to 150 W (by 6 (4) mL dL^{-1} min^{-1}) in *q*F but not *q*L. During self-paced exercise LSR was 0.13 (0.10) mg cm^{-2} min^{-1} higher during HUM than DRY ($p < 0.01$) and continued to increase with time ($p < 0.01$) until the end of exercise, although this tended to be more pronounced in HUM (environment × time: $p = 0.06$), regardless of OCP phase (interaction: $p = 0.43$). Neither onset thresholds nor thermosensitivities of the effector responses were affected by OCP phase or environment (all $p > 0.30$). Water consumption was similar between OCP phases and environments (597 (193) mL; both $p > 0.46$), whilst WBSR was similar between OCP phases and environments (843 (218) g h^{-1}; both $p > 0.28$), resulting in a 1.3 (0.6) % loss of body mass that was similar between OCP phases and environments (both $p > 0.47$).

Between-group analysis revealed no differences between the OCP and eumenorrhoeic groups for LSR at rest or during fixed-intensity exercise (both $p > 0.19$), however LSR was 0.23 (0.21; $p < 0.01$) mg cm^{-2} min^{-1} lower during the self-paced time-trial in the current OCP than previous eumenorrhoeic group. FBF was 4 (3; $p < 0.01$) and 6 (6; $p = 0.01$) mL dL^{-1} min^{-1} lower at rest and during fixed-intensity exercise, respectively, in the current OCP than previous eumenorrhoeic group. The onset threshold (both $p < 0.01$) and thermosensitivity (both $p < 0.01$) of the effector responses revealed differences between the OCP and eumenorrhoeic groups, such that both LSR and FBF occurred at a higher $\bar{T}_b$ and their sensitivities were lower in the current OCP users. Water consumption and percent loss of body mass were similar between groups (both $p > 0.11$), however WBSR was 200 (265; $p = 0.03$) mL lower in the current OCP than previous eumenorrhoeic group in **Chapter Five**.

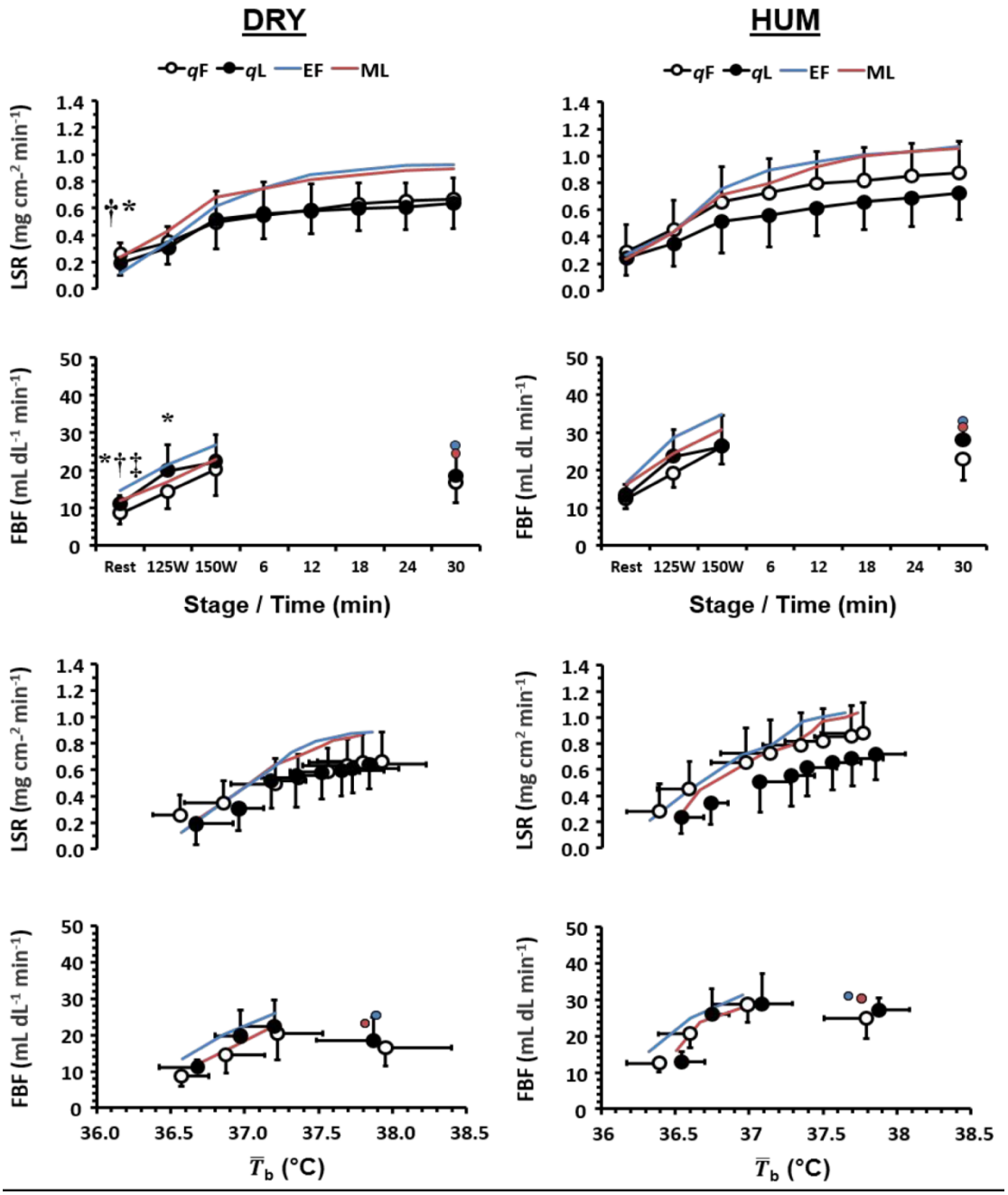

Figure 19: Mean (SD) local sweat rate (LSR, n = 9) and forearm blood flow (FBF, n = 8) against time and mean body temperature ($\bar{T}$b) during exercise in dry (DRY) and humid (HUM) heat during the quasi-follicular (*q*F) and quasi-luteal (*q*L) phase.

* indicates significant difference between *q*F-ML within environment, † indicates significant difference between corresponding *q*F-HUM value, ‡ indicates significant difference between corresponding *q*L-HUM value. Mean early follicular (EF) and mid-luteal (ML) values are provided for the previous eumenorrhoeic cohort in Chapter Five.

6.3.3.3: Thermodynamics

Resting M was 10 (13) $W.m^{-2}$ higher in *q*F than *q*L (p = 0.04) but similar between environments (p = 0.89) whereas resting E was similar between menstrual phases (p = 0.28) but was 23 (9) $W.m^{-2}$ higher in DRY than HUM (p < 0.01), with negligible S (Fig.21). Resting E_{max} was 8 (12) $W.m^{-2}$ lower in *q*F than *q*L (p = 0.05) and was 39 (14) $W.m^{-2}$ higher in DRY than HUM (p < 0.01), whereas E_{req} was similar between OCP phases and environments (both p > 0.18). Thus, the HSI differed between environments as a function of OCP phase (p = 0.04); HSI was higher during HUM (0.23 (0.05) a.u.) than DRY (0.20 (0.04) at *q*F only. During fixed-intensity exercise M was similar between OCP phases and environments (both p > 0.12) but increased with work-rate (p < 0.01), whilst E was similar between OCP phases (p = 0.67) but differed between environments as a function of work-rate (environment × time: p < 0.01), such that differences between work-rates were apparent only during HUM. As a result, S was similar between OCP phases (p = 0.73) but was 30 (15) $W.m^{-2}$ lower in DRY than HUM (p < 0.01) and increased with work-rate (p < 0.01). During fixed-intensity exercise, E_{max} was 44 (14) $W.m^{-2}$ higher in DRY than HUM (p < 0.01) and increased with work-rate (p < 0.01), while E_{req} was similar between OCP phases and environments (both p > 0.57) but increased with work-rate (p < 0.01). Consequently, the HSI was similar between OCP phases (p = 0.71) but was 0.09 (0.06) a.u. lower in DRY than HUM (p < 0.01) and increased with work-rate (p < 0.01). During self-paced exercise, M, E and S were similar between OCP phases (all p > 0.48) but increased with work-rate (all p < 0.01), with only E being 15 (13) $W.m^{-2}$ higher in DRY than HUM (p < 0.01). During self-paced exercise E_{max} was 26 (15) $W.m^{-2\ 2}$ higher in DRY than HUM (p < 0.01) and increased with work-rate (p < 0.01), E_{req} was similar between OCP phases and environments (both p > 0.18) but increased with work-rate (p < 0.01), and consequently the HSI was similar between OCP phases and environments (both p > 0.47) but increased with work-rate (p < 0.01).

Between-group analysis revealed no differences between the OCP and eumenorrhoeic groups for M, E or S at rest or during fixed-intensity or self-paced exercise (all p > 0.12). E_{max}, E_{req} and HSI were similar between groups at all time-points (all p > 0.12) except that resting E_{max} was 13 (13) $W.m^{-2}$ higher in the current OCP than previous eumenorrhoeic group (p < 0.02).

6.3.3.4:Respiratory

Resting ventilation was similar between OCP phases and environments (both $p > 0.27$), but during fixed-intensity exercise was 2.5 (3.1) L min^{-1} higher in *q*L than in *q*F ($p = 0.03$) and increased with work-rate ($p < 0.01$). Resting $P_{ET}CO_2$ was similar between OCP phases and environments (both $p > 0.23$) and similar during fixed-intensity exercise (both $p > 0.56$). Participants were exercising at 61 (11), 72 (12) and 75 (8) % of their $\dot{V}O_2max$ at 125 W, 150 W and during the self-paced time-trial, respectively, and similar between OCP phases and environments (both $p > 0.57$) but increased with work-rate ($p < 0.01$) from 125 W to 150 W only. Between-group analysis revealed no differences between the OCP and eumenorrhoeic groups for ventilation, $P_{ET}CO_2$ or percent $\dot{V}O_2max$ at rest, during fixed-intensity or self-paced exercise (all $p > 0.47$).

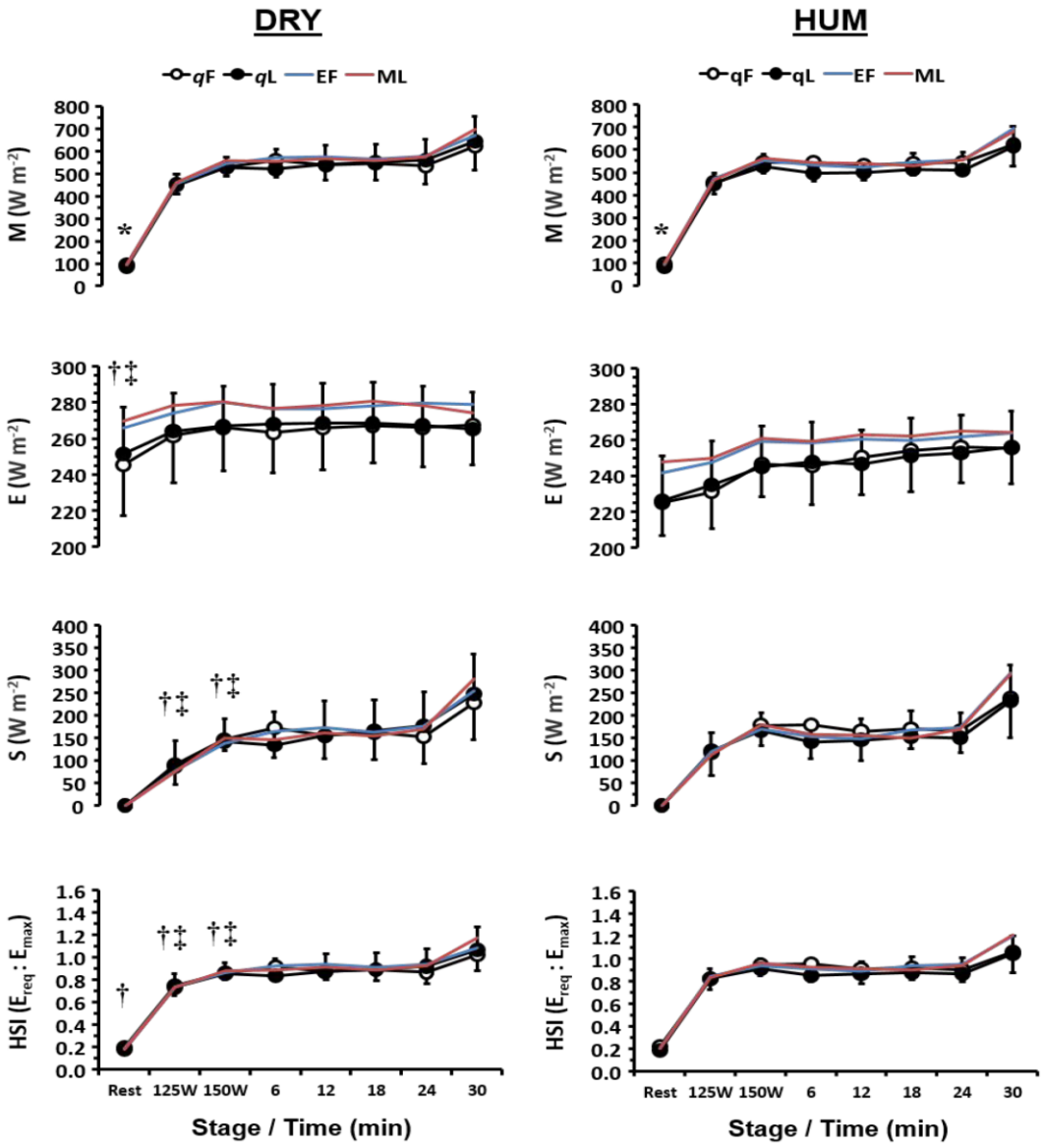

Figure 18: Mean (SD) rate of metabolic heat production (M, n = 10), rate of evaporative heat loss (E, n = 10), rate of heat storage (S, n = 10), maximal evaporative capacity of the environment (E_{max}, n = 10), required evaporative cooling for heat balance (E_{req}, n = 10) and heat strain index (HSI, n = 10) during exercise in dry (DRY) and humid (HUM) heat during the quasi-follicular (qF) and quasi-luteal (qL) phase.

* indicates significant difference between qF-ML within environment, † indicates significant difference between corresponding *q*F-HUM value, ‡ indicates significant difference between corresponding *q*L-HUM value. Mean early follicular (EF) and mid-luteal (ML) values are provided for the previous eumenorrhoeic cohort in Chapter Five.

6.4: Discussion

This chapter tested the hypotheses that in female athletes who are chronic users of the combined, mono-phasic OCP: 1) a small endogenous thermoregulatory rhythm would persist during their active OCP cycle, and would be nullified by behavioural adjustments when faced with the heat stress of exercise in warm environments; 2) there would be an interplay with the different thermal environments; and 3) autonomic responses would be attenuated compared to the matched cohort of eumenorrhoeic athletes in **Chapter 5**. In support of these hypotheses, 1) a small increase in T_{core} occurs during *q*L at rest and fixed-intensity exercise, although this disappears before behavioural adjustments are utilised, 2) autonomic heat loss mechanisms were activated to a greater extent during HUM, whilst behavioural thermoregulation was effective in minimising further strain and did not differ between environments, and 3) chronic OCP use impairs thermoeffector responses, but does not affect total evaporative heat loss or T_{core} during exercise. These results indicate that under the conditions of this investigation, the evaporative capacity of the environment determines endurance performance and, whilst autonomic heat loss responses are attenuated by chronic OCP use, female athletes' behavioural adjustments are not influenced.

A quasi-phase related shift in T_{core}, and to a lesser extent, heat loss mechanisms occurs despite OCP use:

It was observed that a consistent and significant but small *quasi*-phase increase in resting T_{core} by 0.15°C from *q*F to *q*L that persisted into the fixed-intensity exercise (Fig. 19). To the candidate's knowledge, this result during active OC use is unique. Previous investigations suggested this shift be regarded as a strong and residual effect of the endogenous menstrual cycle (Grucza *et al.*, 1993, Martin and Buono, 1997, Rogers and Baker, 1996, Charkoudian and Johnson, 1997, Tenaglia *et al.*, 1999, Sunderland and Nevill, 2003), yet in these studies comparison always occurred when the females were actively taking OC compared to their placebo week. However, this finding is difficult to explain as in the present study both endogenous (Table 4) and exogenous (by design, not measured) concentrations of progestogens and oestrogens remained unchanged between *q*F and *q*L. Nevertheless, it is unlikely to be an oestrogenic effect as it is known that when progestogens and oestrogens are naturally elevated or administered, then a progestogen-dominant thermogenic response ensues (Israel and Schneller, 1950, Rothchild and Barnes, 1952). However, the current design of maintained OC use is sub-optimal for determining causal mechanisms for the apparent "lack

of an OC effect in resetting the thermoregulatory balance point" (Tenaglia *et al.*, 1999). For this, other study designs (i.e. gonadotropin-releasing hormone suppression), mechanism mentioned in (**Section 2.71**) would be required (Stachenfeld and Taylor, 2014), although the candidate is unaware of any such published data on measures of regulated T_{core}. Furthermore, hormone exposure does not necessarily determine its effect, and both central and peripheral thermoregulatory receptors respond differently to synthetic progestin compared to progesterone exposure (Charkoudian and Stachenfeld, 2014).

Interestingly, although present at rest and early during fixed-intensity exercise, the *quasi*-phase related difference in T_{core} had disappeared by 12 min of fixed-intensity exercise. This phenomenon has been observed by some (Tenaglia *et al.*, 1999, Sunderland and Nevill, 2003) but not all investigators (Grucza *et al.*, 1993, Rogers and Baker, 1996), whilst the reverse has also been reported i.e. no difference at rest but with increasing difference during exercise (Martin and Buono, 1997). However, this *quasi*-phase related difference in T_{core} is similar in magnitude to that observed in the matched eumenorrhoeic cohort (**Chapter Five**) and supports previous observations at rest and during heat stress of a smaller difference in the bi-phasic T_{core} in trained women (Kuwahara *et al.*, 2005a, Kuwahara *et al.*, 2005b).

Concurrent to the *quasi*-phase difference observed for T_{core}, LSR during fixed-intensity exercise was lower during *q*L, whilst FBF was indistinguishable between environments and intensity during *q*L yet during *q*F was increased during DRY (*vs*. HUM) and at 150W (*vs*. 125W), indicating a differential response between *quasi*-phases (Fig. 20). However, the activation of heat loss responses relative to $\bar{T}_b$ (onset threshold, thermosensitivity) was unaffected by OCP phase. Therefore, one should consider closely the physiological significance of a shift in resting T_{core} < 0.2 °C that effectively disappears during exercise.

The evaporative capacity of the environment infers greater performance and thermoregulatory strain than does an OCP cycle:

Exercise performance was unaffected by the *quasi*-phase of the OCP cycle but was impaired by the humid tropical environment despite it being WBGT-matched to the dry heat (Fig. 18). This result confirms the observations in eumenorrhoeic athletes (**Chapter Five**) and indicates that a reduction in the evaporative power of the environment is of greater performance consequence to a female athlete than her menstrual/OCP cycle. It is well-known that evaporative cooling rates decrease when the vapour pressure gradient between skin and environment is reduced (Morimoto *et al.*, 1967b, Frye and Kamon, 1983).Therefore, these

females demonstrated a performance-thermoregulatory trade-off, whereby their reduction in (self-paced) exercise work-load (Fig. 18) eased the required evaporation to prevent further thermoregulatory strain (Figs. 19 and 21). That the evaporative capacity of the environment is of greater thermoregulatory consequence than OCP *quasi*-phase is clearly demonstrated by the greater strain observed for most thermometric (Fig. 19), calorimetric (Fig. 21) and effector responses (Fig. 20) at rest and during exercise for HUM compared to DRY. These results are also in agreement with Tenaglia *et al*. (1999) but are in contrast to Sunderland & Nevill (2003) who observed an improved running distance during *q*L in their OCP users. However, both studies compared their females in the active pill *versus* placebo phases.

Chronic OCP use per se impairs autonomic but not behavioural thermoregulatory responses:

The careful matching of groups and the experimental procedures of this chapter and the previous experimental chapter (**Chapter Five)** in eumenorrhoeic females permits the candidate to isolate the effects of chronic OCP use on autonomic and behavioural thermoregulation at rest and during active heat stress. Namely, the use of the same exercise protocols, ambient conditions, phases of the endogenous menstrual cycle and matched the OCP and eumenorrhoeic cohorts for all appropriate physical and functional characteristics. This matching was so successful in as much as comparable self-paced mean power output and work were completed between the groups, which lead to M being similar (Figs. 18 and 21). Thus, that between-group differences were evident for the onset threshold and thermosensitivity of effector responses (Fig. 20) demonstrates that long-term combined, mono-phasic OCP use in endurance athletes affects autonomic thermoregulatory responses. However, that behavioural thermoregulation was maintained (as self-pacing was evident) with no differential effect on regulated body temperature(s), or heat storage observed, indicates that overall thermoregulation and performance were not compromised. Thus, although both LSR and WBSR were reduced in this OCP cohort, either this sweat was not evaporated (dripped) or was of too small a magnitude to affect evaporative heat loss.

The potential mechanism(s) through which chronic OCP use affects autonomic heat loss mechanisms are not well understood, although likely concern a direct central action given the clear upward (i.e. rightward) shift in T_{core} for the onset of sweating and vasodilation (Fig. 20). Oestrogens and progestogens both readily cross the blood-brain barrier and can inhibit temperature-sensitive neurons in the preoptic/anterior hypothalamus (Lincoln, 1967, Nakayama *et al.*, 1975). The direction of this shift implies that this constraint of heat

dissipation (Nakayama *et al.*, 1975) is due to progestin inhibiting warm-sensitive neuronal activity (Nakayama *et al.*, 1975). It is unlikely that this effect is indirectly mediated by a pyrogen or heat shock proteins (Rogers and Baker, 1996, Charkoudian and Johnson, 1999a, Chang *et al.*, 1998), although this data do not allow the candidate to confirm or refute these observations. Equally, these results are unlikely to be due to an interaction with the system(s) that regulate volume of the extracellular fluid (Fortney *et al.*, 1981, Fortney *et al.*, 1983), as although the candidate did not quantify plasma volume, SV and $\dot{Q}$ were similar at rest and during exercise between our OCP and eumenorrhoeic cohorts. However, these findings could be due to changes in osmotic pressure (Fortney *et al.*, 1984) as some (Stachenfeld *et al.*, 1999, Stachenfeld *et al.*, 2000) but not others (Rogers and Baker, 1996) have observed a reduction in plasma osmolality with ≥1 month of OCP use. Interestingly though, despite no reduction in osmolality Rogers and Baker (1996) still observed a delay in the sweating onset whilst Stachenfeld *et al.* (2000) observed no change with OCP use, indicating that the effect of plasma osmolality may be small.

There is evidence to indicate a disparity between the acute and chronic effects of OCP use on thermoregulation (Charkoudian and Johnson, 1997, Stachenfeld *et al.*, 2000). Previous investigations have compared women when actively taking OC compared to their placebo week, even when separate groups of chronic OCP- and non-users were compared (Grucza *et al.*, 1993, Tenaglia *et al.*, 1999, Sunderland and Nevill, 2003). Nevertheless, data comparing these OCP and eumenorrhoeic females supports several previous observations, such as a higher onset threshold for sweating (Rogers and Baker, 1996, Charkoudian and Johnson, 1997) and cutaneous vasodilation (Charkoudian and Johnson, 1997), and an attenuated gain for local sweat rate (Grucza *et al.*, 1993). However, the result of resting (and exercising) T_{core} being similar between groups is in contrast to the upward-shift reported due to OCP use (Grucza *et al.*, 1993, Sunderland and Nevill, 2003). Despite previous reports of a change in the effector onset threshold due to OCP use (Grucza *et al.*, 1993, Rogers and Baker, 1996, Charkoudian and Johnson, 1997), this is the first study to report a change in thermosensitivity for vasodilation and only the second for sweating (Grucza *et al.*, 1993).

The OCP and quasi-phase exert haemodynamic effects:

Use of a combined OCP has been shown to affect both central (Walters and Lim, 1970, Lehtovirta, 1974a) and peripheral (Lehtovirta, 1974b) haemodynamics at rest and during exercise (Lehtovirta *et al.*, 1977), which is likely caused by the oestrogen component

(Lehtovirta, 1974a, Lehtovirta, 1974b) and requires longer than 1 month of OC consumption (Walters and Lim, 1970, Lehtovirta, 1974a, Lehtovirta, 1974b, Stachenfeld *et al.*, 2000). The candidate observed no differences in the central cardiovascular response (SV and $\dot{Q}$) between the OCP and eumenorrhoeic groups. However, FVR was higher in the current OCP compared to eumenorrhoeic cohort, a finding that contrasts with lower peripheral resistance found following two months of combined OCP use (Lehtovirta, 1974a, Lehtovirta, 1974b). Methodological differences likely account for these discrepant results as 1) vascular effects of the synthetic and endogenous hormones may be a confounding factor and the current OCP had a lower [17β-oestradiol] (Table 3), and 2) the current OCP users had been taking the OCP ≥ 12 months, whilst Lehtovirta and colleagues used a within-subject design (pre-post) following 2 months of OCP use.

The central cardiovascular response was similar between *quasi*-phases of the OCP cycle at rest, but showed augmentation of SV (~20-30 mL) and $\dot{Q}$ (~2-4 L min^{-1}) during *q*L compared with *q*F at matched workloads (~70% $\dot{V}O_2$max, measured from the expired gas), and reduced evaporative capacity (in HUM; Table 4). The peripheral vascular response was also affected by the OCP cycle, such that FVR was lower at rest during *q*L in HUM and during exercise at ~60% $\dot{V}O_2$max (measured from the expired gas) *q*L (Table 4). Most previous investigations (Walters and Lim, 1970, Lehtovirta, 1974a, Lehtovirta, 1974b, Stachenfeld *et al.*, 2000) did not compare *across* an OCP cycle such as in the current study. A paucity of data in the literature concerns the active OCP cycle with Littler *et al.* (1974) concluding that any (peripheral) cardiovascular changes that do occur from *q*F to *q*L are insufficient to alter central function. Moreover, the observed *q*L reduction in FVR at rest and during fixed-intensity exercise is similar in magnitude to that demonstrated by the previous eumenorrhoeic cohort in **Chapter Five**, indicating that the menstrual cycle could be exerting a peripheral cardiovascular effect beyond the regulated T_{core} in these OCP users. In support of this, menstrual cycle phase has been demonstrated to modulate vessel conductance and resistance that parallel changes in oestrogen (Williams *et al.*, 2001), NO production (Kharitonov *et al.*, 1994), and eNOS expression (Taguchi *et al.*, 2000), the most likely cause(s) of vascular smooth muscle relaxation (Charkoudian and Stachenfeld, 2016). Unfortunately, the candidate's *a priori* study design does not allow for further mechanistic insight; this would require, for example, cutaneous microdialysis with concurrent pharmacological administration to determine endothelium-dependent and -independent factors.

Considerations

By design, this study tested whether any *quasi*-phase related endogenous thermoregulatory rhythms persist during the active pill phase in chronic OCP users, therefore capturing 75% of their OCP cycle and mimicking real-world use in athletes i.e. competition/performance occurring during active pill use. Therefore, it would be speculation and is beyond the scope of the current data to determine how these responses compare to the 25% of the OCP cycle in which athletes consume a placebo pill; nevertheless, this warrants further investigation. Furthermore, the candidate tested women taking the combined, mono-phasic OCP as this most reflects athlete use, yet there is evidence that these responses could differ in those taking a progestin-only OCP (Stachenfeld *et al.*, 2000).

The same de-limitations are present in this study as in the previous chapter on eumenorrhoeic females (**Chapter Five)**, and were unavoidable in protocol and design due to direct comparison between these cohorts. Namely, the lack of an untrained cohort, periods of fixed-intensity and a variable-intensity exercise that were unequal (and limited) in duration, and inclusion of other physiological measures such as leg blood flow and arterial oxygenation. It is also recognised that homeostatic systems interact, such that the regulation of body temperature is not separate and distinct to that of, for example, fluid, energy substrate and metabolite balance or reproduction. Therefore, representative measures, particularly of plasma osmolality and volume, would have further strengthened the conclusions. Despite careful matching of the groups, the current OCP cohort were on average nine years younger than the previous eumenorrhoeic females in **Chapter Five**. Nevertheless, all other relevant physical and functional characteristics were similar (Table 1), and the candidate is unaware of any research indicating that in such pre-menopausal women this magnitude of an age difference should confound results.

Finally, the results of this investigation should be of interest to those (thermoregulation) researchers whose participants include OC users, as there is now evidence to question the validity of treating the active OCP cycle as a ‘controlled low hormonal phase’.

Conclusion

This study demonstrates that when well-trained women chronically using the combined, mono-phasic OCP exercise in heat-stressful environments, a performance-thermoregulatory trade-off occurs to ensure overall thermoregulation is not impaired. The biggest determinant of this trade-off is the evaporative capacity of the environment. Finally, an endogenous thermoregulatory rhythm persists despite chronic OCP use.

Chapter Seven

7.0: Thermoregulatory, cardiovascular and perceptual responses in men during exercise in differing thermal profiles of heat matched for vapour pressure

Abstract

The purpose of this study was to investigate the contribution of ambient temperature *per se*, when absolute humidity is matched, on the thermoregulatory, cardiovascular and perceptual responses during exercise. Fourteen moderate to highly trained cyclists (age: 31.5 (3.2) years; height: 177.8 (1.5) cm; mass: 76.4 (2.3) kg; $\dot{V}O_{2max}$: 59 (2.45) $mL.kg^{-1}.min^{-1}$; body surface area: 1.93 (0.03) m^2; body fat: 13.5 (4.4)%; peak power output: 392.7 (14.1) watts) were recruited, and they underwent one 30-min self-paced exercise bout in moderate heat (34.9 (0.2)°C, 50.1 (1.1)% relative humidity; 2.80 (0.1) kPa, absolute humidity) and one in mild heat (29.2 (0.2)°C, 69.4 (0.9)% relative humidity, 2.81 (0.05) kPa), with trials counterbalanced. Despite the mean skin temperature (36.3 (0.1) vs. 34.5 (0.2)°C, $p < 0.01$) and thermal perceptions (TD: 3.1(0.1) vs. 2.8(0.1) AU, $p < 0.01$; TS: 6.3(0.1) vs. 5.8(0.1) AU, $p < 0.01$; Skin Wetness: 2.2(0.1) vs. 1.9(0.1) AU, $p = 0.03$; Pleasant: -1.7(0.2) vs. -1.2(0.2) AU, $p < 0.01$) being greater in moderate than mild heat, all other variables, such as rectal temperature (38.0 (0.1) vs. 37.9 (0.1)°C, $p = 0.3$), local sweat rate (1.0 (0.10) vs. 0.9 (0.1) $mg.cm^2.min^{-1}$, $p = 0.28$), cutaneous blood flow (283.4 (26.7) vs 287.3 (22.7) PU, $p = 0.9$), mean power output (206.1 (9.5) vs. 205 (10.4) W, $p = 0.87$), perceived exertion(14.8(0.4) vs. 14.4(0.4) AU, $p = 0.20$) and total work completed (371.1 (63.9) vs. 369.1 (69.8) kJ, $p = 0.77$) did not show a significant difference between environments during the 30-minute time trial. It is concluded that in warm-hot, but compensable, environments matched for evaporative capacity, ambient temperature played a limited role in the physiological responses observed but significantly affected the perceptual responses.

7.1: Introduction

Performing prolonged-duration exercise in the heat exacerbates physiological strain (Galloway and Maughan, 1997, González-Alonso *et al.*, 1999) and thereby escalates the chance of obtaining heat-related illness (Casa, 1999). The most notable physiological strain in the heat is the rise in core temperature (Galloway and Maughan, 1997, González-Alonso *et al.*, 1999, Périard *et al.*, 2011) and subsequent cardiovascular strain (Rowell, 1974b). As a consequence, exercise performance is impaired in order to avoid thermoregulatory catastrophe (Marino, 2004, Noakes *et al.*, 2005). This impairment in exercise performance in the heat is part of our behaviour and has been found to reduce thermoregulatory strain in an uncompensable heat stress environment (Schlader *et al.*, 2011a). This is best exemplified by self-regulating exercise intensity is, as mean power output decreases significantly in the heat, compared to in a temperate environment, in order to maintain the perception of effort and core temperature (Périard *et al.*, 2011, Schlader *et al.*, 2011d, Tucker, 2009).

To date, the underlying mechanism of initiating the behavioural response(s) at rest (Schlader *et al.*, 2017) or during exercise in the heat is attributed to the high ambient temperature, as it induces a greater rise in skin temperature (Schlader *et al.*, 2011c). Higher skin temperature is associated with a change in thermal perception (thermal discomfort, thermal sensation) (Schlader *et al.*, 2011b), and also results in greater cutaneous perfusion, due to a reduced core to skin temperature gradient (Sawka *et al.*, 2012). As a result, exercise intensity must decrease (Cuddy *et al.*, 2014). However, whether skin temperature, as a reflection of high ambient temperature, is associated with altering behaviour and responsible for greater cutaneous perfusion during exercise in the heat, still remains debatable due to three major reasons.

Firstly, all previous studies (Ely *et al.*, 2010, Périard *et al.*, 2011, Schlader *et al.*, 2011d, Tucker *et al.*, 2004) have used two extreme environments, where both ambient temperature and humidity were not equal with each other. In particular, the hot condition was more hot *and* humid than the cool environment. As a result, the combination of temperature and humidity may have caused the elevation in core and skin temperatures and the cardiovascular strain. In fact, humidity may contribute more towards the increase in core and skin temperatures and cardiovascular and perceptual strain, than temperature alone, due to the restriction of evaporative cooling capacity (Che Muhamed *et al.*, 2016a, Maughan *et al.*, 2012). This concept has support from the results of **Chapter Five** and **Six** (in women) as well as in other studies (Che Muhamed *et al.*, 2016a, Maughan *et al.*, 2012, Moyen *et al.*, 2014).

Therefore, to determine whether ambient temperature alone is associated with greater physiological strain, it is necessary to fix the absolute humidity between the environments, so that a firm conclusion can be drawn. Unfortunately, no studies are available in this regard, and therefore this requires further investigation.

Secondarily, although Schlader *et al.* (2011c) eloquently demonstrated that skin temperature is in fact a key regulator of behavioural responses during exercise in the heat, they did so through the open loop approach, whilst neglecting the closed-loop component. The open loop approach manipulates skin temperature, using a water-perfused suit, which is capable of cooling or warming the skin at various temperatures. However, this approach reduces (abolishes) evaporative cooling via sweat from the skin surface and increases the formation of skin wetness, which subsequently changes the heat stress environment to an uncompensable state (Kraning and Gonzalez, 1991a). The resulting skin wetness may incur greater thermal discomfort (Fukazawa and Havenith, 2009) and thereby affect behavioural responses. Furthermore, temperature regulation in real-life is achieved via both closed and open loop control and so this method is not a preferred method for accessing human behavioural thermoregulation. Based on this premise, it would be interesting to investigate whether a high skin temperature, under both open and closed loop approaches, is still the dominant factor in altering perception and thereby affecting behavioural responses when environments are matched for vapour pressure.

Lastly, previous studies did not investigate behavioural thermoregulation from an integrative perspective. That is, all previous studies focused solely on autonomic and perceptual responses (Schlader *et al.*, 2011b, Schlader *et al.*, 2011d) or cardiovascular responses between hot and cool environments (Périard *et al.*, 2011). However, the initiation of behavioural responses during exercise in the heat could be multi-faceted and it is necessary to account for these factors in combination in order for a firm conclusion to be drawn. In particular, whether the initiation of behavioural responses during exercise in the heat is due to the interplay between the perceptual, autonomic and cardiovascular systems remains unknown and this issue can only be resolved by conducting a systematic and integrative investigation. Furthermore, although it has previously been illustrated that the rate of perceived exertion (RPE) is regulated by exercise intensity between hot and cool environments, in which subjects had to reduce their work load in order to maintain the RPE and core temperature, this was done by either the liquid conditioning garment (Schlader *et al.*, 2011b) or using two extreme environments (Schlader *et al.*, 2011d). As mentioned previously, this approach may not be

valid as it poses a few limitations. Furthermore, the inter-connection between thermal perception (thermal discomfort, thermal sensation and pleasantness), RPE and skin wetness on behavioural thermoregulation during exercise in the heat is currently unknown.

Therefore, the aim of this study was to examine the role of skin/environmental temperature on the thermoregulatory, cardiovascular and perceptual responses when both environments were matched for vapour pressure. The candidate hypothesized that high skin temperature is not associated with the changes in thermoregulatory and cardiovascular responses during exercise, despite greater thermal perceptions being observed.

7.2: Methods

7.2.1: Ethical Approval

This study was approved by the Massey University Human Ethics committee: Southern A (16/74).

7.2.2: Participants

Fourteen moderate to highly-trained male cyclists were recruited for this study. The participants' characteristics are outlined in **Table 2** (**Section 4.1**).

7.2.3: Experimental Overview

Seasonal control was mentioned in **Section 4.3**. All participants attended the laboratory on four occasions: (1) preliminary submaximal and maximal aerobic capacity test, (2) experimental familiarization, (3) 35°C temperature trial (35°C, 50.1 (1.1) % relative humidity, 2.8 (0.1) kPa, absolute humidity, WBGT: 29) and 29°C trial (29.2 (0.2)°C, 69.4 (0.9) % relative humidity, 2.81 (0.05) kPa, WBGT: 26). Both trials were different in terms of temperature (34.9 (0.2)°C VS 29.2 (0.2)°C, $p < 0.01$) but were not different in terms of absolute humidity (2.80 (0.1) vs 2.81 (0.05) kPa, $p = 0.7$), with trials counterbalanced. Circadian rhythm was taken into account and was mentioned in **Section 4.4**. Each trial consisted of a six-minute warm up at 125 W and was followed immediately by a 30-min self-paced cycling performance trial. All exercise was performed on an electronically-braked cycle ergometer, as was mentioned in **Section 4.5.3** and set up according to individual preferences (**Section 4.5.3**). Water was recorded and supplied *ad libitum* throughout the entire trial.

7.2.4: Preliminary Testing and Familiarisation

Submaximal and maximal aerobic tests were completed by all participants and described clearly in **Section 4.5.3**. At least 24 h following preliminary testing, familiarization trial (35°C, 50% relative humidity) was undertaken to ensure participants were accustomed to the experimental procedures and to minimise learning effects. These trials replicated entirely, the experimental trials, outlined below.

7.2.5: Experimental Procedure

Both 35°C and 29°C heat stress trials were conducted in the same environmental chamber with a frontal airflow of 19 km h^{-1}. A detailed description of the airflow procedure can be found in **Section 5.2.6**. Hydration status was confirmed using urine specific gravity, as mentioned in **Section 4.4**. Core and skin temperatures were measured by rectal and skin thermistors, as outlined in **Section 4.5.4**. Immediately after the placement of the rectal thermistor, participants entered the environmental chamber wearing only cycling shorts, shoes and socks. Participants rested on the ergometer for 20 min, during which they were instrumented and baseline measurements were recorded. Participants then underwent a six-minute warm up at 125 W to allow sufficient warm-up and fixed-intensity responses to be recorded. Physiological measurements taken during the final 2 min of fixed-intensity included heart rate (HR), blood pressure (BP), cardiac output ($\dot{Q}$) and perceptual responses, whilst rectal (T_{rec}) and skin ($\bar{T}_{sk}$) temperatures, skin blood flow as well as local sweat rate (LSR) were measured continuously. Immediately on completion of the 125 W bout, the ergometer was set to linear mode, in which participants were instructed to perform as much work as possible over 30 min. During this 30 min self-paced period, work completed (kJ), HR, and perceptual responses were recorded every 6 min, whilst T_{rec}, $\bar{T}_{sk}$, skin blood flow and LSR were measured continuously. Blood pressure was measured at the end of the first six-minute stage of the 30-minute self-paced exercise. Total work completed was used as the performance measure, whereas the time profile of power output was used as the behavioural measure.

7.2.6: Measurements

The details of procedures for anthropometric, cardiovascular, body temperature, sudomotor, and perceptual measures were clearly outlined in **Chapter 4 (**anthropometric: **Section 4.5.1**; cutaneous blood flow: **Section 4.5.7.2**; cardiovascular: **Section 4.5.6**; body temperature: **Section: 4.5.4**; sudomotor: **Section 4.5.8**; perceptual: **Section 4.5.9**).

7.2.7: Statistical Analysis

Basic descriptive statistics and the normality of the data were outlined in **Section 4.5.10**. The autonomic, perceptual and cardiovascular data at rest and during the warm up period, as well as the total completed work during the 30-minute time trial, were analysed by paired samples T-tests. However, during the 30-minute time trial, all other data, including the mean power

output and autonomic and perceptual measures were analysed by two-way repeated measure ANOVA (environment × time points); in cases where main or interaction effects occurred, *post hoc* pairwise analyses were performed, using paired samples T-tests with Bonferroni corrections, if appropriate. All data are expressed as mean and standard error estimate (SEE).

7.3: Results

7.3.1: Exercise Performance and Behaviour

Total work completed (Fig. 22) was not different between environments (35°C: 371.1 (17) vs 29°C: 369.1 (18.7) kJ, $p = 0.77$). In line with this observation, the mean power output was not different between environments (35°C: 206.1 (9.5) vs 29°C: 205.10 (10.4) kJ, $p = 0.78$) with a trend over time ($p = 0.06$) and no interaction observed ($p = 0.61$).

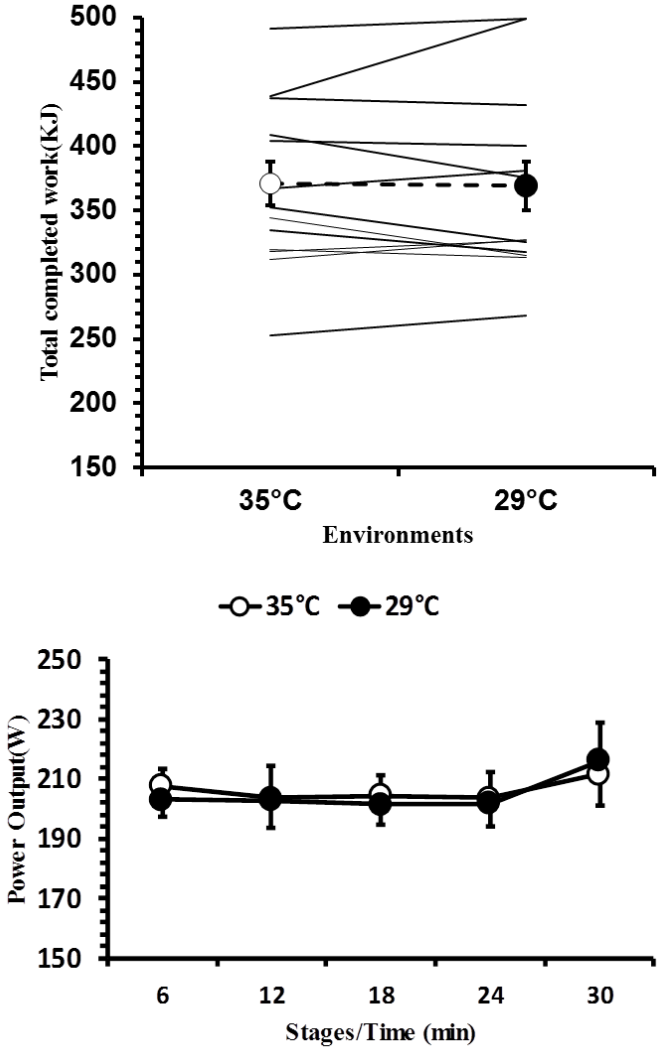

Figure 19: Individual and mean (SEE) work capacity (n = 14), and mean (SEE) power output (n = 14) during exercise in 35 °C and 29°C.

7.3.2: Thermoregulatory Measures

7.3.2.1: Body Temperature

T_{rec} at rest was not different between environments (35°C: 37.2 (0.1) vs 29°C: 37.2 (0.1)°C, p = 1.00) and persisted throughout the warm up period (35°C: 37.3 (0.1) vs 29°C: 37.2 (0.1)°C, p = 0.42) (Fig. 23). Furthermore, the rise in T_{rec} ($p < 0.01$) from rest to warm up was not different between environments. The rise in T_{rec} during the 30 min time-trial displayed a significant environment x time interaction ($p < 0.01$), whereby the change in T_{rec} was greater in 35 °C than in the 29 °C environment (0.1 (0.05)°C, p = 0.05).

Resting T_{Sk} was 2.6 (0.2)°C higher in 35°C than in 29°C ($p < 0.01$) (Fig. 23). Likewise, the rise in T_{Sk} ($p < 0.01$) from rest to warm up was also 2.6 (0.2)°C higher ($p < 0.01$) in 35°C than in 29°C. The rise in T_{Sk} ($p < 0.01$) remained 1.7 (0.1)°C higher at every time point (environments x time point interaction: $p < 0.01$) throughout the 30 min time-trial.

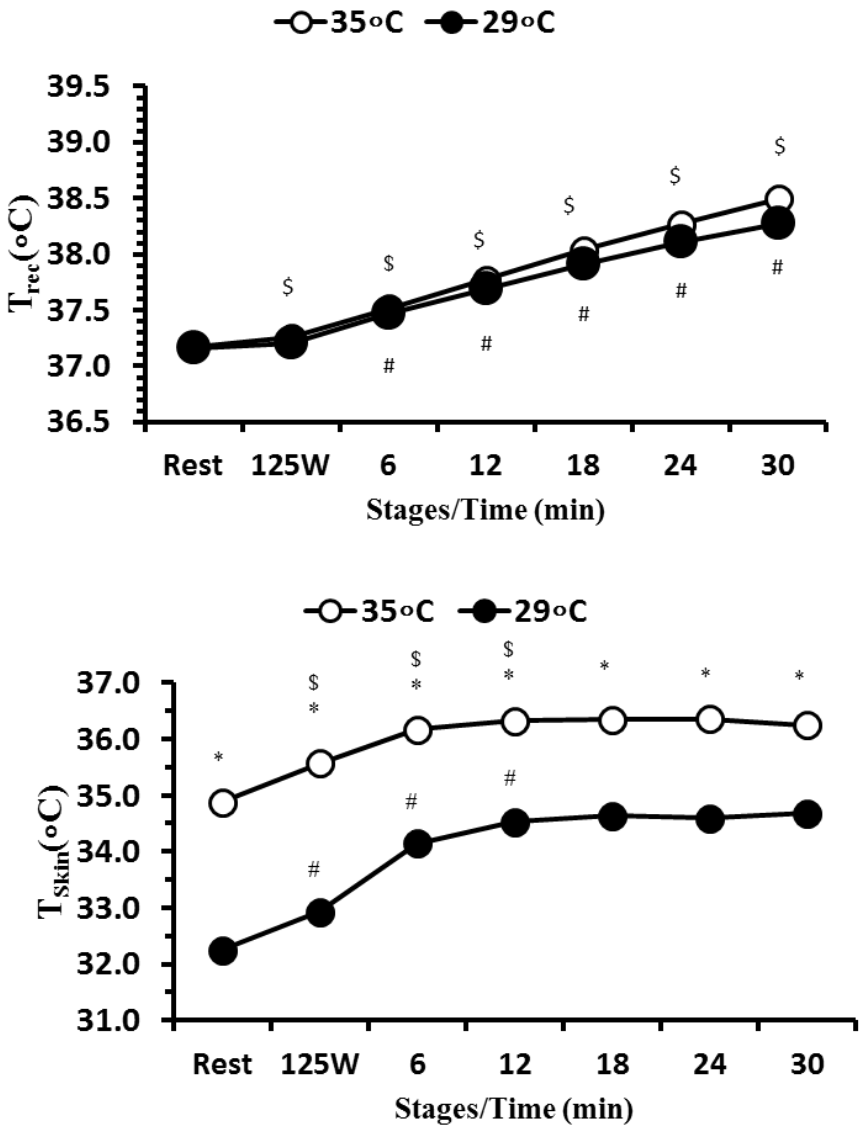

Figure 20: Mean (SEE) rectal temperature (T_{rec}, n = 14) and weighted mean skin temperature ($\bar{T}$sk, n =14) during exercise in 35 °C and in 29 °C environments.

* significant difference between 35°C and 29°C environments**.**

$ Significant difference with preceding time points in 35°C environment.

Significant difference with preceding time points in 29°C environment.

7.3.2.2: Cardiovascular and Thermoeffectors

Resting HR was not different between environments ($p = 0.13$) but the rise ($p < 0.01$) of HR from resting was ~8 beat/min higher ($p = 0.03$) during the warm up phase. This elevation of heart rate persisted throughout the 30 minutes self-paced trial as the rise in HR from warm up ($p < 0.01$) was ~5 beat/min higher in 35 °C than in 29 °C. However, no statistical interaction was observed between environments by time points ($p = 0.62$)

Resting $\dot{Q}$, FVR, SV and MAP were similar between environments (all $p > 0.07$) whilst SKBF was higher in 35°C than in 29°C (Table 5 and Fig. 24, $p = 0.05$). During the warm up period, the rises in $\dot{Q}$, SV and MAP (all $p < 0.01$) were similar between environments (all $p > 0.50$). However, the rise of SKBF ($p < 0.01$) from resting was higher ($p = 0.01$) in 35°C than in 29°C. Therefore, FVR was lower ($p = 0.03$) in 35 °C than in 29°C. During the 30-minute time trial, $\dot{Q}$, FVR, SV and MAP were similar (all $p > 0.10$) between environments and only MAP and $\dot{Q}$ in 29°C were higher ($p < 0.01$) than the warm up period. Similarly, the rise in SKBF ($p < 0.01$) from warm up remained similar between environments ($p = 0.91$) with no interaction observed ($p = 0.12$).

Resting LSR was not different between environments ($p = 0.27$) (Fig. 24) but the rise in LSR ($p < 0.01$) from resting to the end of the warm up stage was higher in 35°C than in 29°C environments ($p = 0.05$). By contrast, the rise in LSR ($p < 0.01$) from warm up to the end of the 30 minutes self-paced exercise was not different between environments ($p = 0.3$) with no interaction observed ($p = 0.20$). Also, WBSR was not different between environments (0.95 (0.09) vs 0.82 (0.06) $g.h^{-1}$), respectively; p = 0.06, possibly type II error). Water consumption was not different between environments (0.55 (0.09) vs 0.48 (0.07) L, respectively; $p = 0.29$).

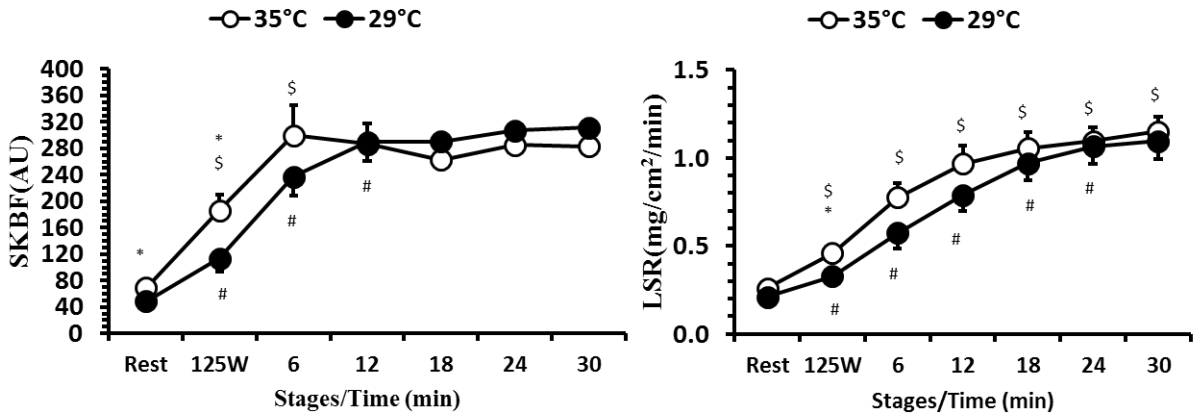

Figure 21: Mean (SEE) local sweat rate (LSR, n = 14) and skin blood flow (SKBF, n = 14) against time during exercise in 35°C and 29°C environments.

$ Significant difference with preceding time points in 35 °C environment.

Significant difference with preceding time points in 29 °C environment.

* Significant difference between 35 °C/29 °C environment.

Table 5: Mean arterial pressure (MAP, n = 13), cardiac output ($\dot{Q}$, n = 11), forearm vascular resistance (CVR, n = 13) and stroke volume (SV, n = 11) at rest, during warm up and 6 minutes after the start of the 30-minute time trial in both 35°C/29°C environments.

	35°C			29°C		
	Rest	**125W**	**6 min**	**Rest**	**125 W**	**6 min**
MAP (mmHg)	86.8(1.70)	96.5(1.60)**#**	103.1(1.8)**#**	88.6(1.8)	95.1(1.6)**#**	102.8(2)**#**
$\dot{Q}$ (L. min^{-1})	10(1.0)	25(1.30)**#**	28(1.7)**#**	9(0.7)	24(1.3)**#**	31(1.1)
CVR (AU. mmHg^{-1})	1.6(0.2)	0.7(0.1)**#***	0.4(0.1)**#**	2.1(0.3)	1.1(0.2)**#**	0.6(0.2)**#**
SV (mL)	140.8(11.6)	204.4(12.4)**#**	185.3(11.2)	128.9(13.9)	204.4(12.1)**#**	209.9(9.3)

Significant difference for the preceding time point

*Significant difference between 35°C and 29°C

7.3.3: Perceptual

At rest, participants felt hotter (TS) ($p < 0.01$) at 35°C than at 29°C, but felt similar skin wetness, PL and TD between environments (Fig. 25) (All $p > 0.10$). During the warm up period, participants felt higher hotter (TS) ($p < 0.01$) and more discomfort (TD) ($p = 0.03$) at 35°C than at 29°C, but felt similar in terms of PL, wetness and RPE between environments (All $p > 0.10$). All measures from the warm up period were significantly different compared to baseline ($p < 0.01$). During the 30-minute time trial, participants felt higher TS, TD, wetness and more unpleasant at 35 °C than at 29 °C (all $p \leq 0.03$) and all measures were significantly different from the warm up period ($p < 0.01$). Furthermore, only TS showed a significant environment x time point interaction ($p < 0.03$); all other measures (TD, PL, Skin Wetness) showed no environment x time interaction (all $p > 0.20$). However, the rise in RPE ($p < 0.01$) from warm up was not different between environments ($p = 0.21$) and showed no environment x time point interaction ($p = 0.94$).

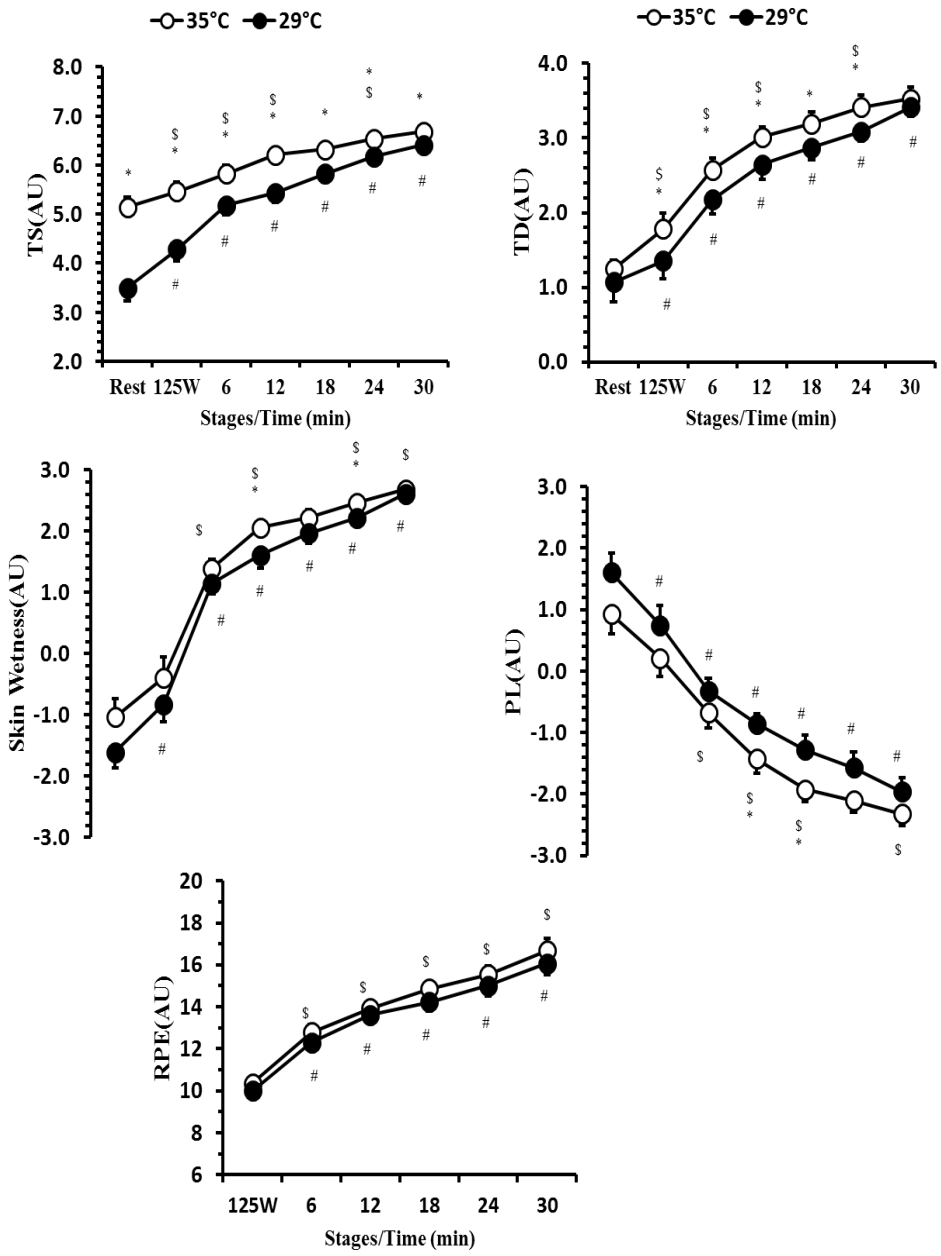

Figure 22: Mean (SEE) rate of perceived exertion (RPE, n = 14), thermal sensation (TS, n = 14), thermal discomfort (TD, n = 14), pleasantness (PL, n = 14) and skin wetness (n = 14).

$ Significant difference with preceding time points in 35 °C environment.

Significant difference with preceding time points in 29 °C environment.

* Significant difference between 35 °C/29 °C environments.

7.4: Discussion

To date, no studies using exercise have quantified the role of T_{sk} on behavioural thermoregulation, using the same vapour pressure between environments and both open and closed loop approaches. Furthermore, the links between thermal perception, autonomic and cardiovascular measures on behavioural thermoregulation remain unclear, as no integrative approaches have been investigated. Therefore, this study investigated behavioural thermoregulation from an integrative perspective, specifically the relationships between perceptual, cardiovascular and autonomic systems on behavioural thermoregulation, in moderate and mild heat environments, with a fixed vapour pressure.

The novel findings of this study are that: (1) behavioural responses in the heat were not affected by high T_{sk}; (2) thermoregulatory autonomic function was unaltered between environments; (3) SKBF was higher at rest and during warm up at 35 °C, but was similar in both environments during self-paced exercise; (4) thermal perception (TS, TD, PL, skin wetness) were directly accompanied by higher T_{sk}; and (5) RPE was disassociated from thermal perception.

Performance was unaffected by high ambient/skin temperature

Unlike the findings from previous studies (Périard *et al.*, 2011, Schlader *et al.*, 2011c, Tatterson *et al.*, 2000, Tucker *et al.*, 2004), this study (Fig. 23) does not support T_{sk} — a reflection of ambient temperature — as a controller of exercise intensity during moderate-duration self-paced exercise. This could be due to several reasons.

Firstly, all previous studies (Périard *et al.*, 2011, Schlader *et al.*, 2011d, Tucker *et al.*, 2006, Tucker *et al.*, 2004) utilised two extreme environments, with the hot trial being more humid and hotter than the cool environment. In such environments, the rate of heat storage increases due to the restriction of evaporative cooling. This results in an anticipatory reduction of skeletal muscle recruitment, in order to prevent a greater rise in HS (Tucker *et al.*, 2006). However, using this approach it is difficult to conclude whether it is the humidity or temperature alone that causes the reduction in exercise intensity.

Secondly, although Schlader *et al.* (2011c) eloquently demonstrated that T_{sk} was the controller of behavioural responses during self-paced exercise, their approach may not be applicable to a real life scenario, as they neglected the closed loop approach of behaviour and temperature

regulation. Their experimental design involved either heating or cooling skin via a water-perfused suit; this approach once again restricted evaporative cooling and thus changed the situation to an uncompensable situation. Therefore, it was expected that the trial that started with the higher T_{sk} would elicit a reduction in power output compared to the cool skin trial. Although the higher skin temperature trial was cooled after 15 minutes of self-paced exercise, the hotter skin at the beginning of the trial may still serve as a protective action to prevent the excessive rise of core temperature, compared to the cooler skin trial, due to higher thermal perception up until 15 minutes of exercise.

Lastly, the protocol used for assessing the effect of T_{sk} on behavioural thermoregulation during exercise was done throughout a prolonged period, using a 60-minute time trial (Schlader *et al.*, 2011c); therefore, all participants would have had to reduce their power output more in the hot skin trial, in order to complete the task without major physiological consequences. This is based on anticipatory theory, which is suggested to act to prevent catastrophic breakdown of the physiological system, as described by Marino (2004). Collectively, the three aforementioned reasons argue the role of T_{sk} as a controller of exercise intensity during self-paced exercise in the heat using both open and closed loop approaches, with environments matched for vapour pressure. Furthermore, this justifies our approach and the finding that behavioural responses in this study were unaltered between the environments.

Thermoregulation is unaltered between environments or nullified by behaviour

The findings from this study indicate that when environments are matched for vapour pressure, no differences occur in terms of regulated T_{rec} (Fig. 23) and sweating (Fig. 24) between environments, at rest and throughout the 30-minute time trial. This may be due to the fact that T_{core} is influenced more by vapour pressure than ambient temperature *per se*, as it reduces evaporative cooling (Che Muhamed *et al.*, 2016a, Moyen *et al.*, 2014). Evaporative cooling is the primary pathway for heat dissipation during exercise and if this is deprived, it increases the rate of heat storage, which alters thermoregulatory function (Che Muhamed *et al.*, 2016b) and ultimately affects physical capacity (Maughan *et al.*, 2012). Likewise, when vapour pressure between the environments is matched, ideally no difference for T_{core} would have been observed. This then results in no change of sweating since T_{core} is the key regulator to initiate the sweating response at rest and during exercise (Saltin *et al.*, 1972, Wyss *et al.*, 1974). The finding of the present study is in line with these studies (Wyss *et al.*, 1974, Saltin *et al.*, 1972), as the sweating response was not different between environments.

The SKBF data (Fig. 24) revealed that SKBF was greater at rest and during the warm up period in 35°C compared to the 29°C environment. This may be because T_{sk} was higher at 35°C than at 29°C. As a reflection of the high SKBF and the lack of change in MAP between the 35°C/29°C environments, FVR was lower during the warm up phase. This finding is also in line with Pergola *et al.* (1994), as they observed that a rising T_{sk} was associated with higher cutaneous vascular conductance (CVC) and higher SKBF, following local heating of the non-glaborous skin. However, interestingly, this did not persist during the 30-minute time-trial. This may due to the fact that vasomotor drive during exercise in the heat is more heavily weighted by T_{core} (Buskirk, 1977, Taylor and Groeller, 2008) and as T_{rec} was not different between 29°C/35°C environments, it is expected that vasomotor drive remains similar between 29°C/35°C environments.

Thermal perception mirrors skin temperature but is disassociated from perceived exertion

The thermal perception data (Fig. 25) are in agreement with Gagge *et al.* (1967), where increasing the T_{sk} was associated with higher TS and TD. However, this study extends this finding to pleasantness and skin wetness. More importantly, this study was able to partition the effect of humidity on thermal perception and to the best of the candidate's knowledge, this study is the first of its kind to investigate the role of ambient temperature *per se* on the effects of thermal perception on behavioural thermoregulation. It is generally agreed that pleasantness corresponds to the restoration of thermal comfort (Attia and Engel, 1981) and so it is expected that having more TD is associated with feeling more thermally unpleasant. However, few studies are available to partition the effects of temperature and humidity on TD and thermal pleasantness. This study is the first of its kind to establish that ambient temperature alone affects thermal comfort and thermal pleasantness. Moreover, traditionally, skin wetness is believed to be regulated by humidity rather than temperature per se (Filingeri and Havenith, 2015). However, this study showed that skin wetness can also be moderated by ambient temperature alone. However, our skin wetness data should be viewed with caution, as we only measured whole body skin wetness, instead of regional skin wetness and this was done through subjective feeling rather than measuring with a hygrometer. Ongoing investigation into this area is therefore required, but this is not the major focus of this chapter.

Another major finding for this study was that RPE remained similar between environments, despite similar power outputs being observed. This finding is in contrast to the findings of Schlader *et al.* (2011b), Schlader *et al.* (2011d), who observed a reduction in power output, despite similar RPE values. However, methodological differences likely account for this discrepancy. Firstly, Schlader *et al.* (2011b) neglected the closed loop component of behavioural and temperature regulation, as they used the water-perfused suit approach. Using a water perfused suit changes the heat stress status to uncompensable and therefore any additional cooling or warming of the face alters thermal perception, because the face has greater thermal sensitivity than other regions of the body (Cotter and Taylor, 2005). In contrast, this study adopted both open and closed loop control, to assess thermal perception and behavioural thermoregulation, which was not the same study purpose as described by Schlader *et al.* (2011b). Furthermore, Schlader *et al.* (2011b) used an untrained population, whereas the participants for this study were moderate to highly trained athletes. Thus, perceptual responses may have differed between our population and theirs, because a greater

aerobic fitness has been linked to lower perceptual responses and higher times to exhaustion, compared to an untrained population (Selkirk and McLellan, 2001).

Secondly, although another study by Schlader *et al.* (2011d), using both open and closed loop designs, concluded that exercise intensity in the heat must decrease in order to maintain the same RPE, the environments used were both extreme, with the hot trial being more humid and hotter than cool trial. As mentioned previously, this approach cannot partition the effect of humidity on behaviour as well as perception, which was contradictory to the main purpose of the study. Nevertheless, study from Schlader *et al.* (2011b), Schlader *et al.* (2011d) and the findings from this studies still agree that thermal perception is altered by T_{sk}, despite no changes in behavioural responses in the current study, in a moderate to highly trained population.

The most likely explanation for no difference in RPE between environments in the current study, is that T_{core} was not different between environments. Elevation of T_{core} has been shown to reduce the arousal level via alterations in brain wave activity (Nielsen *et al.*, 2001). In particular, the α/β wave ratio of the brain wave is elevated during hyperthermia and this is the primary mechanism of reducing arousal and increasing the RPE at a given intensity in the hyperthermic state (Nybo and Nielsen, 2001b). Therefore, it can be concluded that if the T_{core} remains the same between trials, RPE will remain the same, as there is no change in arousal level, due to no change in α/β wave ratio.

One of the limitations for this study is the relatively short exercise duration when compared to more prolonged exercise (i.e. ≥ 60 min). In particular, given the fact that the net rise in T_{core} was greater in the 35 °C than in 29 °C environment, it is expected that if the duration was extended to ~1 hour, even at the same vapour pressure, temperature alone could still possibly induce greater thermoregulatory and cardiovascular strain. This is especially the case as a slow-responding index of T_{core} was used(Mündel *et al.*, 2016).

In conclusion: (1) a higher T_{sk} resulted in greater thermal perception but did not correspond to a reduction in exercise performance when environments were matched for vapour pressure (2) a higher T_{sk} did not result in greater autonomic and cardiovascular strain during a 30-minute time trial when environments were matched for vapour pressure and behaviour allowed.

Chapter Eight

8.0: Autonomic and behavioural thermoregulation during exercise in different thermal profiles when matched for vapour pressure

Abstract

The purpose of this study was to examine the thermoregulatory responses between self-paced and fixed-intensity exercise matched for average workload, in different thermal profiles matched for vapour pressure. Fourteen moderate to highly trained cyclists (age: 31.5 (3.2) years; height: 177.8 (1.5) cm; mass: 76.4 (2.3) kg; $\dot{V}O_{2max}$: 59 (2.5) $mL.kg^{-1}.min^{-1}$; body surface area: 1.93 (0.03) m^2; body fat: 13.5 (4.4)%; peak power output: 392.7 (14.1) Watts) were recruited, where they underwent two self-selected pace trials and two constant-power trials in 35°C (temperature: 34.9 (0.2)°C; relative humidity: 50.1 (1.1)%; absolute humidity: 2.80(0.1) kPa) and 29°C (temperature: 29.2 ± 0.2 °C; relative humidity: 69.4 (0.9)%; absolute humidity: 2.81 (0.05) kPa) with the same vapour pressure. Despite cutaneous blood flow at the forearm region showing counter intuitive results (35°C(Self-paced vs fixed-intensity): 279.1 (28.4) AU vs 227.1(19.1) AU, p = 0.042; 29°C (Self-paced vs fixed-intensity): 301.3 (25.8) AU vs 378 (39.1) AU, p = 0.03), all others variables were not different between self-paced and fixed-intensity or different thermal profiles, such as rectal temperature (35°C: self-paced: 38.0 (0.1)°C vs fixed-intensity: 37.9 (0.1) °C; 29°C: self-paced: 37.9 (0.1)°C vs fixed-intensity: 38.0 (0.1) °C, all $p > 0.2$), skin temperature (35°C: self-paced: 36.3 (0.1)°C vs fixed-intensity: 36.2 (0.1)°C; 29°C: self-paced: 34.5 (0.2)°C vs fixed-intensity: 34.4 (0.2)°C, all $p > 0.4$), local sweat rate (35°C: self-paced: 1.0 (0.1) $mg.cm^{-2}.min^{-1}$ vs fixed-intensity: 0.9 (0.1) $mg.cm^{-2}.min^{-1}$; 29°C: self-paced: 0.9 (0.1) $mg.cm^{-2}.min^{-1}$ vs fixed-intensity: 0.8 (0.1) $mg.cm^{-2}.min^{-1}$, all $p > 0.2$). We conclude that self-pacing does not reduce thermoregulatory strain in mild and moderate heat, when average workload is matched.

8.1: Introduction

Maintaining a desired core temperature during exercise in the heat is preferable for preventing fatigue as well as avoiding hyperthermia and possible heat illness. During exercise in the heat, autonomic thermoregulatory responses have a limited capacity to regulate body temperature, whilst behavioural thermoregulation can be more effective and is virtually limitless in regulating body temperature (Schlader *et al.*, 2010, Parsons, 2014, Benzinger, 1969a). The findings from **Chapters Five**, **Six** and **Seven**, as well as those from several previous studies (Lander *et al.*, 2009, Schlader *et al.*, 2011d, Tatterson *et al.*, 2000, Tucker *et al.*, 2006, Tucker *et al.*, 2004) provide robust evidence that behavioural thermoregulation can reduce thermoregulatory strain in women (**Chapters Five** and **Six**) and in men but to a lesser extent (**Chapter Seven**), under more stressful environments. Schlader *et al.* (2011a) demonstrated that when given the opportunity to behave, thermoregulatory strain was lower in self-paced than in fixed-intensity trials, due to the voluntary reduction of exercise intensity. Collectively, these results highlight the merits of behavioural thermoregulation on thermal homeostasis, and demonstrate that the voluntary reduction of exercise intensity in the heat is, in fact, part of our behaviour.

Although a growing body of evidence has suggested that self-pacing is a proven means to reduce core temperature, this issue still remains incomplete, as the comparison between self-selected pace and fixed-intensity does not have a common starting point. For example, Schlader *et al.* (2011a) eloquently demonstrated that self-pacing reduced thermoregulatory strain in an uncompensable heat stress environment compared to fixed-intensity, yet, the exercise intensity in the fixed-intensity trial was significantly greater than the self-paced trial. This corresponded to higher metabolic heat production, and, therefore resulted in a higher core temperature response. Furthermore, it was conducted in a dry heat environment, something that may not translate to a humid heat setting. Therefore, to provide a definitive assessment of whether self-paced exercise is a means to reducing thermoregulatory strain, it is necessary to match the average workloads (hence, metabolic heat production) between self-paced and fixed-intensity trials and to compare this in differing environments matched for vapour pressure; as even if average workloads are matched, periodic fluctuations in power output, thereby metabolic heat production, could transiently affect thermoregulation.

Therefore, this study aims to investigate different exercise modalities (self-paced vs fixed-intensity), at the same average workload, in both warmer and cooler heat environments

matched for vapour pressure. The candidate hypothesized that self-paced exercise does not modulate thermoregulatory strain when both exercise duration and intensity are matched.

8.2: Methods

8.2.1: Ethical Approval

This study was approved by the Massey University Human Ethics committee (Southern A: 16/74).

8.2.2: Participants

The same group of subjects from **Chapter Seven** also participated in this experiment; however, amongst those fourteen subjects, two subjects were unable to complete the fixed-intensity trial in either 35°C or 29°C due to premature fatigue. Due to this reason, one subject was excluded for the comparison between self-paced and fixed-intensity in 35°C. Likewise, one subject was excluded for the comparison between self-paced and fixed-intensity in 29°C. Thus, the final statistical analysis was carried out using the remaining thirteen participants (n = 13).

8.2.3: Experimental Overview

Seasonal control was mentioned in **Section 4.3**. This chapter carries on from **Chapter Seven**, as all of the self-pacing data from **Chapter Seven** was used to compare directly against the fixed-intensity data from this current chapter. Due to this reason, participants only attended the laboratory on a further two separate occasions, one week apart, as follows: (1) fixed-intensity trial in 35°C (temperature: 34.90 (0.2)°C; relative humidity: 49.7(1.4)%; absolute humidity: 2.80 (0.1)kPa) and (2) fixed-intensity exercise in 29°C (temperature: 29.20 (0.2)°C; relative humidity: 68.8(2.31) %; absolute humidity: 2.81 (0.05) kPa). Temperature and humidity in both 35°C and 29°C environments were very similar to the corresponding environments of the self-paced trials: (temperature (35°C self-paced (SP) vs 35°C fixed-intensity (fix); 29°C SP vs 29°C fix; both $p > 0.4$), relative humidity (35°C SP vs 35°C fix; 29°C SP vs 29°C fix; both $p > 0.3$); absolute humidity (35°C SP vs 35°C fix; 29°C SP vs 29°C fix; both $p > 0.1$). To approximate the rate of metabolic heat production between self-paced and fixed-intensity exercise in 35°C and 29°C environments, the power outputs used for the fixed-intensity trials in 35°C and 29°C were derived from the average power output during the self-paced trials in 35°C and 29°C from **Chapter Seven** (Fig. 26)**.** Due to this design, randomization could be achieved between exercise modalities, and therefore participants were not blinded to which trial they completed. However, all subjects were

unaware of the hypothesis of this study. In addition, since the self-paced trials were randomised (**Chapter Seven**) and because the fixed-intensity trial was conducted one week after the self-paced trial, in either 35 °C or 29 °C, it can be assumed that the order effect would be minimal. Circadian rhythm was taken into account and was mentioned in **Section 4.4**. Each trial consisted of a six-minute warm up, at 125 W, and was followed immediately by a 30-minute fixed-intensity cycling trial. All exercise was performed on an electronically-braked cycle ergometer, as mentioned in **Section 4.5.3**, which was set up according to individual preferences (**Section 4.5.3**).

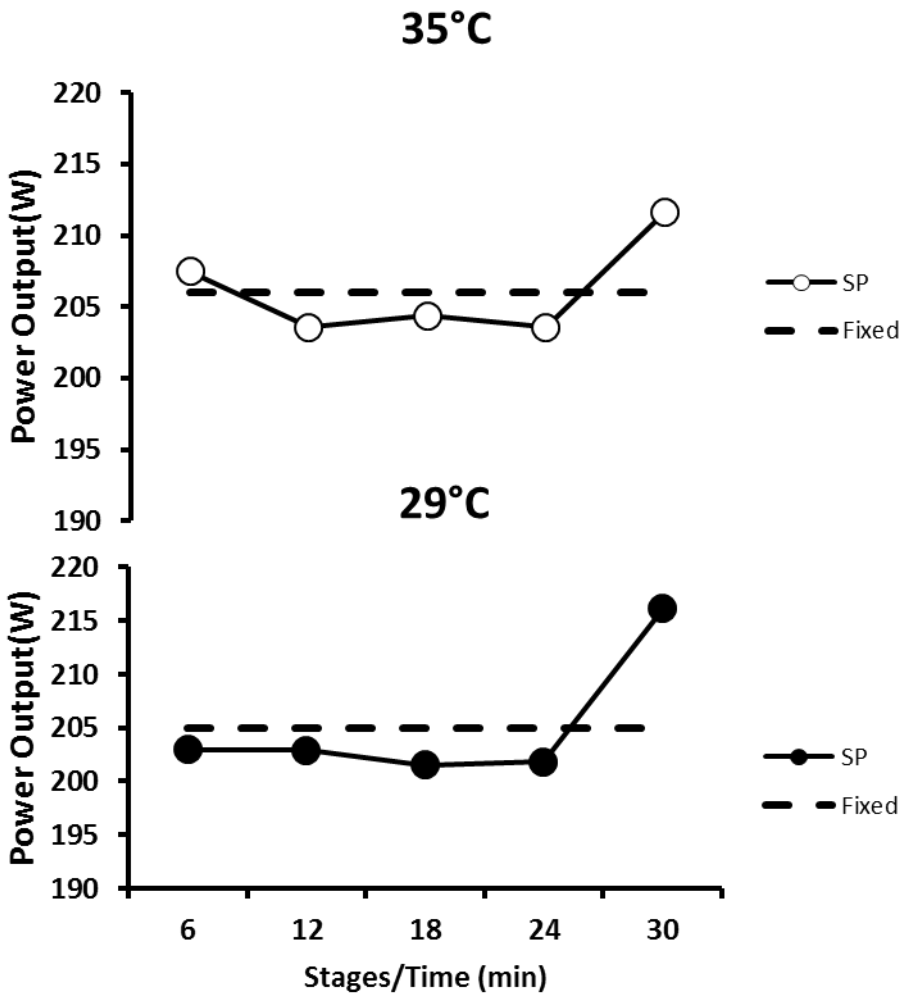

Figure 23: Power output during fixed-intensity and self-paced exercise (n =13) in both 35°C and 29°C environments.

8.2.4: Preliminary Testing

Submaximal and maximal aerobic tests were completed by all participants and are described clearly in **Section 4.5.3**.

8.2.5: Experimental procedure

Both fixed-intensity trials 35°C and 29°C were conducted in the same environmental chamber with a frontal airflow of 19 km h^{-1}. A detailed description of the airflow procedure can be found in **Section 5.2.6**. Hydration status was confirmed using urine specific gravity, as mentioned in **Section 4.4**. Core temperature and skin temperature were measured by rectal and skin thermistors, as outlined in **Section 4.5.4**. Immediately after the placement of the rectal thermistor, participants entered the environmental chamber wearing only cycling shorts, shoes and socks. Then, participants first underwent resting measures, followed by a 6-minute warm up at 125 W, before the 30-minute fixed-intensity exercise. The resting and fixed-intensity measures and protocol were identical to **Section 7.2.5.** Immediately on completion of the 125 W bout, a 30-minutes fixed-intensity exercise bout was performed. During this 30-minute fixed-intensity exercise period, heart rate (HR), and expired gases were recorded every 6 min, whilst rectal temperature (T_{rec}), skin temperature ($\overline{T}_{sk}$), skin blood flow and local sweat rate (LSR) were measured continuously. Blood pressure was measured at the end of the first six-minute stage of the 30-minute fixed-intensity exercise protocol. Water was recorded and supplied *ad libitum* throughout the entire trial.

8.2.6: Measurements

The details of procedures for anthropometric, respiratory, cardiovascular, body temperature, sudomotor, and biophysics measures were clearly outlined in **Chapter 4 (**anthropometric: **Section 4.5.1**; respiratory: **Section: 4.5.2**; cutaneous blood flow: **Section 4.5.7.2**; cardiovascular: **Section 4.5.6**; body temperature: **Section: 4.5.4**; sudomotor: **Section 4.5.8**; biophysics: **Section 4.5.5**).

8.2.7: Statistical analysis

Basic descriptive statistics and the normality of the data were outlined in **Section 4.5.10**. A two-way repeated ANOVA (modality × time points) was used to compare different exercise modalities (self-paced vs fixed-intensity) on thermoregulatory strain, perception as well as

autonomic strain, in both 35 °C and 29 °C environments. A three-way ANOVA (including an environmental factor) was not chosen as i) the previous **Chapter 7** proved that when matched for vapour pressure, physiological strain is approximate, and ii) to minimise duplication of analyses with **Chapter 7**. In cases where main or interaction effects occurred, *post hoc* pairwise analyses were performed, which were clearly outlined in **Section 7.2.7**. The effector responses for sudomotor and vasomotor measures were mentioned in **Section 4.5.10.** All data are expressed as mean and standard error estimate (SEE).

8.3: Results

8.3.1: Thermoregulatory Measures

8.3.1.1: Body temperature

Resting T_{rec} was not different between self-paced and fixed-intensity exercise, at 35°C (p = 0.46) and 29 °C (p = 1.0) (Fig. 27). The rise of T_{rec} (p < 0.01) from resting to the end of the warm up was also not different between self-paced and fixed-intensity exercise at 35°C (p = 0.66) and 29°C (p = 0.56). During the 30-minute exercise period, the rise of T_{rec} from warm up (p < 0.01) was once again similar between self-paced and fixed-intensity exercise, at 35°C (p = 0.29) and 29°C (p = 0.73). Furthermore, no interactions were observed between modality × time points at 35°C (p = 0.44) and 29°C (p = 0.48).

Resting $\bar{T}_{sk}$ was not different between self-paced and fixed-intensity exercise at 35 °C (p = 0.45) and 29 °C (p = 0.54) (Fig. 27). The rise of $\bar{T}_{sk}$ (p < 0.01) from resting to the end of the warm up was also not different between self-paced and fixed-intensity at 35 °C (p = 0.92) and 29 °C (p = 0.72). During the 30-minute exercise period, the rise of $\bar{T}_{sk}$ from warm up was once again similar between self-paced and fixed-intensity exercise at 35°C (p = 0.46) and 29°C (p = 0.63). Furthermore, no interactions were observed between modality × time points at 35 °C (p = 0.82) and 29°C (p = 0.25).

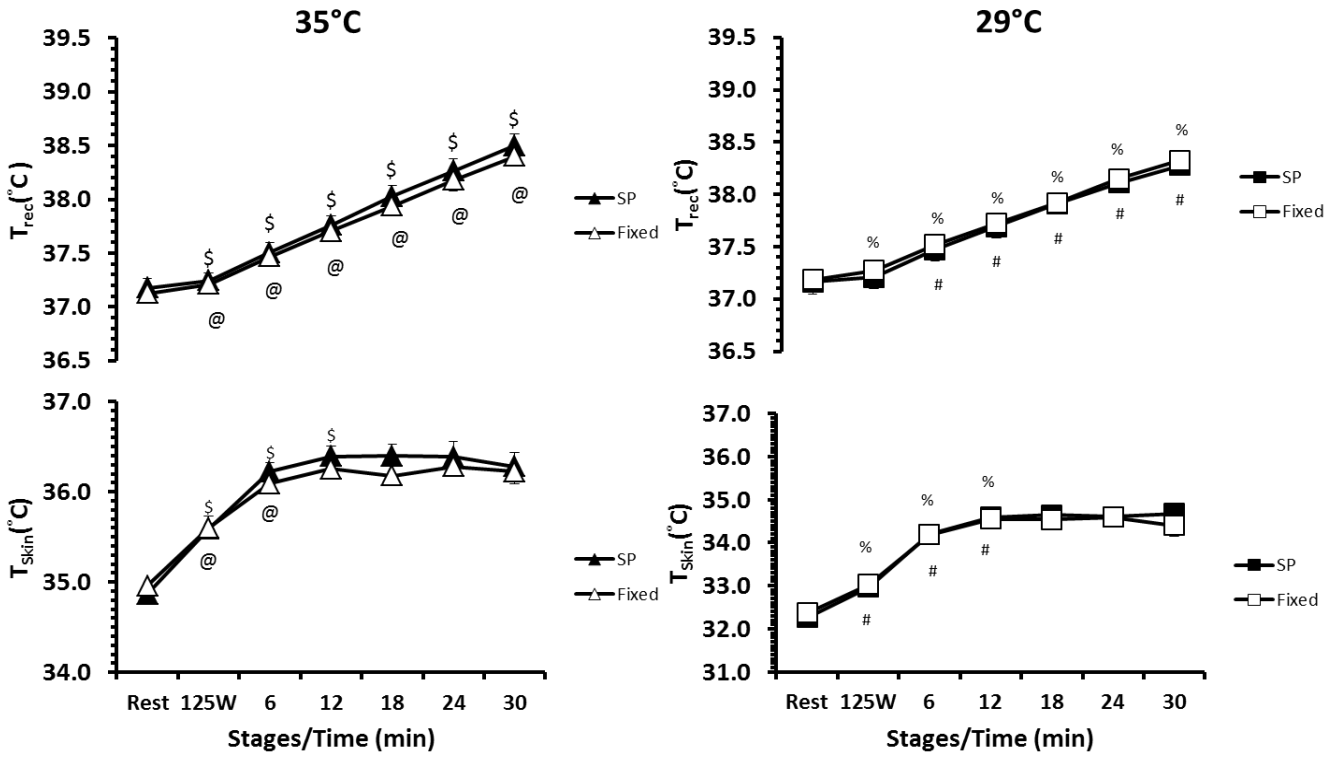

Figure 24: Mean (SEE) rectal temperature (T_{rec}, n = 13) and weighted mean skin temperature (T_{sk}, n =13) during self-paced (SP) and fixed-intensity exercises at 35 °C and 29 °C.

$ Significant difference with preceding time points during self-paced exercise at 35 °C.

@ Significant difference with the preceding time points during fixed-intensity exercise at 35 °C.

Significant difference with preceding time points during self-paced exercise at 29 °C.

% Significant difference with preceding time points during fixed-intensity exercise at 29 °C.

8.3.1.2: Cardiovascular and thermoeffectors

Resting cardiac output ($\dot{Q}$), forearm vascular resistance (FVR), stroke volume (SV), skin blood flow (SKBF) and mean arterial pressure (MAP) were similar between self-paced and fixed-intensity exercise at 35 °C (all $p > 0.4$) and 29 °C (Table 7) (all $p > 0.38$). This remained the same during warm up, as the changes in $\dot{Q}$, FVR, SV, SKBF and MAP from resting ($p < 0.01$) were similar between self-paced and fixed-intensity exercise at 35 °C (all $p > 0.2$) and in the 29 °C (all $p > 0.08$). During the 30-minute exercise period, the changes ($p < 0.01$) in FVR and MAP from warm up did not differ between self-paced and fixed-intensity exercise at 35 °C (all $p > 0.15$) and 29 °C (all $p > 0.08$). Furthermore, the rise in $\dot{Q}$ from warm up was only observed during fixed-intensity exercise at 35 °C ($p < 0.01$) and in self-paced exercise at 29 °C ($p < 0.01$). SV, from the first six-minute of the 30-minute exercise did not differ from the warm up period at 35 °C ($p = 0.63$ and 29 °C ($p = 0.69$). Both $\dot{Q}$ and SV were similar between self-paced and fixed-intensity exercise at 35 °C ($\dot{Q}$: self-paced vs fixed-intensity: $p = 0.78$; SV: self-paced vs fixed-intensity: $p = 0.84$) and 29 °C (Q: self-paced vs fixed-intensity: $p = 0.85$; SV: self-paced vs fixed intensity: $p = 0.78$). SKBF was different between self-paced and fixed-intensity exercise at 35°C and 29°C (Fig. 28). In particular, SKBF was 52.0 AU higher ($p = 0.04$) during self-paced than fixed-intensity exercise at 35 °C but was 76.2 AU lower ($p = 0.03$) than during fixed-intensity exercise at 29 °C. However, no statistical interactions were observed between self-paced and fixed-intensity exercise and time points at 35 °C ($p = 0.69$) and 29 °C ($p = 0.95$). Furthermore, the onset (35 °C SP vs 35 °C Fix: $p = 0.99$; 29 °C SP vs 29 °C fix: $p = 0.47$) and slope (35 °C SP vs 35 °C fix: $p = 0.97$; 29 °C SP vs 29 °C fix: $p = 0.99$) were not significantly different between self-paced and fixed-intensity exercise at both 35 °C and 29 °C (Figure 24).

Resting LSR was similar between self-paced and fixed-intensity at 35°C ($p = 0.38$) and 29°C (all $p = 0.89$) (Fig. 29). This remained throughout the warm up phase as the rises in LSR ($p < 0.01$) from rest to the end of warm up exercise was not different between self-paced and fixed-intensity exercise at 35°C ($p = 0.80$) and 29°C (all $p = 0.55$). In line with this aforementioned observation, the rise in LSR ($p < 0.01$) from warm up to the end of the 30-minute exercise period was not different between self-paced and fixed-intensity exercise at 35°C ($p = 0.28$) and 29°C ($p = 0.22$). Furthermore, no interactions were observed between self-paced and fixed-intensity at 35°C ($p = 0.59$) and 29 °C ($p = 0.97$) for LSR. In line with

this aforementioned observation, the onset (35°C SP vs 35°C fix: p = 0.56; 29°C SP vs 29°C fix: p = 0.) and slope (35°C SP vs 35 °C fix: p = 0.55; 29 °C SP vs 29°C fix: p = 0.48) for sweating were not significantly different between self-paced and fixed-intensity at 35°C and 29°C (Fig. 29). Finally, whole body sweat rate (WBSR) was not different between self-paced and fixed-intensity exercise, at 35°C (p = 0.36) and 29 °C (p = 0.68).

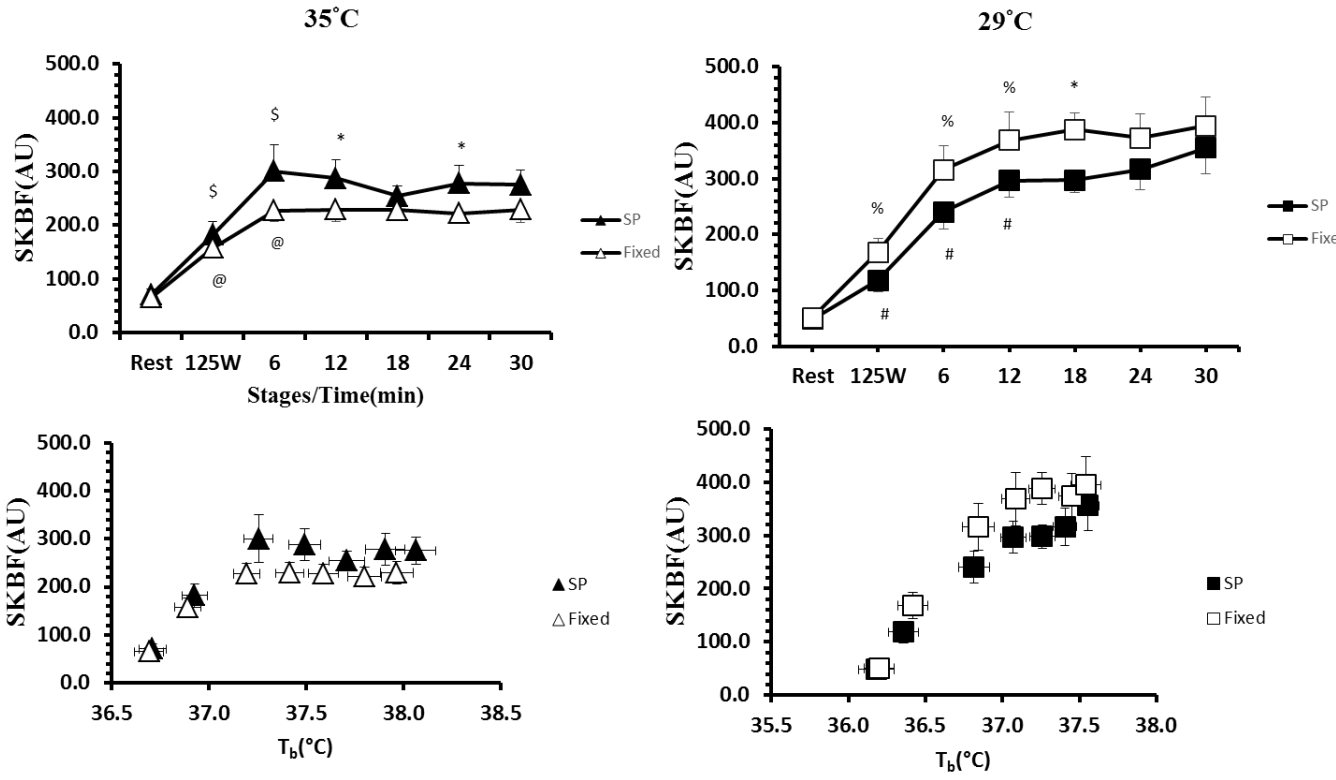

Figure 25: Mean (SEE) skin blood flow (SKBF, n = 13) against time and mean body temperature (T_b) during self-paced (SP) and fixed-intensity exercises in 35°C and 29°C environments.

$ Significant difference with preceding time points during self-paced exercise at 35 °C.

@Significant difference with the preceding time points during fixed-intensity exercise at 35 °C.

Significant difference with preceding time points during self-paced exercise at 29 °C.

% Significant difference with preceding time points during fixed-intensity exercise at 29 °C.

Table 6: Mean arterial pressure (MAP, n = 12), cardiac output (n = 9), forearm vascular resistance (CVR, n = 12) and stroke volume (SV, n = 9) at rest, during warm up and the first 6 minutes off the 30-minute time trial in both 35°C/29°C environments.

	35°C						29°C					
	Self-paced			Fix			Self-paced			Fix		
	Rest	125W	6min	Rest	125W	6min	Rest	125W	6min	Rest	125W	6min
MAP(mmhg)	86.7(1.8)	96.7(1.7)$	103.2(1.7)$	87.1(0.9)	95.5(2.1)@	102.6(1.7)@	88.9(1.9)	96.1(1.3)#	104.1(1.6)#	86.3(2.3)	94.1(1.5)%	99.8(2.0)%
$\dot{Q}$ (liters min)	11(1.2)	25(1.5)$	29(1.9)	10(0.9)	22(1.5)@	30(1.4)@	9(0.8)	23(1.5)#	30(1.2)#	8(1)	23(1.6)%	29(2.9)
CVR(AU mmHg^{-1})	1.6(0.2)	0.7(0.1)$	0.4(0.1)$	1.6(0.2)	0.8(0.2)@	0.5(0.1)@	2.2(0.2)	1.1(0.1)#	0.7(0.2)#	2.1(0.3)	0.8(0.1)%	0.4(0.1)%
SV(mL)	146.1(13.6)	205.4(15.2)$	191.7(12.7)	130.7(11.7)	185.3(14.1)@	196.9(10.5)	129.6(16.9)	196.9(13.6)#	206.4(8.3)#	118.9(11.8)	191.5(13.2)%	199.8(18.3)

$ Significant difference with preceding time points during self-paced exercise at 35 °C.
@Significant difference with the preceding time points during fixed-intensity exercise at 35 °C.
Significant difference with preceding time points during self-paced exercise at 29 °C.
% Significant difference with preceding time points fixed-intensity exercise at 29 °C

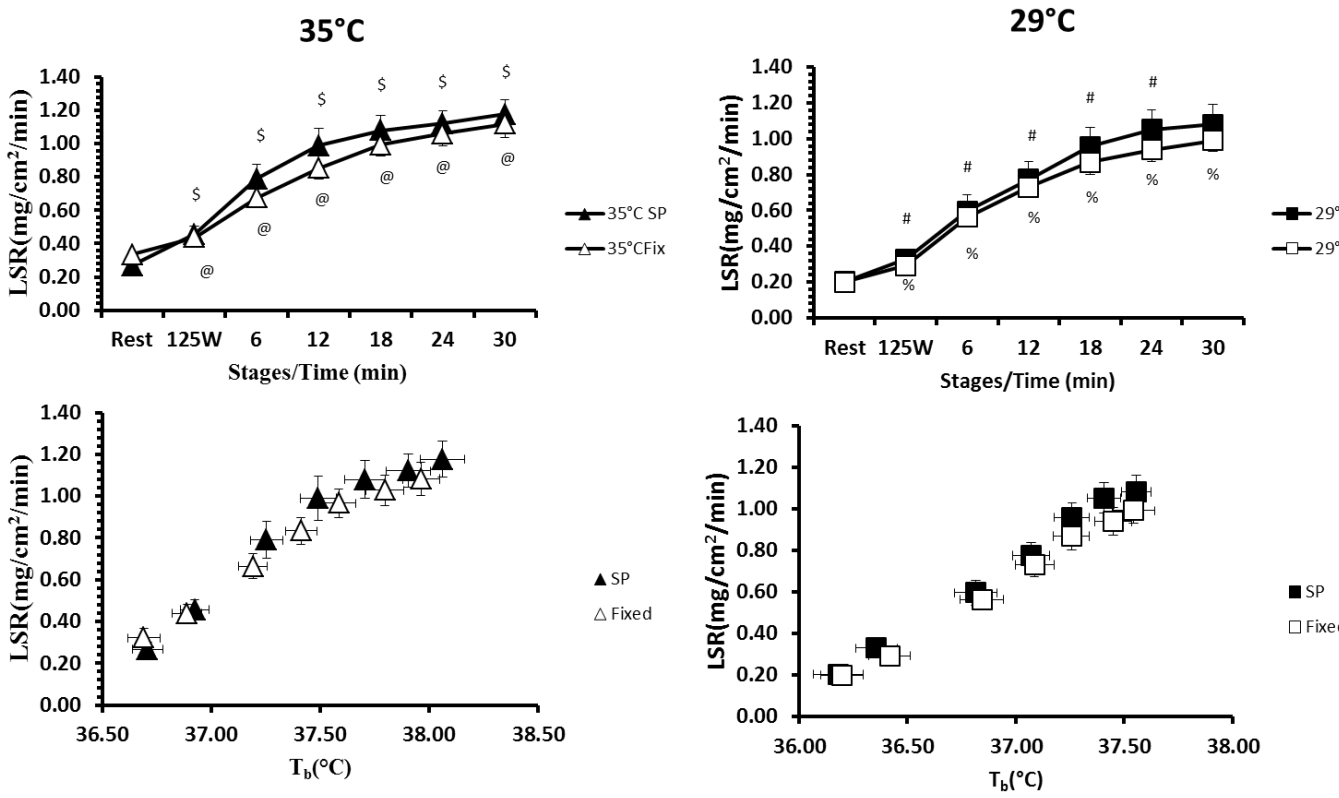

Figure 26: Mean (SEE) Local sweat rate (LSR) against time and mean body temperature (T_b) during self-paced (SP) and fixed-intensity exercises at 35°C and 29°C.

$ Significant difference with preceding time points during self-paced exercise at 35°C.
@Significant difference with the preceding time points during fixed-intensity exercise at 35°C.
Significant difference with preceding time points during self-paced exercise at 29°C.
% Significant difference with preceding time points during fixed-intensity exercise at 29°C.

8.3.1.3: Thermodynamics

Resting metabolic heat production (M) and evaporative heat lost (E) were similar between self-paced and fixed-intensity exercise at 35 ℃ (all $p > 0.10$) and 29 ℃ (all $p > 0.17$) (Fig. 30). This persisted throughout the warm up phase, as the rises in M, E and heat storage (S) (all $p < 0.01$) were not different between self-paced and fixed-intensity exercise at 35℃ (all $p > 0.20$) and 29℃ (all $p > 0.08$). In line with this observation, the rises in M, E and S from warm up ($p < 0.01$) to the end of the 30-minute exercise period were not different between self-paced and fixed-intensity exercise at 35℃ (all $p > 0.20$) and 29℃ (all $p > 0.24$). Furthermore, neither M, E, nor S had any interaction between self-paced and fixed-intensity exercise and time points at 35℃ (all $p > 0.35$) or 29℃ (all $p > 0.28$).

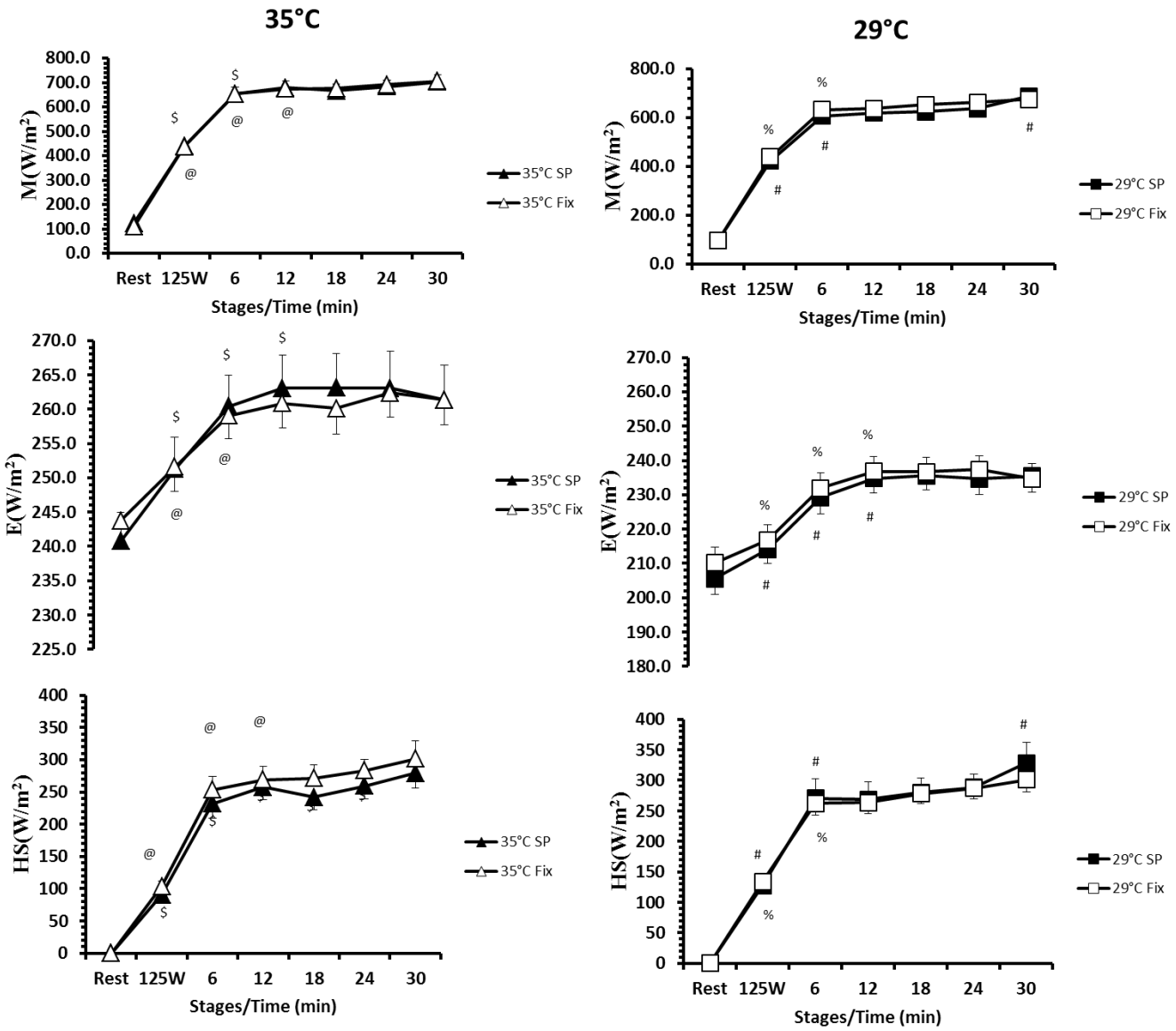

Figure 29: Mean (SEE) rate of metabolic heat production (M, n = 13), rate of evaporative heat loss (E, n =13) and rate of heat storage (S, n = 13) between self-paced (SP) and fixed-intensity at 35°C and 29°C.

$ Significant difference with preceding time points during self-paced exercise at 35 °C.

@Significant difference with the preceding time points during fixed-intensity exercise at 35 °C.

Significant difference with preceding time points during self-paced exercise at 29 °C.

% Significant difference with preceding time points during fixed-intensity exercise at 29 °C.

8.4: Discussion

To date, it is still incompletely known whether self-pacing is a proven means to reducing thermoregulatory strain in the heat (compared to fixed-intensity exercise), as previous studies' average workloads (hence metabolic heat production) have not been matched, exercise duration has not been matched nor have different heat profiles (environments) been investigated. Therefore, the current study sought to determine this. The novel findings of this study are: (1) self-pacing does not modulate temperature regulation, at 35°C vs 29°C, when the rate of metabolic heat production is approximate between different exercise modalities; (2) the control of sweating is not different between self-paced and fixed-intensity in these environments; (3) the vasomotor response displays differential results according to modality and environment, however, the control (onset and slope) is unaffected.

Self-pacing does not modulate temperature regulation in warmer and more mild heat environments, compared to fixed-intensity exercise

Unlike previous research findings from Schlader *et al.* (2011a), which suggested that self-pacing was a proven means to reducing thermoregulatory strain in an uncompensable ($E_{req} > E_{max}$) heat stress environment, the findings from this study in *compensable* heat indicate that when the average rate of metabolic heat production is approximate between self-paced and fixed-intensity exercise, no change in body temperature is observed. Whilst intuitive, this has not previously been demonstrated and further highlights that self-pacing is only valid for regulating body temperature when there is a subsequent reduction of metabolic heat production. This is in accordance with Schlader *et al.* (2011a) and a review article by Flouris and Schlader (2015), who suggest that it is the rate of metabolic heat production that directly contributes towards the rise in core temperature during exercise. Therefore, either small or insufficiently sustained reductions in exercise intensity when self-pacing may not serve a thermoregulatory benefit.

The sudomotor response does not differ between self-paced and fixed-intensity exercise in warmer and more mild heat environments.

In line with the observations for body temperature, WBSR and LSR did not differ between self-paced and fixed-intensity at 35°C vs 29°C. This is aligned with previous studies, which have indicated that core temperature is the key regulator for initiating a sweating response at rest and during exercise (Saltin *et al.*, 1972, Wyss *et al.*, 1974). Furthermore, this was

supported by our calorimetric data, as no changes in heat storage (Fig. 30) and metabolic heat production were observed during the 30-minute exercise trial.

The vasomotor response was different between self-paced and fixed-intensity exercise, depending on environmental conditions, but did not compromise cardiovascular function in either 35 °C or 29 °C.

Overall, this study suggests that self-pacing does not render better cardiovascular stability during exercise at either 35°C or 29°C; despite local cutaneous blood flow responding differently between self-paced and fixed-intensity exercise, according to the environmental conditions. Our cutaneous blood flow data revealed that greater cutaneous vasodilation occurred in the forearm region during self-paced exercise at 35°C compared to fixed-intensity exercise (Fig. 28). However, it was the opposite at 29°C, as there was greater cutaneous vasodilation in the forearm region during fixed-intensity exercise. It is unclear why this occurred as all other thermoregulatory and cardiovascular variables were unchanged, as was the control of the cutaneous blood flow (onset and slope). Therefore, either the greater power (increased sample size and reduced measurement variance) resulted in a type 1 error, or this effect is too small to have physiological consequence.

Considerations and limitations of the study

Certain limitations from this study must be highlighted. First, the combination of exercise and environmental heat load in the current study was compensable. As it has been demonstrated that self-pacing is a proven means to reducing thermoregulatory strain in uncompensable heat stress environments (Schlader *et al.*, 2011a), whether these results could be inferred from such an experimental conditions remains unknown. Another limitation of this study was the relatively short exercise duration, in combination with a slower-changing index of core temperature. This exercise duration has previously demonstrated differences between exercise modalities (Schlader *et al.*, 2011a), but it is acknowledged that were the exercise duration more prolonged, it would be expected that self-pacing could potentially attenuate physiological strain compared to fixed-intensity exercise, even where the rate of metabolic heat production is approximate. This was partially supported by the subjects who did not complete the fixed-intensity trial at either 35°C (n = 1, time to exhaustion: 25 minutes) or 29°C (n = 1, time to exhaustion: 11 minutes). Both subjects demonstrated a greater rise in rectal temperature compared with the self-paced trial.

In conclusion, this study concludes that when the rate of metabolic heat production is approximate between self-paced and fixed-intensity exercise bouts, self-pacing does not attenuate thermoregulatory strain compared with fixed-intensity exercise, as no differences were found in terms of autonomic or cardiovascular responses in compensable heat stress environments (35°C and 29°C).

Chapter Nine

9.0: General Discussion

This thesis had two broad aims: to clarify the role of the endogenous and exogenous female reproductive hormones on exercise thermoregulation in both dry and humid heat, and the effect of ambient temperature *per se* on exercise thermoregulation when vapour pressures are matched in men. An overarching goal was to highlight the efficacy of behavioural thermoregulation in comparison to autonomic control. The results obtained would advance the existing knowledge of human (exercise) temperature regulation, thereby reducing the incidence of heat-related illness, and providing guidance on optimal performance.

9.1: General aim I

The findings from **Chapters Five** and **Six** indicated that trained women use behavioural thermoregulation to reduce/limit physiological strain to counter the (relatively small) effects of fluctuations in endogenous and exogenous female reproductive hormones, as well as in a more stressful environment i.e. humid heat. Collectively, the results from **Chapters Five** and **Six** provided clear evidence that physiological strain was higher in humid than dry heat, and so even trained women had to reduce their power output to prevent the greater rise of heat storage in a humid heat environment. Further, the findings from **Chapter Six** clearly showed that chronic consumption of exogenous female reproductive hormones (OCP) induces greater autonomic strain compared with naturally-fluctuating endogenous female reproductive hormones (eumenorrheic), in both dry and humid heat environments, at rest and during exercise. However, and interestingly, although autonomic strain was higher in women using the OCP compared with eumenorrheic women, rectal temperature was similar during exercise in both dry and humid heat environments. Previously, behavioural thermoregulation was only believed to nullify physiological strain between environments (hot vs cool) (Schlader *et al.*, 2011a, Schlader *et al.*, 2011d), but previous studies did not investigate whether it can reduce the physiological strain from the fluctuations of both endogenous and exogenous female reproductive hormones. The findings from **Chapters Five** and **Six** clearly indicated that behavioural responses during exercise are not only a means to modulate physiological strain between environments, but are also a means to modulate physiological strain from the

fluctuation of female reproductive hormones.

However, although **Chapters Five** and **Six** provide unique and worthwhile data, questions still remain as to which environmental factors (temperature or humidity) contribute to the initiation of behavioural responses during exercise in the heat and the efficacy between self-pacing and fixed-intensity exercise. Findings from previous studies (Che Muhamed *et al.*, 2016b, Maughan *et al.*, 2012, Moyen *et al.*, 2014) and **Chapters Five** and **Six** clearly indicate that vapour pressure is one of the driving factors of behaviour thermoregulation during exercise in the heat. However, ambient temperature alone may also contribute to initiating a behaviour response during exercise in the heat. Furthermore, although previous studies demonstrated that self-pacing is a means to modulate physiological strain in the heat compared with fixed-intensity exercise, the rate of metabolic heat production between self-paced and fixed-intensity was not equal and therefore the efficacy of self-pacing remains unknown.

9.2: General aim II

Two major conclusions were derived from the second aim. First, the findings from **Chapter Seven** clearly indicated that ambient temperature alone had a minimal influence on behavioural and autonomic responses during self-paced exercise across different thermal profiles. This reaffirms vapour pressure as the main contributor to initiating behavioural responses during exercise in the heat. Furthermore, the findings from **Chapters Five, Six** and **Seven** support the notion that the validity of wet-bulb globe temperature (WBGT) in the prediction of physiological strain is questionable, as it neglects the role of vapour pressure on physiological strain. These findings, therefore, provide invaluable information for sporting organisations (e.g. ACSM, NATA etc.), to allow them to develop a better consensus for exercise in the heat, so that the incidence of heat-related illness can be reduced for both sexes. The last conclusion from this area is that self-pacing alone cannot be sufficient to modulate body temperature, unless there is a reduction in the rate of metabolic heat production. This was clearly outlined in **Chapter Eight,** as its findings indicated that when the rate of metabolic heat production was equivalent between self-paced and fixed-intensity exercise across different thermal profiles, no autonomic differences were found between different exercise modalities. Together, this highlights the importance of metabolic heat production on dictating the thermoregulatory demands during exercise in the heat.

9.3: Hypotheses

Following the General Aims of this thesis, specific hypotheses were formed and tested in the experimental chapters. Whether these are accepted or rejected is stated below:

1.) The menstrual cycle will not affect female athletes autonomic and behavioural responses in both dry and humid heat (**Chapter Five**). *Alternative hypothesis accepted.*
2.) Thermoregulatory strain will be nullified by thermoregulatory behaviour in eumenorrheic women and in women taking hormonal contraception **(Chapters Five** and **Six).** *Alternative hypothesis accepted.*
3.) Self-selected pace will be lower in humid heat, but it will not be different between eumenorrheic women and women taking combined hormonal contraception **(Chapters Five** and **Six)**. *Alternative hypothesis accepted.*
4.) Autonomic strain will be greater in women taking combined hormonal contraception than eumenorrheic women **(Chapters Five** and **Six)**. *Alternative hypothesis accepted.*
5.) The thermoregulatory and cardiovascular response is not different between environments matched for absolute humidity, despite differing perceptual responses **(Chapter Seven).** *Alternative hypothesis accepted.*
6.) Thermoregulatory strain will not differ between fixed and self-selected pace, when metabolic heat production is equivalent **(Chapters** and **Eight)**. *Alternative hypothesis accepted.*

9.4: Limitations and Future Direction

All experimental work has assumptions and limitations that need to be acknowledged; this thesis is no different. Firstly, all participants used were highly trained (relative to the general population), therefore it is unlikely that their responses could be generalised to less trained populations. This is especially the case as: 1) a higher aerobic capacity (and training status) confers effects analogous to partial heat acclimation (Cheung and McLellan, 1998) , thereby skewing the thermoregulatory alters response; 2) trained individuals display altered perceptual responses to those less trained (Selkirk and McLellan, 2001, Tikuisis *et al.*, 2002); and 3) trained females display a reduced absolute and fluctuations in reproductive hormone concentrations (Kuwahara *et al.*, 2005a, Kuwahara *et al.*, 2005b) that would affect several physiological systems, including thermoregulation.

Secondly, the current body of work has only investigated the acute responses in men and

women that are not heat acclimated. Therefore, the extent that the factors contained in this thesis should affect the adaptive response remains unknown and is a worthy line of enquiry since exercise heat acclimation is the single most effective counter-measure to environmental heat stress/strain (Buono *et al.*, 1998). Yet there is (still) a paucity of data on acclimation to humid heat stress despite a majority of the global population living and training in such an environment (Hue, 2011), and whether a female's greater sweating (and convective heat loss) efficiency relative to a man's (Kenney, 1985) infers any (dis)advantage remains unexplored. In support of the adaptive response in women, future studies should also investigate whether heat shock protein (HSP) expression is different between different phases of the menstrual cycle before and immediately after heat acclimation as the HSP 90 and 72 superfamily independently contribute to cutaneous vasodilation (Fujii *et al.*, 2017) and the reduction in inflammatory response during the recovery period (Sawka *et al.*, 2011).

It was beyond the scope of the current thesis to directly compare the male and female cohorts, although this would be worthwhile given the current knowledge on sex-differences for exercise thermoregulation are at its infancy, especially the behavioural responses (Gagnon and Kenny, 2012). However, it would be difficult to achieve, as this would require the matching of women with men of the same physical and functional characteristics, a likely difficult task.

A final consideration must be given to the actual protocol and conditions. The relatively short duration of exercise used for these participants may likely yield different responses to more prolonged endurance exercise, NS could be different if intensity were different (i.e. simulated occupational modes such as treadmill walking for several hours), or if the conditions of heat stress were different i.e. more extreme.

In addition to the possible work introduced above (i.e. responses following exercise heat acclimation, directly comparing men and women, longer exercise duration and different ambient conditions) several avenues of research present themselves following the body of work contained in this thesis. For example, although not a focus of this thesis, results from **Chapters Five** and **Six** indicate that the menstrual and OCP cycles affect the ventilatory response to exercise, such that a hypocapnia might occur secondary to hyperventilation. Therefore, of particular interest would be the cerebrovascular response to such exercise, and whether this confers any constraints on orthostatic tolerance. Similarly, evidence from *in vitro* and animal studies (Voss *et al.*, 2003) indicates that the heat shock protein response to exercise heat stress is altered by oestrogen, such that the adaptive response and subsequent

cellular protection may be significantly altered depending on the hormonal milieu in females. As alluded to in **Chapters Five** and **Six**, the interaction of multiple related physiological systems (i.e. reproductive, thermoregulatory and osmo- and/or baro-regulation) remains studied in isolation yet too often do they overlap in the real world. Whereas, by examining the blood flow to the active musculature in concert with the systemic cerebral circulations would provide a more complete picture and reference for such integrated and stressful exercise.

9.4.1: Special consideration of this thesis

Originally, this thesis aimed to address the acute and the adaptive response (i.e. exercise heat acclimation) to heat stress for male and female athletes in both dry and humid heat environments. However, data collection for **Chapters Five** and **Six** took approximately 2 years, and with a maximum of 4 years allowed for thesis submission a change in focus for the remaining chapters was deemed necessary so that the candidate could complete the degree within the time frame. However, the change of research direction still provide a valuable input to the current literature. Moreover, despite the major contribution concerning the effects of female reproductive hormones on behavioural thermoregulation in both DRY and HUM **(Section 9.1 and 9.2)**, the candidate also noticed that the current experimental protocols were predominately derived from males and whether it could be applied to a female population remains questionable. This, therefore warranted further investigation. For example, originally for **Chapter Five and Six**, the candidate believed that fixed-intensity exercise in both DRY and HUM should be based on percentage of $\dot{V}O_{2max}$ rather than the absolute workload (125W and 150W). However, the candidate observed that even in highly trained females, this approach resulted in a higher $\dot{V}O_2$ response per individual and subsequently resulted in a much larger change of thermoregulatory behaviour during the 30 minute time trial. Using an absolute workload approach was more appropriate as this was not associated with a large change of thermoregulatory behaviour during the 30 minute time-trial in both DRY and HUM, and resulted in a similar $\dot{V}O_2$ per stage (125W: 60% and 150W: 70%) . This, therefore indicates that the experimental protocols should be sex-specific instead of a "one size fits all" approach. Furthermore, this approach should be further extended to dietary control as reproductive hormones may alter substrate utilisation during endurance exercise (Tarnopolsky, 2008).

9.5: Conclusions

This thesis investigated human temperature regulation during exercise in the heat, and how this was affected by the menstrual (and OCP) cycle and the ambient thermal profile. This was achieved by completing four experimental chapters, from which the following conclusions can be drawn:

1. Endogenous and exogenous female reproductive hormones do not influence exercise performance in both dry and humid heat in trained females.
2. Exercise performance is lower in humid heat compared to dry heat in both eumenorrheic athletes and athletes using the OCP.
3. Autonomic thermoregulatory strain is higher in humid heat compared to dry heat for both cohorts of women.
4. Chronic consumption of the OCP results in greater autonomic thermoregulatory strain compared with matched eumenorrheic females in both dry and humid heat conditions.
5. Ambient temperature alone has a minimal influence on behavioural and autonomic responses in the heat.
6. Self-pacing does not modulate body temperature in different thermal profiles when the rate of metabolic heat production is equivalent between different exercise modalities.

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APPENDIX 1: Ethical approval for Chapter Five and Six

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA

28 April 2015

Joseph Tze-Huan Lei
School of Sport & Exercise
PN621

Dear Joseph

Re: HEC: Southern A Application – 14/99
Acute physiological responses in dry and humid: Effects of biological sex and menstrual cycle

Thank you for your letter dated 25 April 2015.

On behalf of the Massey University Human Ethics Committee: Southern A I am pleased to advise you that the ethics of your application are now approved. Approval is for three years. If this project has not been completed within three years from the date of this letter, reapproval must be requested.

If the nature, content, location, procedures or personnel of your approved application change, please advise the Secretary of the Committee.

Yours sincerely

Mr Jeremy Hubbard, Chair
Massey University Human Ethics Committee: Southern A

APPENDIX 2: Ethical Approval for Chapter Six and Seven

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Date: 16 February 2017

Dear Joseph Lei

Re: Ethics Notification - **SOA 16/74 - The physiological response of self-paced versus fix-intensity exercise acute dry and humid heat tolerance among male athletes**

Thank you for the above application that was considered by the Massey University Human Ethics Committee: **Human Ethics Southern A Committee** at their meeting held on **Thursday, 16 February, 2017**.
On behalf of the Committee I am pleased to advise you that the ethics of your application are approved.

Approval is for three years. If this project has not been completed within three years from the date of this letter, reapproval must be requested.

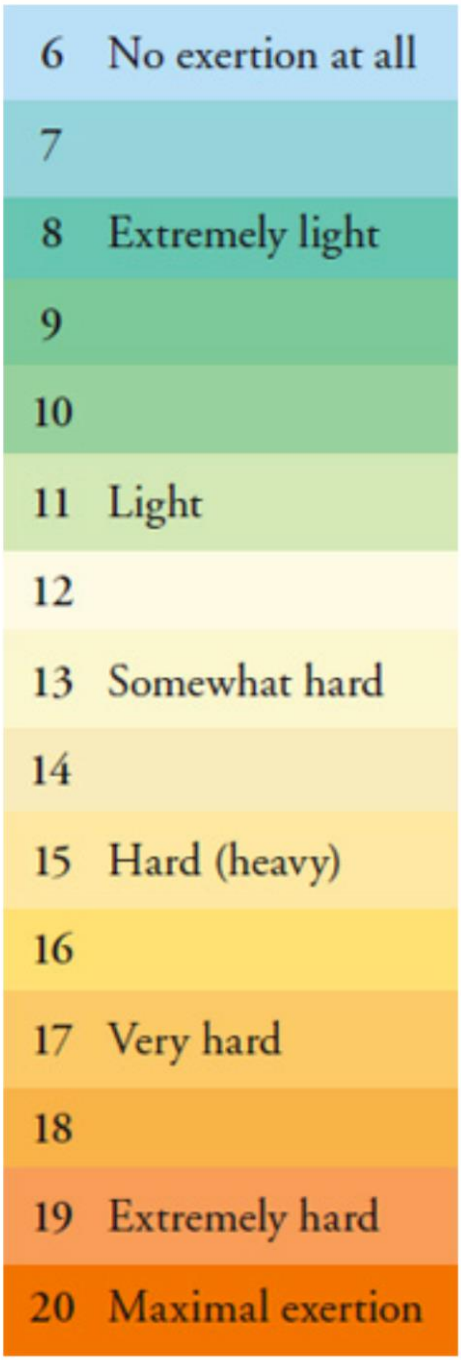
If the nature, content, location, procedures or personnel of your approved application change, please advise the Secretary of the Committee.

Yours sincerely

Dr Brian Finch
Chair, Human Ethics Chairs' Committee and Director (Research Ethics)

APPENDIX 3: RPE Scale

How hard does the exercise fee



APPENDIX 4: Thermal Discomfort Scale

How comfortable does your body temperature feel?

1.0 ..... Comfortable
1.5
2.0 ..... Slightly uncomfortable
2.5
3.0 ..... Uncomfortable
3.5
4.0 ..... Very uncomfortable

APPENDIX 5: Thermal Sensation Scale

How does the temperature of your body feel?

1.0	Cold
1.5	
2.0	Cool
2.5	
3.0	Slightly cool
3.5	
4.0	Neutral (neither cool nor warm)
4.5	
5.0	Slightly warm
5.5	
6.0	Warm
6.5	
7.0	Hot

APPENDIX 6: Pleasant scale

How pleasant does the temperature of your body feel?

-3.0 ..... Very Unpleasant
-2.5
-2.0 ..... Unpleasant
-1.5
-1.0 ..... Slightly Unpleasant
-0.5
0.0 ..... Neutral (neither pleasant nor unpleasant)
+0.5
+1.0 ..... Slightly Pleasant
+1.5
+2.0 ..... Pleasant
+2.5
+3.0 ..... Very Pleasant

APPENDIX 7: Skin Wetness Scale

How wet does your skin feel?

-3.0 …….. Very Dry
-2.5
-2.0 …….. Dry
-1.5
-1.0 …….. Slightly Dry
-0.5
0.0 …….. Neutral (neither wet nor dry)
+0.5
+1.0 …….. Slightly Wet
+1.5
+2.0 …….. Wet
+2.5
+3.0 …….. Very Wet

APPENDIX 8: Publication for Chapter Five

DRC 16

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Tze-Huan Lei

Name/Title of Principal Supervisor: Dr Toby Mündel

Name of Published Research Output and full reference:

Influence of menstrual phase and arid vs. humid heat stress on autonomic and behavioural thermoregulation during exercise in trained but unacclimated women

In which Chapter is the Published Work: Chapter Five

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate:

  and / or

- Describe the contribution that the candidate has made to the Published Work:

  The candidate was responsible for all the data collection, analyses and drafting of the manuscript.

Tze-Huan Lei — Digitally signed by Tze-Huan Lei, DN: cn=Tze-Huan Lei, o=Massey University, ou=School of Sport and Exercise, email=t.h.lei@massey.ac.nz, c=NZ, Date: 2018.05.07 10:24:33 +12'00'

Candidate's Signature

07/05/2018

Date

Toby Mundel — Digitally signed by Toby Mundel, Date: 2018.05.09 11:47:19 +12'00'

Principal Supervisor's signature

09/05/2018

Date

GRS Version 3– 16 September 2011

APPENDIX 9: Publication for Chapter Five

DRC 16

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Tze-Huan Lei

Name/Title of Principal Supervisor: Dr Toby Mündel

Name of Published Research Output and full reference:

Humid heat stress affects trained female more than does their menstrual phase

In which Chapter is the Published Work: Chapter Five

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate:

  and / or
- Describe the contribution that the candidate has made to the Published Work:

  The candidate was responsible for drafting the manuscript.

Tze-Huan Lei — Digitally signed by Tze-Huan Lei DN: cn=Tze-Huan Lei, o=Massey University, ou=School of Sport and Exercise, email=t.h.lei@massey.ac.nz, c=NZ Date: 2018.05.07 10:31:13 +12'00'

Candidate's Signature

07/05/2018

Date

Toby Mundel — Digitally signed by Toby Mundel Date: 2018.05.09 11:47:43 +12'00'

Principal Supervisor's signature

09/05/2018

Date

GRS Version 3– 16 September 2011