On the Edge of Thermoregulation:

a Matter of Physiology and Physics

by

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Abstract

This thesis describes experiments designed to investigate the point of uncompensable heat stress in healthy exercising males by manipulating variables in the heat balance equation.

The addition of minimal clothing did not have a clear influence on the mean skin temperature ($\overline{T}_{sk}$) and thermoeffector responses (local sweat rate and skin blood flow) during incremental exercise at 40 °C (it did at 30 °C). A comparison between the two different ambient temperatures however, showed a significant effect on $\overline{T}_{sk}$, which was on average 1.55 (0.29) °C higher in 40 °C compared to 30 °C. Subsequently, a protocol was developed where ambient temperature was incrementally increased. This was shown to be a reliable and valid method to evoke uncompensable heat stress, and allowed the comparison of variables (humidity, work rate) and groups (fitness). Further experiments in the thesis show that uncompensable heat stress occurs at a similar deep body temperature (T_c) for males with a range of aerobic fitness in high and low humidity environments. It was also shown that high aerobic fitness (compared to low aerobic fitness): 1) does not offer any benefit in terms of delaying the transition to uncompensable heat stress when exercising at a matched absolute work rate (60 Watts) in a low and high humidity environment, or at a matched relative work (40 % of VO_{2max}) in a low humidity environment, 2) may cause uncompensable heat stress to occur at a lower ambient temperature when working at matched relative work rate in a humid environment.

Importantly, in these experiments, local sweat rate continued to rise beyond the point of uncompensable heat stress – or upper limit of the "thermoregulatory zone", regardless of the work rate, humidity or aerobic fitness status of the individual, indicating a thermoeffector reserve remained. It is therefore concluded, that the transition into uncompensable heat stress is not synonymous with maximal thermoeffector output and the sweating and skin blood flow responses of an individual are determined by the thermal profile. An elevated and increasing T_c may be necessary to facilitate increased levels of thermoeffector output. This is an important and novel contribution to the understanding of the thermoregulatory response to heat stress.

Declaration

Whilst registered as a candidate for the degree of doctor of philosophy, I have not been registered for any other research award. The results and conclusions embodied in this thesis are the work of the named candidate and have not been submitted for any other academic award.

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To Jo, Jim and Mike:

Kipling had his honest men,

Who taught him all he knew,

While those chaps have been good to me,

I couldn't have done it without you.

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Dissemination

Presentations arising from the work undertaken in this thesis:

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Glossary and Abbreviations

A

ANOVA	Analysis of variance

B

$b \cdot min^{-1}$	Beats per minute
bf %	Body fat percentage
Biological zero	Short term occlusion of the brachial artery by inflating a manual sphygmomanometer cuff to 240 mmHg to measure a baseline in SkBF
BSA	Body surface area ([m^2] calculated by the DuBois formula - $A_D = 0.202 * W^{0.425} * H^{0.725}$)
BSREC	BioSciences Research Ethics Committee

C

C	Convective thermal exchanges
°C	Degrees centigrade
Category ratio scale	A scale by which the absolute intensity of a sensation can be measured by the selection of pre-determined descriptors by an individual
CNS	Central nervous system
CV_{Drift}	Cardiovascular drift
CV % / CV	Coefficient of variation

D

D_{max}	Method used to locate an inflection point in data by fitting a quadratic and linear equations to the data and selecting the maximal perpendicular distance between the two line lines.

E

E	Evaporative thermal exchanges

E_{max}	Maximal rate of evaporative cooling for heat balance
E_{req}	Required rate of evaporative cooling for heat balance

F

F_IO_2	Fraction of inspired oxygen
FU	Flux (arbitrary) units.

H

h	Hour
HF	Heat flux ($W \cdot m^{-2}$)
HR	Heart rate (measured in $b \cdot min^{-1}$)
Hypobaric hypoxia	Where: F_IO_2 = 20.9 % and P_B < 760 mmHg

I

ICC	Intra-class correlation
Inflection in T_{re}	A distinct breakpoint in T_{re} as determined by either visual, D_{max}, or set value assessment

K

K	Conductive thermal exchanges
°K	Degrees Kelvin (where 0 °K is absolute zero)
kg	Kilogram
kPa	Kilopascal

L

LoA	Limits of Agreement

M

m	Meter
M	Metabolic heat production ($W \cdot m^{-2}$)
min	Minute
mL	Millilitre
mmHg	Millimetres of mercury

N

Normobaric hypoxia	Where: $F_IO_2 < 20$ % and $P_B \sim 760$ mmHg

O

O_2	Oxygen

P

P_a	Partial pressure of the water vapour in the ambient air
P_B	Barometric pressure
POA	Preoptic area of the hypothalamus
PSI	Physiological Strain Index (Moran, Avraham, Pandolf, & Shitzer, 1998)
P_s	Partial water vapour pressure at the skin surface
P_{sa}	Saturated vapour pressure at a given temperature
$P_{sk,s}$	Saturated water vapour pressure at the skin

R

R	Radiative thermal exchanges
RCI	Reciprocal cross inhibition
RER	Respiratory exchange ratio

RH	Relative humidity (%)
RPE	Rating of perceived exertion (Borg, 1982)
rpm^{-1}	Revolutions per minute

S

s	Second
S	Stored thermal energy
SD	Standard deviation
SFEC	Science Faculty Ethics Committee
SG	Specific gravity (dimensionless)
SkBF	Skin blood flow (initially measured in Voolts and then converted to FU or normalised as a percentage of the average highest 5 minute period unless stated otherwise stated)
$SkBF_{Forearm}$	Skin blood flow as measured at the dorsal aspect of the forearm
$SkBF_{Finger}$	Skin blood flow as measured at the finger pad of the index finger
SR	Sweat rate (measured in $\mu L \cdot min^{-1}$ and converted into $L \cdot m^{-2} \cdot hr^{-1}$)
SR_{Back}	Local sweat rate on the back
$SR_{Forearm}$	Local sweat rate on the forearm
SW	Skin wettedness

T

T_a	Ambient temperature
T_{ac}	Insulated aural / auditory canal temperature
$\overline{T}_b$	Mean body temperature (using rectal temperature – Chapter Five onwards)
$\overline{T}_{b\ ac}$	Mean body temperature (using insulated auditory canal temperature)
$\overline{T}_{b\ re}$	Mean body temperature (using insulated auditory canal temperature)
T_c	Deep body temperature

TC	Thermal comfort
TEM	Typical error of the mean
T_{oes}	Oesophageal temperature
T_{re}	Rectal temperature
TS	Thermal sensation
$\overline{T}_{sk}$	Mean skin temperature

V

v	Air velocity
VAS 20 cm scale	Visual analogue scale of 20 centimetres in length
$\dot{V}O_2$	Rate of oxygen uptake
$\dot{V}O_{2max}$	Maximal rate of oxygen uptake
$\dot{V}O_{2peak}$	Peak rate of oxygen uptake

W

w	External work
W	Watt
$W \cdot m^{-2}$	Watts per metre squared
WBGT	Wet bulb globe temperature (° C)

1. Chapter One – Introduction

Humans are tachymetabolic homeotherms (Romanovsky, 2007a), that is, they possess high basal metabolic rates (compared to poikilothermic organisms) and regulate deep body temperature within a relatively narrow range (typically 35 to 40 °C). Whilst extreme environmental conditions can pose a challenge to survival, behavioural and physiological thermoregulation enables thermal homeostasis to be maintained in the face of a wide range of external temperatures and levels of metabolic heat production, meaning that humans are able to live and thrive in a wide range of environmental conditions. An often quoted story that depicts man's amazing ability to tolerate extreme temperature is as follows: "*One morning toward the end of the eighteenth century, the Secretary of the Royal Society of London, one Mr. Blagden, ventured into a room heated to 105 °C, taking with him some eggs, a piece of raw steak and a dog. A quarter of an hour later, the eggs were baked hard and the steak cooked to a crisp but Blagden and his dog walked out unharmed*" (Ashcroft, 2000). While the methodology of this experiment may seem unorthodox to the modern reader, it clearly demonstrates how well humans tolerate dry heat.

In 1938, Nielsen's seminal study demonstrated that, during exercise, deep body temperature becomes elevated relative to resting conditions and that the equilibrium rectal temperature (T_{re}) is dependent on work rate, and independent of a wide range of ambient conditions (Nielsen, 1938). However, environmental conditions impairing the capacity to lose heat to the environment affect thermoregulation by influencing the effectiveness of heat loss pathways from the body (Mekjavić & Eiken, 2006). For example, when water vapour pressure rises, sweat evaporation is limited (Maughan, Otani, & Watson, 2012). Likewise, heat flow gradients between the core, the skin, and air, govern heat loss by conduction and convection; with an increase in air temperature, skin temperature increases and heat loss is reduced by a narrowing of the skin to environment temperature gradient (Périard, Caillaud, & Thompson, 2012). Indeed, heat is gained from the environment if air temperature is above skin temperature; if this occurs, evaporation is the only viable heat loss pathway (Havenith *et al*., 2008) as it is determined by a water vapour rather than thermal gradient. An inability to lose sufficient metabolic heat to the environment will lead to an inexorable rise in deep body temperature and will limit the potential duration and / or intensity of exercise as well as lead to heat illness if exercise does not cease, and in extreme cases, heat stroke and death (Coris, Ramirez, & Van Durme, 2004).

In 1963, Lind defined the upper environmental limits at which an equilibrated T_{re} could be achieved (Lind, 1963a). He demonstrated that over a select range of environmental conditions where an equilibrated T_{re} was possible, the equilibrated T_{re} was dependent on metabolic heat production and independent of ambient temperature. This was termed the 'prescriptive zone' and though equilibrated T_{re} could be achieved beyond the upper limit of the 'prescriptive zone' (Lind, 1963a), this occurred at an elevated absolute T_{re} value (see Figure 2.3). Building on Lind's work, Belding and Kamon (1973) designed a discontinuous protocol to define the environmental conditions beyond which an equilibrated T_{re} was *not* possible, and an inflection point in T_{re} was induced. Thereafter, a number of studies focused on defining the 'critical environmental limits' inducing an upward inflection in the equilibrium T_{re} in a variety of continuous protocols by incrementally increasing either temperature (Kenney, Mikita, & Havenith, 1993; Kenney & Zeman, 2002), or water vapour pressure (Belding & Kamon, 1973; Dougherty, Chow, & Kenney, 2010; Kamon, Avellini, & Krajewski, 1978; Kamon & Avellini, 1976; Kenney & Zeman, 2002; Kenney *et al.*, 1987, 1993) after an initial stable period. The inflection in T_{re} was taken to represent the transition from a "compensable" thermal environment where the required heat loss to achieve thermal balance is not achieved (Chen, Fan, & Zhang, 2003; Kraning & Gonzalez, 1991). Although the 'critical environmental limits' as classically defined is not the same as the 'prescriptive zone', it is worth noting that there has been some inconsistency in the application of terminology, which may have caused confusion in the literature. For example, Kenney, DeGroot, and Holowatz (2004), incorrectly associate moving out the prescriptive zone with uncompensable heat stress.

With increased thermal stress the human body will increase sweat rate (Sawka, Montain, & Latzka, 2001), skin blood flow (Charkoudian, 2003), heart rate (Givoni & Goldman, 1973) and oxygen uptake (Arngrímsson *et al.*, 2003) to try and achieve thermoregulatory balance. However, this 'physiological cost' (Corbett, Barwood, & Tipton, 2014; Lind, 1963b) of thermoregulation is seldom considered, and could be used to explain the influence of various factors including clothing, humidity, work rate and aerobic fitness on physiological and perceptual responses to exercise in the heat. Clearly an increase in physiological cost will have a negative impact on the exercising individual in the short or long term. However, the thermophysiological responses are often not considered when appraising thermally stressful environments, with the physical aspects of the environment (ambient temperature, relative humidity) given greater emphasis when defining the upper boundary of the thermoregulatory zone (TZ). This is exemplified by the use of E_{max} and E_{req} (Cheung & McLellan, 1998a; Givoni & Goldman, 1973) which describe the maximum evaporative potential of the environment and the required rate of evaporative cooling for heat balance, respectively. Indeed, it is assumed in some didactic models of thermoregulation that at the point of this upper boundary of the TZ sweating and skin blood flow have reached maximum values, see Figure 2.2 and 9.1 (Mekjavić, Tipton, & Eiken, 2003; Werner, Mekjavić, & Taylor, 2008). However, it is known that the thermoregulatory system responds dynamically and proportionally to thermal afferent information (Werner, 2010), therefore it may be the case that the rate of rise and absolute temperature does not permit sweating and skin blood flow to peak until deep body temperature has increased beyond the equilibrium value.

The physiological and thermal factors at the upper limit of thermal balance are poorly understood, there is no clear picture as to how these parameters change as an individual transitions from compensable to uncompensable heat stress. As Mekjavic and Eiken (2006) discuss, it is important to consider the influence of both non-thermal factors (*e.g.* aerobic fitness) and thermal factors (*e.g,* humidity and work rate) when considering the thermal strain placed on an individual. As it is unclear from the present literature how different thermal and non-thermal factors influence this point of uncompensability, this thesis offers an important contribution to the literature in this regard. Therefore the work presented in this thesis is important and novel as it will investigate the thermal responses and physiological cost associated with the restriction in evaporative cooling (clothing and high humidity), increased metabolic heat production and altered evaporative dissipation capacity (aerobic fitness and matched work rates). Put simply, parameters of the heat balance equation (see section 2.1.1) will be manipulated in an experimental setting to find the ‘edge’ of thermoregulatory control. The thermophysiological responses at the point of uncompensable heat stress will be examined and quantified. Quantifying the physiological cost of achieving thermal balance and understanding the thermophysiological response at the tipping point of thermal compensability could be an important factor for defining environments, as well as for preventing heat stress, heat related illness and improving exercise strategies for individuals who exercise and perform in the heat.

1.1. Summary of individual chapters

This chapter presents the context and purpose of the research described in this thesis.

Following an outline of the physical laws of heat exchange and human thermoregulatory system, Chapter Two discusses the different approaches used in defining the TZ and uncompensable heat stress. The thermal and non-thermal factors which influence temperature regulation are then discussed with a view to highlighting the key gaps in the understanding of how these factors influence the transition from a compensable, to an uncompensable, heat stress.

Chapter Three details the equipment and general procedures employed in all of the studies presented in this thesis, with the specific methods used in individual studies described in the appropriate chapter.

Chapter Four examines the extent to which clothing imposes an additional ‘physiological cost’ on males exercising at a range of work rates in two ambient temperatures (30 °C and 40 °C), and the way in which this impacts upon the upper boundary of the TZ. However, although a number of individuals were able to achieve uncompensability, in the main, participants terminated exercise due to volitional exhaustion as a consequence of the high work rates and prolonged work duration. Thus, an alternative protocol for eliciting thermal uncompensability was required.

The aim of the work described in Chapters Five and Six was to develop an alternative experiment protocol, designed to allow participants to become uncompensable (by inducing an inflection in T_{re}) before fatigue or excessive discomfort caused exercise termination, and to investigate the reliability (Chapter Five) and face validity (Chapter Six) of this approach.

In Chapter Seven, the influence of ambient humidity on the ambient temperature eliciting an inflection point in deep body temperature was explored. The thermal profile, thermophysiological responses and perceptual responses at the inflection point in deep body temperature were investigated. Although it is well established that a high ambient humidity will reduce the capacity for heat loss by evaporation, and could therefore potentially lower the critical ambient temperature eliciting an inflection in deep body temperature, the way in which humidity influences the thermal profile at these thermoregulatory ‘limits’, and the associated thermo-physiological responses is poorly understood.

In Chapter Eight the influence of aerobic fitness and work rate on the environmental conditions, thermal profile, thermophysiological responses and perceptual responses at the inflection point in deep body temperature during exercise in high and low humidity environments were examined. This study employed participants of Low or High aerobic fitness who were well matched for anthropometric measures, and made comparisons across matched *absolute* and *relative* work rates.

Finally, the last two Chapters of the thesis consist of a General Discussion (Chapter Nine), and highlight the Assumptions, Limitations, and Delimitations associated with the work presented in this thesis (Chapter Ten).

2. Chapter Two – Literature review

2.1. Physical principles of heat transfer

Humans utilize an array of behavioural and autonomic mechanisms to maintain deep body temperature within a narrow range (35 to 40 °C) in the face of a wide range of external temperatures and levels of metabolic heat production (Lind, 1963a; Nielsen, 1938). Regardless of the regulatory mechanism, the relationship of heat transfer between the body and the environment is governed by the laws of thermodynamics. The first law states that the potential kinetic energy of molecules and atoms cannot be created or destroyed, only transferred from one form to another. For instance, when humans perform external work by pedalling on a bicycle, chemical energy liberated by respiration within the cells of the body is transferred to kinetic energy and heat energy. The second law describes temperature (the average kinetic energy in the system) and that heat will always flow from a system of high temperature to a system of lower temperature. The magnitude and direction of heat flow between humans and their environment is dictated by the thermal gradients and the water vapour pressure surrounding the individual; it is these factors which govern sensible (convective, conductive, and radiant) and insensible (evaporative) heat transfer. The third law describes enthalpy, which is the total amount of energy possessed by an object. Thermal energy results from movement and collisions of particles at the sub-atomic and cellular level and is quantified by measurement of temperature and calorimetry (Taylor, Tipton, & Kenny, 2014).

2.1.1 Heat transfer pathways

The heat balance equation is classically used to describe the net rate at which humans generate and exchange heat with the surrounding environment:

$$M - (W) = R + C + K + E \qquad [W \cdot m^{-2}] \qquad \textit{Equation 2.1}$$

and so the heat storage equation is the following, for heat balance S must equal 0:

$$S = M - (\pm W) \pm R \pm C \pm K - E \qquad [W \cdot m^{-2}] \qquad \textit{Equation 2.2}$$

Where S = storage of body heat, M = metabolic energy transformation, W = mechanical work (this is positive when performing measureable external work and negative when work is performed on the subject), R = radiant heat exchange, C = convective heat transfer, K = conductive heat transfer and E = evaporative heat transfer (IUPS Thermal Commission, 2001; Nishi, 1981).

Radiation is the rate of transfer of heat energy between a system and the environment through electromagnetic waves (Kerslake, 1972) and so does not require a medium and is minimally effected by the temperature of the air the heat energy is transferred through. Above 0 °K (absolute zero), all matter emits and absorbs thermal radiation (IUPS Thermal Commission, 2001). When outdoors, the sun becomes an important consideration as a source of radiative heat for humans (Macpherson, 1962) as well as surrounding objects. Heat transfer by radiation is driven by the fourth power of the difference in absolute temperatures, and so is gradient driven.

Convection is the rate of conduction of heat from a system to or from a moving gas or fluid (in humans, usually air, water [during immersion] and blood) and can either be natural or forced. Natural convection occurs along a thermal gradient, where changes in density drive the movement of the gas or fluid. This density change is a result of heat exchange (Kerslake, 1972) and occurs constantly between the human body and the ambient air. For example, when the ambient air temperature is cooler than the skin, heat transfers from the skin to the adjacent air layer. This reduces the density of the air, causing it to rise, and to be replaced by air cooler than the skin, thus allowing the process to repeat. This convection current can, of course, work in the opposite direction. Forced convection occurs when the movement of the gas or fluid surrounding the system is driven by ‘forced movement’ rather than changes in density (Kerslake, 1972). Forced convection, through the circulatory system, is an important means of heat transfer from deep body tissues to the skin for dissipation to the ambient environment (IUPS Thermal Commission, 2001) and an often underappreciated function of the cardiovascular system (González-Alonso, 2012). Heat flow gradients between core, skin and air therefore govern heat transfer by conduction and convection. With an increase in air temperature, skin temperature increases and heat loss is reduced as these gradients narrow (Périard *et al.*, 2012). Indeed, heat is gained from the environment if air temperature and surrounding objects are above that of skin temperature, if this occurs, evaporation is the only viable heat loss pathway (Havenith, Richards, *et al.*, 2008).

Conduction, is the rate of transfer of heat through non-moving gas or fluid along a thermal gradient, or by direct contact between a system and a solid material (IUPS Thermal

Commission, 2001). Because air has a relatively high thermal resistance, unless the skin is in direct contact with an object, conduction in the context of human thermophysiology is often considered negligible and / or combined with radiation in heat balance calculations (Parsons, 2003). It is important to note however, that conduction plays an important role in transferring heat from deep tissues to the skin surface and from the skin to clothing (Parsons, 2014).

Evaporation is a change of state from liquid to vapour below the boiling point (latent heat of vaporisation), and in humans occurs from all moist surfaces including the respiratory system, which accounts for approximately 10 % of resting heat loss (Parsons, 2003). The heat required to change the sweat to vapour comes from the skin, which in turn is heated by cutaneous blood flow and the deep body tissues respectively. As it is the liquid molecules with the highest kinetic energy which escape into the vapour form, the result is a lowering of the average kinetic energy of the molecules in the liquid and so consequently a fall in temperature. The resulting heat dissipation from sweat is equivalent to 2.43 $kJ \cdot g^{-1}$ and the energy remains within the sweat molecules and is released upon condensation. However, the amount of heat lost by evaporation of sweat is dependent upon the absolute humidity gradient between the skin and the ambient environment, as well as the latent heat of vaporization (2427 $J \cdot g \cdot sweat^{-1}$ at 30 °C [Wenger, 1972]) and the amount of sweat available for evaporation (IUPS Thermal Commission, 2001), which is controlled by sudomotor activity (see section 2.4.1). In thermoneutral air temperatures (33 to 35 °C [Brengelmann & Savage, 1997; Savage & Brengelmann, 1996]) and below, radiation and convection are the main heat loss pathways (preserving body water), however with increasing air temperature, evaporative cooling becomes dominant (Armstrong, 2000). Heat is distributed around the body by the cardiovascular system and conduction between tissues, then subsequently exchanged with the environment through the combination of skin blood flow and sweat production.

2.1.2 Heat storage in humans

There is no heat storage when metabolic heat production is balanced with total heat loss. Therefore, it follows that heat storage occurs when the heat transfer pathways, in isolation (evaporation) or combination, are not sufficient to offset metabolic heat production or if they serve as heat gain pathways. For example, situations of heat storage can occur in instances of: high radiant heat load, high surrounding air or water temperature (with or without a sustained high metabolic rate), high relative humidity (RH) of surrounding air and when wearing clothing with high insulative properties or limited vapour permeability (Aoyagi, McLellan, & Shephard, 1997). Heat storage will eventually lead to increases in deep body temperature (hyperthermia), although unlike hypothermia which is defined as a deep body temperature of 35 °C and below by the Royal College of Physicians, there is no agreed deep body temperature at which hyperthermia begins, though the IUPS Thermal Commission (2001) state that hyperthermia is a condition where core temperature is above its range specified for the normal active state of the species, it is generally accepted that a T_c above 40.5 °C is present in those who have suffered heat stroke (Coris *et al*., 2004).

2.2. Autonomic mechanism of control

Autonomic thermoregulation is achieved by the simultaneous action of active and passive systems which together determine the heat exchange between man and the environment. The passive system is simply the physical heat exchange between the body and the surrounding environment, dictated in part by the anthropometric characteristics of the individual as, for the same total body mass, those with a greater relative amount of fat mass will have a greater change in core temperature for a given change in body heat content (Cheung, McLellan, & Tenaglia, 2000) as the specific heat of adipose tissue is approximately half that of fat-free mass (Bar-Or, Lundegren, & Buskirk, 1969). The active system consists of thermoreceptors (non-uniformly distributed centrally and peripherally), ascending and descending neural pathways, and effector organs distributed throughout the body (Werner, 2010). These form independent thermoeffector loops which have their own afferent and efferent branches influencing the heat loss and heat gain pathways of the body (Romanovsky, 2007b). This multi-sensor, multi-processor, multi-effector system, creates a distributed, feedback controlled, thermoregulatory system which responds dynamically and proportionally to thermal insults (Werner, 2010). This response is instigated by the sympathetic nervous system through four sets of effectors (Parsons, 2014):

- Vasodilation / vasoconstriction of skin arterioles, principally through the presence of the neurotransmitter norepinephrine
- Activation of eccrine sweat glands, principally through the presence of the neurotransmitter acetylcholine (sympathetic cholinergic stimulation)
- Increased muscle tone and shivering in skeletal muscle, activated by a reduction in temperature

- Endocrine glands, with the release of thyroxine and also epinephrine and norepinephrine which influence the metabolic rate of all body cells

Despite some valid objections (Jay, Reardon, *et al.*, 2007; Webb, 1995), it is the convention to view the combination of shell (peripheral) and deep body (brain and viscera) temperatures (mean body temperature) as the integrated, spatially distributed temperature signal that is the regulated variable in the thermoregulatory system (Romanovsky, 2007b, 2014; Werner, 1980).

2.3. Thermoreceptors

Thermoregulation does not occur in isolation from other homeostatic processes. It works in concert with other regulatory mechanisms, for example osmoregulation and blood pressure regulation (Taylor, 2006b). As with any other physiological regulatory process, thermoregulation requires specific sensory-receptor organs to register the physical or chemical changes in the system (Benzinger, 1959). Thermoreceptors are thermally specific free nerve endings which interact with other non-thermal and non-specific nerves to provide thermoafferant information (Mekjavić & Eiken, 2006). They are located centrally (hypothalamus, spinal cord, stomach and viscera) and peripherally (skin, oral and urogenital mucosa) and relay sensory information regarding external and internal temperatures respectively (Romanovsky, 2007b). Over a specific range of temperatures, different thermoreceptors will increase and decrease their neural firing as temperature increases. When ambient temperature changes, thermoreceptors respond with a burst of activity (dynamic response), which then reduces and levels off (static response) if the new temperature is maintained (Bligh, 1998) see Figure 2.1.

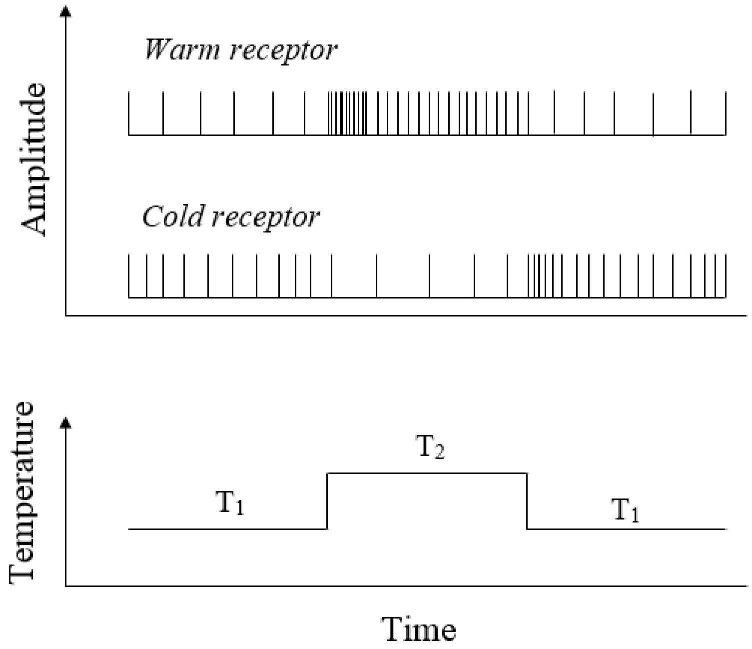


Figure 2.1. Nerve impulse from single warm and cold receptors set up by temperature stimulus (redrawn from Hensel, 1981).

This has an important implication for the comparison of thermoregulatory responses during a dynamic versus stable temperature environments. In the stable environment, the thermoafferant signal will be reduced after the initial burst, however, in the dynamic environment it may not have the opportunity, and so it is conceivable that for a matched temperature profile (of the individual) the thermoafferent signal could be different between a constant and a dynamic environment. Central thermoreceptors are predominantly warm sensitive, which is logical considering humans are endothermic homeotherms and regulate their deep body temperature far closer to the lethal hyperthermic temperature than the lethal hypothermic limit (Romanovsky, 2007b). It appears that both centrally evoked cold- and heat-defence responses are predominately triggered by changes in the activity of warm sensors. This is due to the observation that the activity of the less abundant central cold thermoreceptors appears to be primarily due to synaptic inhibition from the nearby warm thermoreceptors, rather than specific activity (Romanovsky, 2007a). Peripherally, there are four primary skin thermoreceptor types; two mediate nociceptive responses to painfully cold or hot stimuli and the other two, comprising of distinct populations of cold and warm receptors, are involved in thermoregulation. As opposed to central thermoreceptors, the majority of peripheral thermoreceptors are cold sensitive (Romanovsky, 2007b). Cold sensors are located in or immediately beneath the epidermis, with signals conducted by thin myelinated Aδ fibres (Romanovsky, 2007b). The less prevalent warm sensors lie slightly deeper in the dermis and their signals are conducted by unmyelinated C fibres (Romanovsky, 2007a, 2007b)

When thermoreceptors are excited by thermal stimuli (Bligh, 1998; Taylor, Kondo, & Kenney, 2008) the resulting neural information is integrated to elicit an appropriate thermoregulatory response (Benzinger, 1959). There is, however, no single neural area that acts as the centre for thermoregulation, but a hierarchy of structures extending through the hypothalamus, brain stem, and spinal cord (Boulant, 2000). Nevertheless, the preoptic hypothalamus, consisting of the medial and lateral parts of the preoptic nucleus, as well as regions of the nearby septum and anterior hypothalamus, is a significant neural structure in homeothermic regulation (Boulant, 2000). It is well established that many central thermoreceptors are located in the preoptic anterior hypothalamus (POA) (Romanovsky, 2007a), where a substantial portion of thermal integration is thought to occur (Boulant, 2000). Experimental observations (Mekjavić & Eiken, 2006) and theoretical reasoning (Bligh, 2006) suggest that the sensor to effector pathways for heat loss and heat gain, overlap and mutually inhibit each other. This ‘reciprocal cross inhibition’ takes place between the thermoafferent signals from thermoreceptors and the POA.

Ultimately, the integration of thermal information results in the central nervous system sending efferent signals, through the autonomic nervous system, to the appropriate effector organs (*e.g.* skin vasculature, sweat glands) to control the balance between heat loss and gain. It is this balance between heat loss and heat gain pathways which result in a stabilised body temperature, and as such there is no need for the classical ‘set-point’ temperature. Indeed, the concept of a unified control system with a single controller, unvarying reference signal and the term ‘set-point’ has been rejected by some (Kanosue & Crawshaw, 2010; Mekjavić & Eiken, 2006; Romanovsky, 2004, 2007a, 2007b). As a theory, reciprocal cross inhibition has been with us since the early twentieth century. Sherrington’s 1906 description of the reciprocal cross inhibition of agonist and antagonist muscle activity, changed the course of neurophysiology (Burke, 2007) and was subsequently incorporated into the human thermoregulatory models of Bligh (1984 & 1998), and to some extent Boulant (1981). Indeed, it has been suggested that set point theory may not have prevailed within the thermophysiology literature for so long, had this elegant mechanism been more widely recognised in other physiological systems (Cabanac, 2006). It is, perhaps, telling that one of the scientists who first provided the idea of an unvarying reference signal at a set level in human thermoregulation, and so the concept of the POA acting as a thermostat, was James Hardy who was primarily a physicist with a particular interest in ventilation engineering (Bligh, 1998).

2.4. Thermoeffector responses during heat stress

The recruitment of thermoeffector responses (vasomotor activity, sudomotor activity and shivering [Bligh, 1998; Hardy, 1961]) is driven by the hierarchy of thermoregulatory centres and the activity of each effector mechanism is initiated by deviations in mean body temperature from different temperature thresholds (Bligh, 2006; Kanosue & Crawshaw, 2010). This process alters rates of heat exchange from within the body to the environment, allowing individuals to thermoregulate over a wide range of ambient temperatures and levels of metabolic heat production (Lind, 1963a; Nielsen, 1938). Specifically, it is sudomotor activity and cutaneous vasodilation which are the autonomic thermoeffectors which contribute to heat loss (Benzinger, 1969). These effector mechanisms are not necessarily activated simultaneously, but do work in concert to increase to heat loss potential up to the point where maximal vasodilation and maximal sweating are reached. Typically, sudomotor activity will initiate at a higher threshold temperature, compared to cutaneous vasodilation. Sweating is, therefore, critical in situations where increases in skin blood flow alone are insufficient to prevent dangerous increases in body temperature. While 'delayed' sweating may be less effective for maintaining optimal deep body temperature, overall, may be of benefit due to demands from other homeostatic systems, such as during dehydration (Kanosue & Crawshaw, 2010). Various physical (water vapour pressure, thermal gradient [Kerslake, 1972]) and non-thermal factors (dehydration, exercise, fever, blood glucose, sleep, motion sickness, inert-gas narcosis [Mekjavić & Eiken, 2006]) also influence heat exchange and, by extension, thermoeffector response.

2.4.1 Eccrine sweat production

Sweat glands are part of the endocrine system and humans have three types: 1) apocrine, 2) apoeccrine and 3) eccrine, but only eccrine sweat glands produce sweat to facilitate evaporative cooling and, as such, will be the focus of this section. Humans have an estimated 1.6 to 4.0 million sweat glands with the highest concentrations found on the forehead, scalp, axillae, palms of the hands and soles of the feet (Kondo *et al*., 1998; Shibasaki, Wilson, & Crandall, 2006; Silverthorn, 2007). Gland densities around the body vary, from 64 glands$\cdot$cm^{-2} on the back to 700 glands$\cdot$cm^{-2} on the palms and soles (Saga, 2002). The eccrine glands are tubular structures with a bulbous secretory coil and dermal duct which pass through the dermis to a spiralled duct (where some minerals are reabsorbed) through the epidermis to enable the delivery of sweat onto the surface of the skin (Shibasaki *et al*., 2006). It is important to note that it is the number of sweat glands activated and the amount of sweat released per gland that determines the sweat rate for an individual (Shibasaki *et al*. 2006). Work by Kondo *et al.* (2001) comparing exercise (117.5 ± 4.8 W) and passive heating (lower-leg immersion into hot water of 42°C) in an ambient temperature of 25°C and relative humidity of 50 % showed that initially, after crossing the temperature threshold for sweating, sweat rate is elevated by increased sweat gland activation which becomes maximal within approximately 8 minutes, then subsequent increases are driven by the amount of sweat per gland produced. Therefore, after approximately 8 minutes, any increase in sweating is mediated by the increase in sweat output per gland, rather than the activation of further sweat glands (Kondo *et al*., 1998, 2001).

The more stressful the environment or exercise intensity, the more dependent humans become on evaporative cooling (Armstrong, 2000; Bain, Deren, & Jay, 2011; Sawka *et al*., 2001); eccrine sweat glands can produce large quantities of sweat when attempting to thermoregulate (Shibasaki *et al*., 2006). Sweating has the potential to provide a very effective avenue for heat loss due to the high latent heat of vaporisation (2.43 $kJ.g^{-1}$ sweat at 30 °C) (Wenger, 1972). However, the efficacy of this heat loss pathway is influenced by many factors including the environmental conditions (Cheung & McLellan, 1998a; Givoni & Goldman, 1973), hydration status (Sawka, Montain, & Latzka, 2001), circadian rhythm (Reilly & Waterhouse, 2009), ethnicity (Taylor, 2006) and acclimation (Buono, Martha, & Heaney, 2009). Maximal sweat rate values of over 3 $L\cdot hr^{-1}$ have been recorded, although the average upper limit appears to be approximately 1.5 $L\cdot hr^{-1}$ (Taylor *et al*., 2008), although these rates cannot be maintained indefinitely. Hidromeiosis (reduction in sweating due to swelling of epidermal layer with wetting and physical blockage of sweat gland duct [Candas, Vibert, & Vogt, 1980]) causes reduced sweat rates despite prolonged elevated deep body temperature, with skin wettedness and dehydration thought to be important contributing factors (Kerslake, 1972; Shibasaki *et al*., 2006). The evaporation of sweat is determined by the water vapour pressure gradient between the skin and the ambient air (Kerslake, 1972). High sweat rates cause a concurrent increase in the water vapour pressure at the skin (until saturation), which in turn increases the skin to ambient air vapour pressure gradient, promoting an increase in evaporative heat loss (Kerslake, 1972).

Eccrine sweat gland secretion is thought to be regulated around a threshold of either deep body temperature or mean body temperature (Candas, Libert, & Vogt, 1979; Kondo *et al*., 1998; Nadel, Bullard, & Stolwijk, 1971; Werner, 2010). Sweat glands are innervated through sympathetic cholinergic nerves and the subsequent sweat production rates are mediated by both central and local factors (Nadel *et al*., 1971). With a rise in deep body temperature, thermoefferent activity from the central nervous system increases and induces the release of acetylcholine (ACh) at the sudomotor junction, ACh binds to muscarinic receptors on the sweat gland where it is broken down by acetylcholinesterase (Shibasaki *et al*., 2006) causing the initiation of sweating. Additionally, non-thermal input from mechanoreceptors and baroreceptors also influence sweating, for example attenuating mean arterial pressure drop post-exercise enables sweat rate to be maintained at elevated levels (Journeay, Reardon, Martin, & Kenny, 2004; Journeay, Reardon, McInnis, & Kenny, 2005; Kenny & Jay, 2013). But the interaction and hierarchy of these thermal and non-thermal factors are still not clear (Flouris & Cheung, 2010). Some authors believe that the level of sudomotor activity achieved during exercise is determined by the required evaporation for heat balance (E_{req}), unless this rate is higher than the maximal evaporation possible within the given environment (E_{max}) (Cramer, Bain, & Jay, 2012; Jay, Bain, Deren, Sacheli, & Cramer, 2011). However this reasoning is flawed when viewed from a neurophysiological perspective because the body is unable to sense the E_{max} and cannot even sense humidity (Newton, 2011); it can only evoke an effector response (*e.g.* sweating) which is in proportion to the absolute and rate of change of body temperature at the time.

Although the secretion of sweat onto the skin surface occurs across the whole-body (Kondo *et al*., 1998), there are differences in sweat rate between body segments (Kondo *et al*., 1998; Weiner, 1945) and within segments themselves (Machado-Moreira, Smith, van den Heuvel, Mekjavić, & Taylor, 2008; Machado-Moreira, Wilmink, Meijer, Mekjavić, & Taylor, 2008). There is also large variation between individuals; Sato and Dobson (1970) suggest that this is due to functional differences in the sweat glands *per se*, rather than the number of active sweat glands. However, the data presented indicates that variability within an individual between regions is caused by differences in the number of active sweat glands (Sato & Dobson, 1970; Sato & Sato, 1983).

2.4.2 Cutaneous vasodilation

In addition to evaporation of sweat from skin, physical exchange of heat between an individual and their environment is also influenced by an alteration of the conductance of the superficial tissues by the redistribution of blood flowing through them (Richards, 1973). Vasodilation has been shown to increase during cholinergic nerve activity that signals the onset of sweating (Kellogg *et al*., 1995; Shibasaki, Wilson, Cui, & Crandall, 2002). Indeed, sweating does not start when cutaneous vasodilation is maximal, rather, sweating and cutaneous blood flow work side by side to control heat balance. Heat transfer to the environment is most effectively regulated at the skin surface. To dissipate heat, blood flow is directed in close proximity to the body's surface, and conversely to conserve heat, blood flow is redirected away from the body's surface. This does not imply a uniform temperature across the body's surface; heat flow will vary depending on the location (central *vs*. distal) or proximity to specific anatomical locations and organs (*e.g.* forehead and the brain) (Romanovsky, 2014). Indeed, during normothermia and hyperthermia, the regulation of cutaneous blood flow provides the only controllable mechanism by which heat can be transferred from deep within the body and exchanged to the environment (González-Alonso, 2012). An increase in cutaneous vasodilation enables substantial heat loss through vascular convective and conductive heat transfer from within the body to the surface of the skin, and so to the environment (Charkoudian, 2010).

The cutaneous vasculature contains both vasoconstrictor and vasodilator nerves and is under autonomic nervous control (Edholm, Fox, & Macpherson, 1957) and so dual sympathetic neural control of skin blood flow is required (Kellogg, 2006). It is this sympathetic neural control of skin blood flow, mainly by the active vasodilator system, that is responsible for up to 90 % of cutaneous vasodilation, which occurs during heat stress (Charkoudian, 2003). During heat stress, elevation in mean body temperature causes cutaneous vasodilation through cholinergic nerve innovation (Kellogg, 2006). However, work by Aizawa and Cabanac (2000) showed that reversal of temperature gradients between layers of skin at the cheek did not alter the local cutaneous blood flow, which suggests that, in this area at least, cutaneous blood flow is dependent primarily on deep body temperature.

There are two distinct types of skin in the human body: glabrous (hair-less) and non-glabrous (hairy) (Parsons, 2014). The differences in their vasculature and control pathways by the sympathetic nervous system allows them to work in concert to achieve fine control of heat transfer from the body. Glabrous skin (palms, souls of feet, lips) is dense with arteriovenous anastomoses (AVA) which allow blood to bypass surface capillary beds. These AVA allow high blood flow directly from arterioles to venules and so substantial changes in skin blood flow are possible. The AVA in glabrous skin are innervated by sympathetic vasoconstrictor nerves (Charkoudian, 2003), meaning that the control of SkBF is by the introduction and withdrawal of vasoconstrictor tone. In contrast, non-glabrous skin (rest of the body) has negligible levels of AVA but does have deep and superficial capillaries. The most efficient heat transfer with the environment occurs when blood is directed through these surface capillaries. In non-glabrous skin control of blood flow is accomplished by both the adrenergic vasoconstrictor system and cholinergic vasodilator system (Kellogg, 2006).

Whole body resting skin blood flow in a thermoneutral environment (T_a of 26 to 28 °C and a $\overline{T}_{sk}$ of 33 °C [Parsons, 2003]) is approximately 250 $mL \cdot min^{-1}$, which results in ~80 to 90 $kcal \cdot h^{-1}$ of heat dissipation. In these ambient temperatures, vasomotor changes alone will regulate an individual's temperature in what is termed the 'vasomotor' (Werner *et al*., 2008) or 'null zone' (Bligh, 1998, 2006; Mekjavić, Sundberg, & Linnarsson, 1991) with tonic control. This is homeostatically advantageous to an individual because stable internal temperatures are maintained with minimal loss of water (no sweating) or use of energy (no shivering). However, during hyperthermia skin blood flow can increase up to 6 to 8 $L \cdot min^{-1}$ (Charkoudian, 2003) with stable maximal skin blood flow thought to be achieved at skin temperatures of 42 °C in the forearm (Taylor, Johnson, O'Leary, & Park, 1984) and 45 °C in the hand (Roddie & Shepherd, 1956). During prolonged exercise, it has been shown that once T_c reaches 38 °C, skin blood flow stops increasing and reaches an upper limit that is below expected maximal skin blood flow (Brengelmann, Johnson, Hermansen, & Rowell, 1977; Johnson, 2010) as well as that expected to be present during hyperthermia at rest (Taylor, Johnson, O'Leary, & Park, 1984). To achieve this high skin blood flow heart rate and cardiac output increase, and other vascular beds (*e.g.*, renal and splanchnic) vasoconstrict (see Charkoudian, 2003 for review). When exercising in the heat, the large increases in blood flow to the skin as well as the perfusion requirement of the active muscle causes an additional circulatory strain and places a high demand on cardiac output (Simmons, Wong, Holowatz, & Kenney, 2011). This 'competition' for blood flow has been cited as one reason for early cessation of exercise in the heat (Sawka, Cheuvront, & Kenefick, 2012).

2.5. Thermoregulatory zone and the prescriptive zone

The thermoregulatory zone (TZ) describes the range of ambient temperatures across which thermoregulation is possible by physiological regulation, and within it the primary physiological effector response responsible for heat loss are used to describe separate discrete zones (Werner *et al*., 2008) see Figure 2.2.

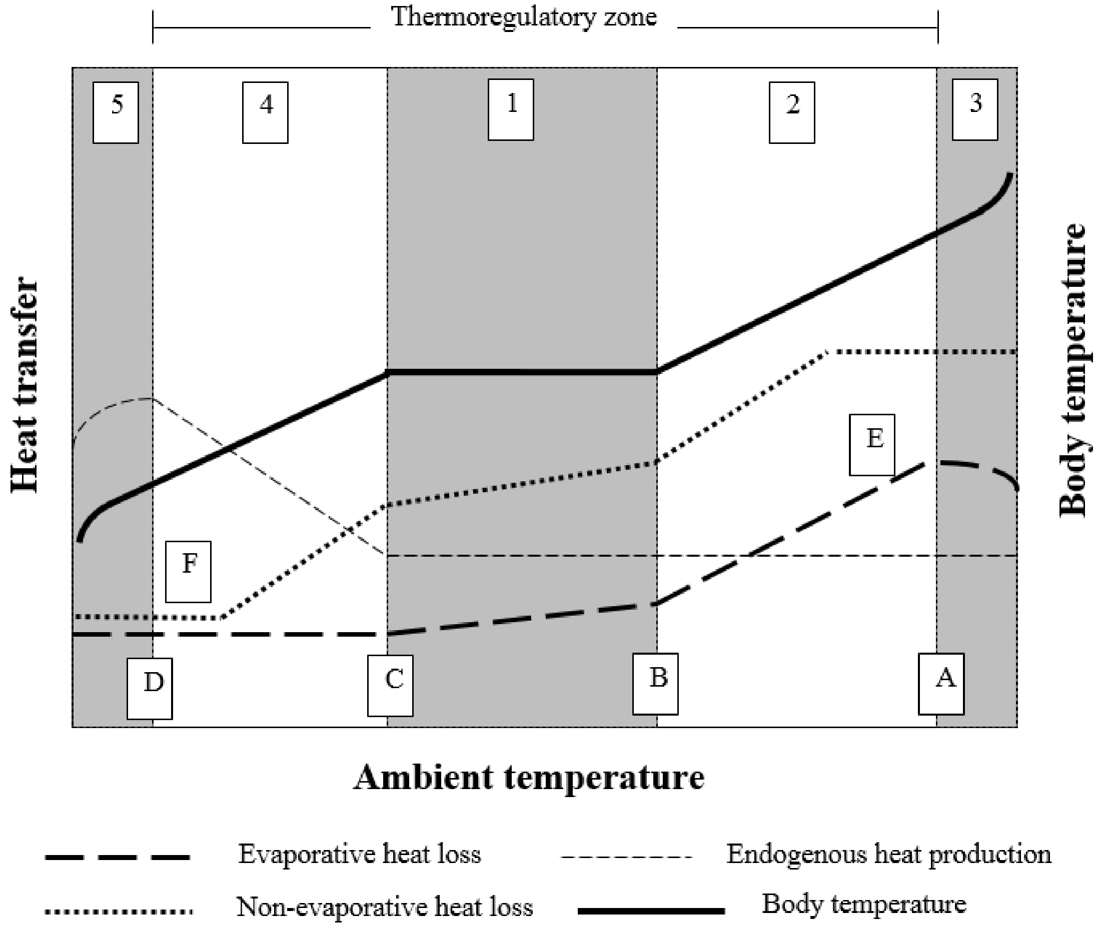


Figure 2.2. Overview of thermoeffector and body temperature response across a wide range of air temperatures. Where: 1= Null zone, 2= sudomotor zone, 3= uncompensable zone (hyperthermic), 4= metabolic regulatory zone, 5= uncompensable zone (hypothermic). A= peak sweat secretion, B & C= thermoeffector thresholds, D= maximal shivering thermogenesis, E= maximal vasodilation, F= maximal vasoconstriction. (redrawn from Werner *et al*., 2008).

Missing from this typical didactic model of the thermoregulatory zone is: 1) mention of any behavioural thermoregulation, which in many cases is far more powerful than autonomic thermoregulation (Parsons, 2014; Schlader, Stannard, & Mündel, 2010); and 2) inclusion of the prescriptive zone (PZ), as depicted in Figure 2.3 (Region A). In the PZ, rectal temperature equilibrates at a value that is dependent on work rate, and independent of a wide range of ambient conditions (Lind, 1963a). Therefore, in diagram in Figure 2.2, the PZ would be within the TZ (4, 1 and 2), but importantly would not reach the boundary with zone 3 or 5. Here thermoregulation is possible, but is dependent on ambient temperature as well as work rate (see Region B in Fig. 2.3). Additionally, an important characteristic of the thermoregulatory system is that regulation does not always denote constant maintenance of a variable, which is conceptually similar to some other regulated physiological responses in the body, for example the elevation in mean arterial pressure occurring during exercise (Taylor *et al.*, 2008). As such, beyond the PZ a steady state rectal temperature may still be achieved, albeit at an elevated value and following a delay (see Region B in Fig. 2.3).

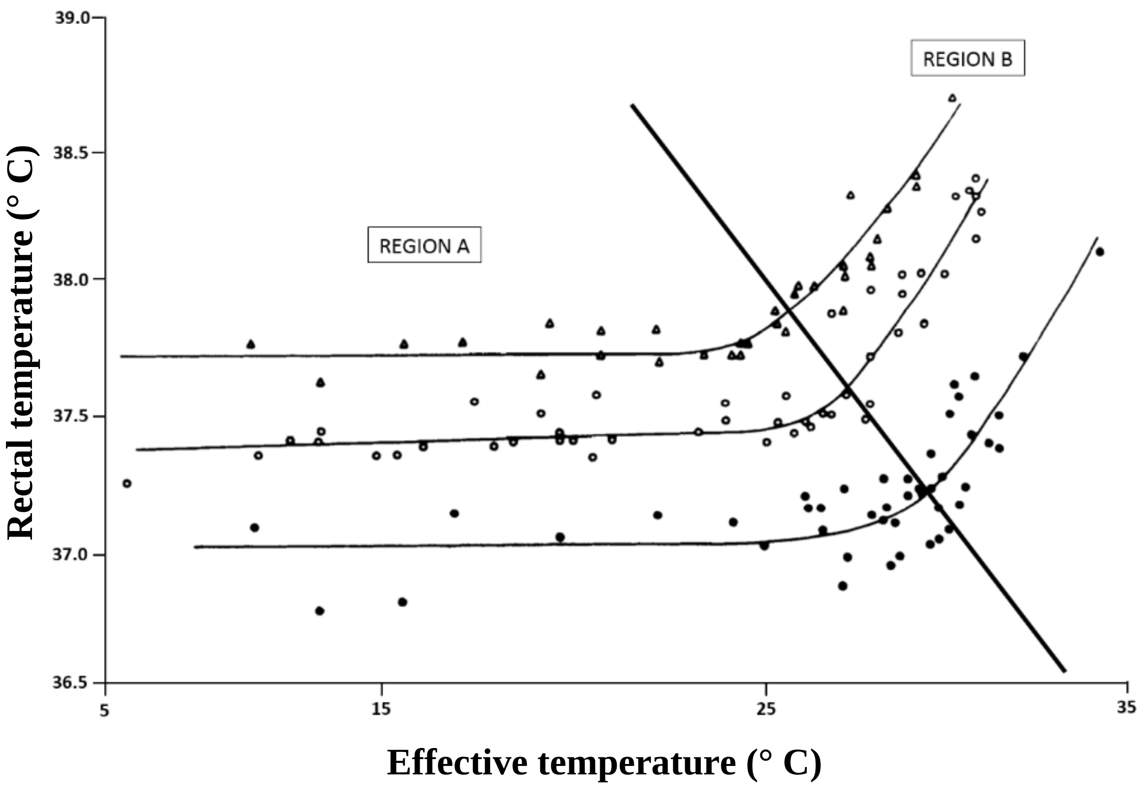


Figure 2.3. The equilibrium rectal temperature of one participant working at energy expenditures of 180 (closed circles), 300 (open circles) and 420 (closed triangles) kcal·hr^{-1} over a wide rate of ambient temperatures. Region A = Prescriptive zone, Region B = Outside Prescriptive Zone, but still able to thermoregulate (redrawn from Lind, 1963a).

There appears to be some inconsistency in the application of the terminology. For instance Kenney, DeGroot, and Holowatz (2004), associate moving out the PZ (Region A in Fig. 2.3) with uncompensable heat stress: *"Thermal environments above this designated ''prescriptive zone'' (Lind, 1963) do not allow thermal balance either because of excessive dry heat gain or limited evaporative heat loss, and the result is a continuous rise in T_c."* p480) thereby ignoring the fact that, as classically proposed by Lind (1963a), at combinations of work rates and environmental conditions outside the PZ (Region B in Fig. 2.3), deep body temperature (T_c) does and can still equilibrate, albeit after a longer time period and at a higher absolute temperature. These authors are not alone in misrepresenting Lind's original definition of the prescriptive zone: Mora-Rodríguez (2012) incorrectly reproduces the classic prescriptive zone graph and the associated interpretation by 1) stating that rectal temperature can only reach equilibrium within the prescriptive zone, and 2) joining data points on the graph together, making it appear that the data are continuous rather than discreet data collections from separate tests, on separate individuals. Taylor (Taylor, 2006a) states that if T_c varies by more than 2 °C above or below 37 °C, it can be assumed that thermal balance has been lost, or thermoregulatory failure has occurred. Figure 2.3 (taken from Lind *et al.* [1963a]) shows an equilibrated rectal temperature as high as ~ 38.75 °C for one participant, confirming that even when you leave the PZ, you are still in the TZ.

2.6. Compensable and uncompensable heat stress during exercise

The magnitude of heat stress can be classified as either: 1) compensable – where thermoregulation is effective and a thermal steady state can be achieved *i.e.* within the 'thermoregulatory zone'; or, 2) uncompensable – where thermoregulation is insufficient and so a thermal steady state is unobtainable (Kraning & Gonzalez, 1991). Uncompensable heat stress occurs when the rate of endogenous heat production exceeds the capacity to lose heat to the environment (Givoni & Goldman, 1972; Robinson, Turrell, & Gerking, 1945). Uncompensable heat stress has also been defined as the point where the required evaporative cooling necessary to achieve thermal balance (E_{req}) exceeds the maximum evaporative potential of the environment (E_{max}) (Cheung & McLellan, 1998a; Givoni & Goldman, 1973). However, this definition disregards the other heat transfer pathways (see 2.1) or situations of reduced sweating *e.g.* dehydration (Sawka *et al*., 2001), and assumes that the thermoafferent input is sufficient to initiate maximal sweating (see 2.4). Regardless of the mechanism, the result is that deep body temperature rises inexorably (Cheung *et al*., 2000; Kraning & Gonzalez, 1991), often to the point of exhaustion (González-Alonso *et al*., 1999). The increase in deep body temperature into hyperthermia has been shown to reduce exercise tolerance in the heat (Cheung, 2007; González-Alonso, Crandall, & Johnson, 2008; Hargreaves, 2008; Nybo, 2008), impair exercise performance (Hettinga *et al*., 2007), and lead to heat illness if exercise does not cease (Coris *et al*., 2004).

The combination of thermal (*e.g.* environmental conditions and work rate) factors immediately preceding the point of uncompensable heat stress defines the upper boundary of the ‘thermoregulatory’ zone (Tipton, 2006); this point can be influenced by a variety of non-thermal factors (*e.g.* aerobic fitness, [de]hydration, clothing, anthropometrics). This boundary is not fixed and is characterised by the upper limit of a range of combinations of environmental and exercise conditions in which individuals can achieve thermoregulatory balance. However, in an attempt to define critical environmental limits for work and / or exercise in the heat, researchers (Belding & Kamon, 1973; Dougherty *et al*., 2010; Kamon *et al*., 1978; Kamon & Avellini, 1976; Kenney & Zeman, 2002), have manipulated water vapour pressure and / or dry bulb temperature to identify the critical ambient condition which leads to an inflection point in deep body temperature at a given work rate in a range of populations (Dougherty *et al*., 2010; Kamon *et al*., 1978; Kenney & Zeman, 2002) and clothing conditions (Berglund & Gonzalez, 1977; Kenney *et al*., 1987, 1988, 1993) based on adaptations of the method originally developed by Belding and Kamon (1973).

Belding and Kamon (1973) designed a discontinuous protocol requiring each participant to exercise under a given set of environmental conditions for up to one hour on each occasion to allow for sufficient time to enable a plateau in deep body temperature to become evident; multiple visits were required to determine the combination of work rate and ambient conditions under which deep body temperature could not achieve a plateau. More recent research in this area has favoured continuous protocols where either T_{db} or water vapour pressure is progressively increased after an equilibration period (Dougherty *et al*., 2010; Kamon *et al*., 1978; Kamon & Avellini, 1976; Kenney & Zeman, 2002), presumably this approach is adopted because of the substantial logistical benefits which allow multiple combinations of work-rate and environment to be tested in a larger number of participants, as well as reducing the confounding effects of inter-day participant biological variability.

With reference to the description of the prescriptive zone (Lind, 1963a), it is however possible that if the ambient heat load does not increase continuously once the inflection point occurred (thereby preventing the thermoregulatory system from adapting and achieving a new steady state), deep body temperature may rise temporarily and then plateau for a second time at a higher absolute temperature. Indeed, it may be the case that an inflection in deep body temperature does not necessarily indicate thermoregulatory ‘failure’, but instead represents a permissive rise in attempt to maintain a gradient for heat loss (Lind, 1963a) or a function of the continuous increase in ambient temperature or water vapour pressure after inflection of deep body temperature.

It therefore remains to be seen if the inflection point observed in studies employing continuous type protocols is ‘true’ uncompensability, or a product of the experimental approach employed. The doubt arises because human thermoregulation (as with all biological and mechanical regulatory systems) has an inherent time lag due to the physical properties of heat transfer kinetics (thermal inertia), and thresholds at which the initiation of response occurs, when responding to increases in temperature, as well as the impulse frequency of warm thermosensors, which subsides from brief but intense activity to a steady level when temperature is raised in a step-wise fashion (Bligh, 1998). If these steady state impulses are plotted against temperature (as it is in many models of cold and warm thermosensor activity), there is a linear relationship (Bligh, 1998). It is proposed that if the temperature change is dynamic, as in the case of the second portion of the aforementioned studies (Belding & Kamon, 1973; Dougherty *et al*., 2010; Kamon *et al*., 1978; Kamon & Avellini, 1976; Kenney & Zeman, 2002), impulse frequency may never attain a steady state and the corresponding effector response, may have to play ‘catch up’ with the effector response demanded by the constantly changing afferent input. This could mean that the inflection in deep body temperature seen in these studies are artefacts of the methodology, not an indication of uncompensable heat stress.

A similar issue that has received little attention relates to the level of output of the various effector responses at the point of uncompensability, and whether this is maximal. The majority of studies examining the limits of thermal compensability do not measure sweat rate or skin blood flow and so are unable to provide insight into this important matter (Dougherty *et al*., 2010; Kamon *et al*., 1978; Kenney & Zeman, 2002; Kenney *et al*., 1988, 1993). The effector response is proportional to and permitted by the nature of the change in and the absolute body temperature. In a specific environment, if the integrated thermoafferent input is insufficient to evoke a maximal effector response, a maximal response will not be evoked, irrespective of the consequences for deep body temperature. Thus, it may be that not all effector responses are maximally evoked as an individual moves from the thermoregulatory zone to uncompensable heat stress (Tipton, 2013). It follows that an inflection in deep body temperature may not necessarily indicate an incidence of thermoregulatory failure, but rather, the peak response permitted by the thermoafferent input at the time and/or an increase in deep body temperature in an attempt to maintain a gradient for heat loss from the core to the shell. Maintaining or improving the thermal gradient between core, skin and environment would have the benefit of reducing the additional homeostatic burden of increased water loss through sweat.

2.7. Thermal factors which influence temperature regulation

2.7.1 Metabolic heat production

Metabolic heat production is the difference between the total energy produced and the total external work performed by the body, *i.e.* $\dot{M} \pm \dot{W}$ (Parsons, 2003). Due to low mechanical efficiency, approximately 70 to 80 % of the energy liberated by substrate oxidation is lost as heat (Parsons, 2003; Taylor, 2006). When energy demand is increased by exercise the metabolic heat production of the individual rises; this leads to increased muscle temperature, which along with increased muscle blood flow, allows greater conductive and forced convective (mass flow in circulation) heat exchange between the working muscle and the blood and to the surrounding tissues and compartments (Hardy, 1961). As a consequence, deep body temperature typically becomes elevated relative to resting conditions (Nielsen, 1938), and heat loss responses (cutaneous vasodilation and sweat production) are initiated and will continue to rise unless heat balance is established (Webb, 1995). Indeed, as Kenny, Webb, Ducharme, Reardon, and Jay (Kenny, Webb, Ducharme, Reardon, & Jay, 2008) and Webb (1995) have demonstrated, the increased heat production of exercise can achieve a steady level in ~5 minutes, but this is not balanced by heat loss mechanisms at the same initial pace. This results in a rapidly increasing muscle temperature and initial heat storage (Nadel, 1985). However, this should not be seen as a thermoregulatory failure (Nielsen & Nielsen, 1962) as deep body temperature remains regulated, but at an elevated level that is primarily dependent on work rate when in the thermoregulatory zone (Lind, 1963a; Nielsen, 1938) and increased muscle temperature also improves the ability for muscles to undertake work.

2.7.2 Influence of work rate

Historically, data from Saltin and Hermansen (1966) suggested that deep body temperature was best determined by the relative workload expressed as a percentage of maximal oxygen uptake. The deep body temperature (oesophageal) responses to cycle exercise were shown to be varied amongst participants with wide ranging aerobic fitness ($\dot{V}O_{2max}$ 38 to 76 $mL \cdot kg^{-1} \cdot min^{-1}$) when reported in absolute terms, but when presented as a given percentage of $\dot{V}O_{2max}$ this variation was reduced by 65 %. The influence of the relative work rate on the thermal response to exercise has since be challenged by studies using hypoxia (reduced O_2 concentration in ambient air [Rowell, Freund, & Brengelmann, 1982]) and simulated altitude (reduction in ambient air pressure [Greenleaf, Card, & Saltin, 1969]) to acutely reduce participant's blood oxygen saturation. These interventions temporarily lower the $\dot{V}O_{2max}$ thereby allowing comparison of the deep body temperature response at the same fixed absolute workload, which would represent different relative work rates under the experimental and control conditions, for the same person. These studies found that reducing $\dot{V}O_{2max}$ did not have an effect on exercising deep body temperature and thus that deep body temperature is not directly linked to the relative work rate expressed as a percent of $\dot{V}O_{2max}$. Acute changes in VO_{2max} using graded ischaemia in the working muscles (Kacin *et al.*, 2005) and intermittent hypoxic exposures (Kacin, Golja, Eiken, Tipton, & Mekjavić, 2007) indicate that the increase in relative work rate is associated with an augmented sweating response and attenuated skin blood flow during dynamic exercise (Kacin, Golja, Tipton, Eiken, & Mekjavić, 2008). Nonetheless, studies are still undertaken with the assumption that using a relative work rate will allow equitable comparison between groups of different gender, fitness and age (Fritzsche & Coyle, 2000; Gant, Williams, King, & Hodge, 2003; Gass, McLellan, & Gass, 1991; Mora-Rodríguez, Del Coso, Hamouti, Estevez, & Ortega, 2010; Nadel, Pandolf, Roberts, & Stolwijk, 1974; Périard *et al*., 2012; Roberts, Wenger, Stolwijk, & Nadel, 1977).

The results of studies comparing the thermal responses of untrained with aerobically trained individuals will be influenced by the method used to standardise exercise intensity *i.e.* the use of relative or absolute intensity work rates. If equal work efficiencies are assumed, which has been shown to be an appropriate assumption in cycling (Marsh, Martin, & Foley, 2000; Moseley, Achten, Martin, & Jeukendrup, 2004), metabolic heat production should be equal between untrained and trained individuals if working at the same absolute work rates. Therefore, any thermoregulatory advantage afforded by aerobic training to those individuals should be evident as reduced deep body and skin temperatures (Havenith, Coenen, Kistemaker, & Kenney, 1998). However, when exercise intensity is standardised to a given relative level, usually expressed as a percentage of $\dot{V}O_{2max}$, metabolic heat production will differ, as trained individuals will be exercising at a higher absolute work rate than untrained individuals and will be generating more metabolic heat (Gagnon, Jay, Lemire, & Kenny, 2008). It has been reported that when working at matched relative work rate and so an higher absolute workload, cutaneous blood flow (Fritzsche & Coyle, 2000) and sweat rate (Nadel *et al*., 1974; Roberts *et al*., 1977) in aerobically trained individuals is higher compared to untrained individuals. However, when considering the heat balance equation, the trained group - with the greater $\dot{V}O_{2max}$ - require a greater rate of sweat evaporation to balance the greater rate of metabolic heat production elicited by the protocol. This limitation has been recognised by others (Bar-Or, 1998; Fritzsche & Coyle, 2000; Schwiening *et al*. 2011). Nevertheless, Havenith *et al.* (1998) propose that if the increased heat dissipation capabilities (Mora-Rodríguez, 2012) and the increased levels of heat production in trained individuals 'equal out' then the higher metabolic heat production is compensated by the higher heat dissipation resulting in heat balance and so there will be no additional burden for trained participants, relative to untrained individuals, working at a given relative work rate (Périard *et al*., 2012).

Of course, it is important to remember that in situations of heat stress, endurance performance in both aerobically trained and untrained individuals is significantly impaired and high levels of aerobic fitness cannot protect individuals from heat illness (Mora-Rodríguez, 2012). There are situations where the improved thermoregulatory ability and increased heat production or heat loss capabilities of fitter individuals are mismatched. For example, environments with high water vapour pressures reduce the evaporative capacity of the environment or microenvironment (also seen in encapsulating clothing) in which the individual is exercising (Cheung & McLellan, 1998a); in these situations the increased metabolic capacity of fitter individuals may not be 'compensated' by their increased heat dissipation. Furthermore, high relative work rates in a dry environment can also put trained individuals at a thermoregulatory disadvantage. When exercising at 80 % $\dot{V}O_{2peak}$ in hot-dry environment (36 °C, 25 % RH, airflow 2.5 $m \cdot s^{-1}$) the metabolic heat production from both trained and untrained participants exceeded the individuals heat loss capacity (limited by both physiological and physical factors), but with the inherently higher absolute work rates for the trained participants their increase in, and final deep body temperatures, were higher than those of untrained participants (Mora-Rodríguez *et al*., 2010). Therefore, if the metabolic heat production is sufficiently high, dry environments can become uncompensable, potentially leaving trained individuals most at risk of heat illness due to their higher absolute workloads when exercise intensity is set as a percentage of $\dot{V}O_{2max\,/\,peak}$ (Mora-Rodríguez *et al*., 2010).

2.7.3 Environmental heat stress

Environmental heat stress also can also result in thermoregulatory disturbance through heat gained from the external environment. Ambient temperature (mean temperature of the air surrounding the human body) influences heat flow between the human body and the air (Parsons, 2003). Therefore, when air temperature exceeds that of the skin, the human body gains heat from the environment (Parsons, 2003). Consequently, mean body temperature rises and cutaneous vasodilation and sweating are initiated. When ambient temperature is greater than skin temperature, increased skin blood flow means that blood flow from cooler deep body tissues can provide potential cooling to the skin surface but this is countered by the heat gained from skin surface which is influenced by the ambient temperature, overall this leads to a net heat gain and will cause an increase in deep body temperature. Thus, heat loss though evaporation is the only means by which the body can lose heat when environmental temperatures exceed that of skin temperature (Hardy, Du Bois, & Soderstrom, 1937).

The mechanisms underlying hyperthermia-induced exhaustion are not well understood. As a research methodology, passive heating is useful when attempting to delineate the effects of hyperthermia on the central nervous system directly, without the confounding influence of exercise induced hyperthermia (Morrison, Sleivert, & Cheung, 2004; Thomas & Cheung, 2006). For the same reasons, it can also be used for heat acclimation protocols. Exposure to passive heat stress (45 °C 24 % RH) for 90 minutes on 9 successive days has been shown to cause an earlier onset of sweating and decreased rectal temperatures and heat storage (Henane & Bittel, 1975).

2.7.4 Combination of heat stress and exercise

‘‘Perhaps the greatest stress ever imposed on the human cardiovascular system (except for severe haemorrhage) is the combination of exercise and hyperthermia. Together these stresses can present life-threatening challenges, especially in highly motivated athletes who drive themselves to extremes in hot environments.’’ (Rowell, 1986)

It is widely acknowledged that exercise performance is reduced in the heat relative to cooler conditions and that certain combinations of exercise intensity and environments have the potential for inducing serious heat illness (Sawka, Leon, Montain, & Sonna, 2011). When heat stress is combined with exercise, there is competition placed on the cardiovascular system to simultaneously support the competing thermoregulatory demands for cutaneous blood flow and metabolic demands of the contracting skeletal muscles. Indeed this competition can be observed from the moment of initiation of exercise. In hyperthermic conditions, there is an initial reduction in cutaneous blood flow, which is due to enhanced active vasoconstrictor tone (Kellogg, Johnson, & Kosiba, 1991). This was demonstrated by Kellogg, Johnson and Kosiba (1991) using iontophoresis of bretylium to selectively block noradrenergic vasoconstrictor nerves in the skin. This technique has also been used to show that the active vasoconstrictor system is also responsible for the attenuated maximal skin blood flow observed during exercise in the heat (Kellogg, Johnson, Kenney, Pérgola, & Kosiba, 1993). It appears that the exercise inhibits the active vasodilator system independent of vasoconstrictor activity (Kellogg *et al*., 1993). These responses, alongside the active vasodilator system elevating the deep body temperature threshold for cutaneous vasodilation (Kellogg & Kosiba, 1991), suggest that heat loss is not the initial priority during exercise in the heat. Despite the thermal load that exercise places on the body, cardiac output is preferentially directed to meet the increased metabolic demands of active skeletal muscle rather than heat dissipation.

The competition for blood flow as a consequence of thermal stress eventually has a negative effect on maximal (or peak) oxygen uptake (Arngrímsson, Petitt, Borrani, Skinner, & Cureton, 2004; González-Alonso, 2012; Nybo, Jensen, Nielsen, & González-Alonso, 2001), accelerating the decline in cardiac output and mean arterial pressure, which lead to decrements in exercising muscle blood flow, O_2 delivery, and O_2 uptake (González-Alonso & Calbet, 2003). $\dot{V}O_{2peak}$ has been shown to decrease progressively with an increase in ambient temperature (Arngrímsson *et al*., 2003). However, the extent of the effect ambient temperature on $\dot{V}O_{2\ max}$ varies between studies (Rowell, Brengelmann, Murray, Kraning, & Kusumi, 1969); some studies have shown negligible effect (Pirnay, Deroanne, & Petit, 1970), a marginal reduction of 3 % (Rowell *et al*., 1969) or more substantial reduction of 25 % when participants are preheated (Pirnay *et al*., 1970) in hot environments. Additionally, the occurrence of cardiovascular drift during exercise in the heat is associated with a decreased $\dot{V}O_{2max}$ and an increased relative metabolic intensity (Wingo, Lafrenz, Ganio, Edwards, & Cureton, 2005).

The extent of the reduction in $\dot{V}O_{2max}$ during exercise in the heat appears to be directly proportional to changes in body temperatures, such that when only skin temperature is elevated it is marginally affected; bigger reductions are seen when both deep body and skin temperatures are elevated (Arngrímsson *et al*., 2004, 2003; Nybo *et al*., 2001). González-

Alonso and Calbet (2003) attribute the impaired systemic and skeletal muscle aerobic capacity that precedes fatigue (with or without heat stress) largely to the failure of the heart to maintain cardiac output and O_2 delivery to locomotive muscle.

The more strenuous the exercise intensity and hotter the climate, the greater the dependence on evaporative cooling and therefore sweating (Sawka *et al*., 2001). If this fluid loss is not replaced the person will dehydrate, this in turn reduces local sweating (Bittel & Henane, 1975) and lowers skin blood flow (Kenney *et al*., 1990) for a given deep body temperature, leading to a decreased capacity for heat loss. Montain and Coyle (1992) found that the magnitude of increase in core temperature and heart rate and the decline in stroke volume observed during cycling in the heat were directly related to the increase in serum sodium ($r = 0.81\text{-}0.98$, $P < 0.02\text{-}0.19$) from 5 to 120 minutes of exercise, indicating that dehydration directly impacts thermoregulation and cardiovascular function. Rehydration during exercise in the heat has been shown to reduce cardiovascular strain (Montain & Coyle, 1992).

2.8. Non-thermal factors influencing temperature regulation

2.8.1 Clothing

Clothing generally imposes a barrier to heat transfer and evaporation from the skin surface by altering the total exposed skin surface (Gonzalez, 1987; Jeong & Tokura, 1989) and providing additional layers of insulation and impermeability (Watkins, 1984). As a consequence the thermoregulatory system must balance the interaction between the skin surface, clothing, and ambient air (Pascoe, Shanley, & Smith, 1994). The additional layer of insulation is provided by the materials themselves, as well as the trapped air layers, and typically presents a barrier to all avenues of heat transfer (Kerslake, 1972). This insulation can be affected by six factors (Gavin, 2003):

- Wind speed - higher speeds disrupts the zone of insulation
- Body movements - action of limbs disrupts the zone of insulation
- The chimney effect - loose fitting clothing ventilates trapped air layers (Pascoe, Bellingar, & McCluskey, 1994)
- The bellows effect - vigorous body movements disrupts the zone of insulation
- Water vapour transfer - clothing resists the passage of water vapour through it so reduces evaporative cooling (Aoyagi, McLellan, & Shepard, 1995)

- Permeation efficiency factor - how well clothing absorbs liquid by capillary action. Sweat transfers through clothing, and can be absorbed, condensed, ventilated and diffused (Dai, Imamura, Liu, & Zhou, 2008; Havenith, Richards, *et al*., 2008)

Importantly, the evaporation of sweat, the primary heat loss mechanism for exercise in a hot environment (Monteith & Mount, 1973; Sawka *et al*., 2007) is negatively affected by clothing (resistive to evaporation and creation of humid microclimate beneath clothing layers), because sweat can only contribute to heat loss if it is able to evaporate (Gavin, 2003). Any retention of sweat or wicking away from the skin by garments reduces its cooling efficiency (Havenith *et al*., 2009). Typically, clothing alters the vapour pressure at the skin in a manner that reduces the evaporative potential of sweat,, the E_{max} of the microclimate between the skin and garment will also become diminished (Aoyagi *et al*., 1995), causing earlier uncompensable heat stress (Cheung & McLellan, 1998a; Givoni & Goldman, 1973). Thus, wearing additional layers of clothing likely alters the balance between E_{max} and E_{req}, resulting in an earlier inflection in deep body temperature (Belding & Kamon, 1973). If the efficiency of a heat loss pathway is reduced, it is logical that exercise tolerance in the heat whilst wearing clothing may also be negatively affected, if the heat is limiting. For example, Belding and Kamon (1973) have shown that a “moderate” clothing assembly (shorts, sock and shoes, and cotton khaki work shirt and trousers) reduced the critical ambient water vapour pressure which elicited an earlier inflection in deep body temperature (from 18.4 (3.8) to 11.4 (2.5) $W \cdot m^2 (m \cdot sec)^{0.6} \cdot mmHg$) compared to a semi-nude condition (shorts, socks and shoes) when exercising at a series of fixed external work rates between 123 and 198 $W \cdot m^{-2}$.

While comparisons of typical sporting assemblies have shown that some team sport players may be a higher risk of hyperthermia due to their clothing (Armstrong *et al*., 2010; Shin-ya, Nakai, Yoshida, & Takahashi, 2005), not all clothing assemblies have significant impact on thermoregulation (see Table 2.1). Low levels of clothing such as the addition of a short sleeved t-shirt (cotton or synthetic material) and cycling shorts compared to a semi-nude (swimsuit) in 30 °C 35 % RH did not measurably affect thermoregulatory responses or comfort when running, walking or resting post exercise (Gavin *et al*., 2001). However, it may be that the environmental conditions (alongside the exercise intensity) were not arduous enough to cause the participants to move out of the prescriptive or thermoregulatory zone, so no differences in T_{re} were to be expected. Additionally, sweat evaporation rates were nearly 100 % for all conditions (probably assisted by the forced convective cooling provided by fans) and sweat loss were similar across clothing conditions, this meant that skin temperatures were also similar. The relationship between clothing the upper boundary of the thermoregulatory zone is not entirely clear in regards to thermoeffector response and the 'physiological cost' of thermoregulation (see section 2.9).

Many fabrics have been introduced to the market with claims from the manufacturers' of improved evaporative characteristics, as a result of improved vapour permeability. This would lead to increased sweat evaporation, and therefore potentially increase the heat stress tolerated before the individual became uncompensable. Broadly, fabrics can be classified by fibre type (natural or synthetic), but yarn type, fabric construction, and applied finishes also influence heat transfer (Pascoe, Shanley, *et al.*, 1994). However, the majority of studies comparing natural with new synthetic fibres have found no difference in thermoregulation or clothing comfort (Davis & Bishop, 2013) see Table 2.1. Of the nine papers presented in Table 2.1, six estimated sweat loss by pre and post weight (either of the participant or of the clothing), and none measured skin blood flow. Therefore the dynamics of the sweating response throughout exercise (*i.e.* onset of sweating, peak sweat rate) cannot be determined and no comment can be made on the influence of the garments on the thermoeffector response of skin blood flow.

Table 2.1. Thermoregulatory comparison of natural versus synthetic fabrics worn during exercise in warm and hot environments

Study	**Garment**	**Protocol**	**Measures showing no difference**
(Stapleton, Hardcastle, & Kenny, 2011).	Four clothing ensembles: 1) cotton underwear and shorts; 2) Activewear (two-piece sweat wicking undergarment with long legs and long sleeves [93 % polyester, 7 % spandex]); 3) Coveralls and Cotton undergarments; 4) Coveralls and Activewear. Additionally in conditions 3 & 4 a hard hat with earmuffs, gloves, and socks with closed toe shoes were worn	n=8, 60 min of cycling at rate of heat production of 400 W followed by 60 min of recovery in a whole-body calorimeter at 40 °C, 15 % RH	T_c, $\bar{T}_{sk}$ & HR
(Brazaitis, Kamandulis, Skurvydas, & Daniusevičiūtė, 2010)	Comparison of two long-sleeved garments 1) 94 % cotton, 6 % elastaine; 2) 93 % polyester, 7 % elastaine.	n=8, 20 min exercise bouts of 8 km·hr^{-1} at 1 % grade, with 5 min rest between each, and then seated rested for 60 min, 25 °C, 60 % RH.	T_c, $\bar{T}_{sk}$ & HR
(Wickwire *et al*., 2007)	Comparison of two undergarments garments worn under a bullet-proof vest and standard clothing: 1) Synthetic fabric, short-sleeved t-shirt (80 % cationic polyester and 20 % elastane) (Under Armour; Baltimore, MD); 2) 100 % cotton short-sleeved t-shirt with high moisture regain (Life; Jamaica).	n=10, walking exercise on a treadmill at a speed of 4.8 km/h and 0 % grade for 12 min, then 10 biceps curls with 14.3 kg, this cycle continued for approximately 2 hrs WBGT 35.10 (0.37) °C, air velocity of 6.4 km·hr^{-1}	T_c, $\bar{T}_{sk}$ & HR
(Wingo & McMurray, 2007)	A comparison of: 1) long-sleeve synthetic shirt; 2) cotton shirt; 3) or no shirt all while wearing cotton sweatpants.	n=9, running on a treadmill at 65 % $\dot{V}O_{2peak}$ for 45 min in 22 °C	T_c, $\bar{T}_{sk}$ & HR

(Gavin *et al*., 2001)	Comparison of cotton and synthetic clothing ensembles (crew neck, short sleeve T-shirts, cycling shorts, and anklet socks) and a semi-nude condition which consisted of a Lycra swim suit, polyester socks, and running shoes.	n=8, 15 min seated rest, 30 min running at 70 % $\dot{V}O_{2max}$, 15 min walking at 40 % $\dot{O}_{2max}$, and 15 min seated rest, at 30 (1)°C and 35 (5) % RH.	T_c, $\overline{T}_{sk}$ & HR
(Heus & Kistemaker, 1998)	Comparison of traditional construction work outfit (cotton) with new outfit (coolmax and cordura).	n=6, cycling exercise work rest cycles at 30°C, 70 % RH, solar radiation 700 W.m-2.	T_c & $\overline{T}_{sk}$
(Kwon, Kato, & Kawamura, 1998)	Comparing traditional construction work outfit (cotton) with new outfit (coolmax and cordura).	n=6, cycling exercise work rest cycles at 30°C, 70 % RH, solar radiation 700 W.m-2.	T_c & $\overline{T}_{sk}$
(Roberts, Waller, & Caime, 2007)	Comparison of 'base-layer hot' garment, 'base-layer cold' garment, a 100 % cotton t-shirt and bare-chested.	n=7, intermittent treadmill protocol, 20.6 °C (0.2), 47.5 (7.7) %.	T_c only
(Kaplan & Okur, 2012)	Comparison of 5 different garments, 2 x polyester, 1 x 70/30 % cotton / polyester mix, 1 x 95/5 % polyester / elastane mix, 1 x 100 % cotton.	n=5, work rest cycles, 24 (0.5) °C and 60 (5) % RH	$\overline{T}_{sk}$ & HR

Abbreviations: T_c = deep body temperature, $\overline{T}_{sk}$ = mean skin temperature, HR = heart rate

Under certain conditions, clothing provides protection from extreme environments, such as those where substantial radiative heat gain would occur (Nielsen, 1990) or where the ambient temperature is in excess of the skin temperature, clothing may act as a barrier to heat gain. For instance when fire fighters wore protective clothing during a live firefighting exercise (mean [SD] ambient temperature 74 [42] °C), mean abdominal microclimate temperature was 32 °C between the skin and underclothing, compared to 38 °C mean chest skin temperature (Eglin, Coles, & Tipton, 2004). Thus, skin temperature was determined more by the deep body temperature (which on average was elevated to 38.5 °C) than the environment, due to the insulating properties of the clothing.

2.8.2 Aerobic fitness

Endurance training results in beneficial adaptations to the neuromuscular, metabolic, cardiovascular, respiratory and endocrine systems, which in turn manifest as improvements in the key parameters of aerobic fitness, namely $\dot{V}O_{2max}$, exercise economy and the lactate / ventilatory threshold (Jones & Carter, 2000). Another consequence of endurance training (long term) is improved thermoregulatory function, even when the training takes place in thermoneutral environments (Baum, Bruck, & Schwennicke, 1976; Buono & Sjoholm, 1988; Gisolfi & Robinson, 1969; Piwonka, Robinson, Gay, & Manalis, 1965; Selkirk & McLellan, 2001). Short term exercise training has also been shown to result in improvements in the capacity to dissipate heat (Henane, Flandrois, & Charbonnier, 1977; Ichinose, Inoue, Hirata, Shamsuddin, & Kondo, 2009; Okazaki *et al*., 2002). There are several mechanisms which may contribute to this improved thermoregulatory capacity including adaptations related to regular exposure to high core temperatures during exercise (Avellini, 1982), plasma volume expansion, lower resting T_c, raised threshold for endotoxin leakage and inflammatory activation during exertional heat stress (Selkirk, McLellan, Wright, & Rhind, 2008), and a reduction in the deep body temperature threshold for skin vasodilation and sweating activation (Henane *et al*., 1977). Taken together, these adaptations result in an attenuated cardiovascular response due to physiological adaptations similar to partial heat acclimatization leading to in enhanced heat dissipation and therefore tolerance of exercise in the heat. The similarity between the effects of physical training and that of heat acclimation have been summarised by Aoyagi, McLellan, and Shephard (1997 p194), see Table 2.2.

Table 2.2. Influence of physical training or heat acclimation on selected physiological, psychological and biophysical variables during submaximal, unskilled exercise in the heat while wearing minimal clothing (adapted from Aoyagi, McLellan, and Shephard, 1997, p194).

Variable	Physical training	Heat acclimation
$\dot{V}_E$	↓ or ↔ (for heavy or light exercise)	↓
$\dot{V}O_2$	↔	↓
$\dot{V}CO_2$	↓ or ↔ (for heavy or light exercise)	↓
RQ	↓ or ↔ (for heavy or light exercise)	↓
% $\dot{V}O_{2MAX}$	↓	↓
Sweat secretion	↑ or ↔	↑ or ↔
Plasma volume	↑	↑
T_c	↓ (during exercise)	↓ (at rest and during exercise)
$\overline{T}_{sk}$	↓	↓
HR	↓	↓
Subjective ratings	↓	↓
M	↔	↓
W	↔	↔
C_{res} and E_{res}	↓or ↔	↓
R, C and K	↑ or ↓(at $\overline{T}_{sk} > T_a$ or $\overline{T}_{sk} < T_a$)	↑ or ↓(at $\overline{T}_{sk} > T_a$ or $\overline{T}_{sk} < T_a$)
E_{sk}	↑or ↔ (in a dry or wet environment)	↑or ↔ (in a dry or wet environment)
S	↓	↓
Tolerance time	↑	↑

Abbreviations and symbols: C_{res} = rate of convective heat exchange through the respiratory tract; E_{res} = rate of evaporative heat loss from the respiratory tract; E_{sk} = rate of evaporative heat loss from the skin surface; HR = heart rate; M = rate of metabolic heat production; R, C, and K = rates of radiant, convective, and conductive heat exchange through the skin surface; RQ = respiratory quotient; S = rate of body heat storage; T_a = ambient temperature; T_c = core temperature; $\overline{T}_{sk}$ = mean skin temperature; $\dot{V}CO_2$ = rate of carbon dioxide production; $\dot{V}_E$ = rate of expiratory ventilation; $\dot{V}O_2$ = oxygen uptake; % $\dot{V}O_{2MAX}$ = relative work intensity (expressed as percentage of maximal aerobic power); W = rate of external work.; ↔ = unchanged value; ↑ = increased value; ↓ = decreased value.

2.8.3 Anthropometric considerations

A significant and often overlooked confounding issue with many studies comparing the thermoregulatory responses of trained and untrained individuals is the influence of mass, body surface area and body fat percentage. Havenith and van Middendorp (1990) have shown that the variables which have the greatest relative influence on the variance in heat storage and deep body temperatures between individuals were body fat percentage and surface area to mass ratio when exercising at 25 % and 45 % of $\dot{V}O_{2max}$. When creating mathematical models of human thermoregulation and comfort, it is known that that total body weight and surface area and segment dimensions affect the heat flow within the body and to the environment (Fiala, Lomas, & Stohrer, 1999; Huizenga, Hui, & Arens, 2001). This is because the physical characteristics of the human body affect the exchange and distribution of heat, *e.g.* taller and larger individuals have greater areas for dry heat exchange. (Havenith *et al*., 1998; Havenith, Luttikholt, & Vrijkotte, 1995) and the production of heat. Body mass and anthropometric characteristics govern the heat storage capacity of the body, influencing the threshold of heat gained / lost before deep body temperature changes with a given thermal challenge (Havenith, 2001b). Individuals with a greater body mass typically have smaller increases in deep body temperature during heat stress (Havenith *et al*., 1998, 1995). Because adipose tissue has a lower heat capacity compared with lean tissue (such as blood, muscle, water, and bone), variations in the relative amounts of fat *vs*. fat-free mass determine the specific heat capacity of the human body, defined as the amount of heat energy required to increase 1 kg of body weight by 1 °C. Individuals with a greater relative amount of fat mass have a greater change in core temperature for a given change in body heat content if they are the same total mass (Cheung *et al*., 2000). However, whether exercise is weight bearing or non-weight bearing also needs to be considered because heat production is independent of mass during non-weight bearing exercise (Selkirk & McLellan, 2001), therefore during weight supported exercise, *e.g.* cycling, a larger body mass would represent a thermal advantage.

Havenith *et al.* (1998) noted that $\dot{V}O_{2peak}$ and body mass are strongly correlated, meaning the effects of mass and fitness on deep body temperature cannot be fully separated even when using a multiple regression approach (Havenith *et al*., 1995; Havenith & van Middendorp, 1990), and so if body mass is not accounted for when comparing different groups, it may affect the outcome (Jay *et al*., 2011). In response to this problem, Jay *et al.* (2011) compared two independent groups matched for body mass, body surface area (BSA), sex, age, acclimation status, and ethnicity, but with substantial differences in fitness ($\dot{V}O_{2peak}$), during exercise at the same absolute heat production (520 W) and a relative exercise intensity of 60 % $\dot{V}O_{2peak}$. They demonstrated that independent of metabolic heat production, body mass and BSA, the large differences in $\dot{V}O_{2peak}$ did not influence changes in deep body temperature or sweating during exercise at a fixed rate of metabolic heat production. Additionally, when mass and BSA are similar, exercise at the same relative intensity resulted in a much greater change in core temperature and sweating in the trained group due to differences in heat production (834 W *vs*. 600 W). At present the influence of aerobic fitness on the critical inflection point in T_c is unclear, but any such investigation must ensure that mass and BSA are not different between comparison groups.

2.9. Physiological cost of thermoregulation

Methods for quantifying physiological strain experienced by the human during exercise in the heat typically include some measure of deep body temperature, such as the physiological strain index (Moran et al., 1998) or cumulative heat strain index (Frank, Belokopytov, Shapiro, & Epstein, 2001). However, as deep body temperatures at a given work rate have been shown to be independent of ambient temperature within the prescriptive zone (Lind, 1963a), the use of scales that emphasise the measurement of a deep body temperature (usually rectal temperature) may not be as useful or discriminatory in these conditions as they are under conditions of uncompensable heat stress (Gonzalez *et al*., 1997). Given that during prolonged exercise athletes will typically self-select work rates that allow thermal compensability in their environment (Schlader, Stannard, & Mündel, 2011), this may apply to many typical exercise scenarios. Indeed, failure to stay within the thermoregulatory zone would result in an inexorable rise in deep body temperature until exercise termination (González-Alonso *et al*., 1999).

Macpherson (1962) and Budd (2008) highlight the requirement for careful selection of heat strain indices during experimentation, to ensure the relevance and specificity of the index to the intended use. Under compensable conditions, increases in environmental temperature are balanced by increased thermoeffector responses, which are necessary to achieve thermoregulatory balance. This will likely manifest in an increased heart rate (Givoni & Goldman, 1973), sweat rate (Sawka, Montain, & Latzka, 2001), oxygen uptake (Arngrímsson *et al*., 2003) and skin blood flow (Charkoudian, 2003). However, this 'physiological cost' of thermoregulation is seldom considered. When this occurs, deep body temperature may be similar between conditions and differences in the magnitude of thermal strain will solely be reflected in effector output (Corbett *et al*., 2014). Therefore, if the participant remains within the thermoregulatory zone (through self-pacing or fixed by research design) the quantification of thermal strain by measures of deep body temperature alone, may lack the ability to adequately discriminate between the groups, conditions or garments. However, it is known that increased thermoeffector responses are associated with impaired aerobic performance due to their increased energetic and water costs (Sawka, Cheuvront, & Kenefick, 2012). These effector responses can be regarded as the 'equivalent compensatory effort' (Frank, Belokopytov, Shapiro and Epstein 2001) or 'physiological cost' (Corbett *et al*., 2014; Lind, 1963b) of achieving thermoregulation in the thermoregulatory zone. Comparing the magnitude of the 'physiological cost' required to achieve thermoregulation is therefore a viable method to assess heat strain within the thermoregulatory zone.

2.10. Summary

The thermoregulatory zone defines the upper and lower boundaries of human thermoregulation and has important applications in work and sport. Therefore, it is important to be able to identify these boundaries in a reliable and valid manner and to understand the factors influencing them. The studies reported in this thesis have focused on the hyperthermic boundary. Because humans regulate their deep body temperature closer to the lethal hyperthermic temperature than the lethal hypothermic limit, previous literature has often been concerned with the measurement of deep body temperature at the point of 'failure', the rate of rise of deep body temperature over the exposure, and quantifying tolerance times. However, this literature review has identified inconsistencies in the terminology used to define the upper boundary of the thermoregulatory zone and has highlighted potential weaknesses with the methodologies employed previously to find the point of uncompensability. In particularly, it is unclear whether the continuous-type protocols that are commonly employed evoke a 'true' uncompensable state, or if the inflection in deep body temperature seen in these studies are artefacts of the continuously rising ambient temperature or humidity. Additionally, the reliability of these protocols and the associated thermal and thermoeffector responses is poorly understood.

There has been substantial focus on the physics of the environment and heat exchange in the transition from compensable to uncompensable conditions. Taken together, this gives a somewhat distorted view of thermoregulation and often misses the key aspect of the physiological thermoeffector response. Indeed, in this literature review it has been postulated that there may be instances of sub-maximal rates of skin blood flow and sweat rate at the upper limit of the thermoregulatory zone, if there has not been a sufficient stimulus to evoke thermoafferant drive for maximal thermoeffector response (as seen in hypothermia). This hypothesis is in contrast to some didactic models of thermoregulation, which equate maximal thermoeffector response with thermoregulatory 'failure' and the transition to uncompensability. Accordingly, one of the primary aims of this thesis was to provide insight into the thermoeffector responses at the point of uncompensable heat stress and beyond.

Finally, it is clear that there still remain a number of gaps in the collective knowledge as to how key parameters such as, clothing, environment (humidity and air temperature), work rate and fitness separately or in concert, influence the transition from compensable to uncompensable conditions at the upper limit of the thermoregulatory zone. Previous work has mainly focused on the 'critical environment' for a range of adults and children (Belding & Kamon, 1973; Dougherty *et al*., 2010; Kamon *et al*., 1978; Kamon & Avellini, 1976; Kenney & Zeman, 2002), but no measure of thermoeffector response (*i.e.* local sweat rate or skin blood flow) has been reported. Therefore, important insights as to *why* a participant became uncompensable often remain without a clear physiological mechanism; it is then inevitable that authors will focus on the physics of the environment rather than the physiology of the human within that environment.

3. Chapter Three – General Methods

The general methods used for the experiments in this thesis are described in this section, with specific details relevant to each particular study presented in the appropriate chapter.

3.1. Ethical considerations

All experiments detailed in this thesis complied at all times with the Declaration of Helsinki (World Medical Association, 2013). All experimental protocols were peer-reviewed within the Department of Sport and Exercise Science and given favourable opinion, prior to commencing the research, by the University of Portsmouth BioSciences Research Ethics Committee (BSREC) and its successor committee, the Science Faculty Ethics Committee (SFEC), favourable opinion codes were: BSREC 12/050b, BSREC 12/077b, SFEC 2014-041b and SFEC 2014-043a (Appendix A and E).

3.2. Participants

3.2.1 Informed consent

Written informed consent was obtained from each volunteer participant after they had read a participant information sheet and received a full verbal briefing by the experimenter informing the individual of the purpose, procedure and known risks associated with the study. In the majority of cases, individuals were able to visit the laboratory to see the experimental set up and the equipment to be used prior to volunteering. It was made clear that they could withdraw from the study at any point, without reason and without penalty or coercion.

3.2.2 Inclusion and medical criteria

Healthy, 18 to 39 year old males participated in the experiments described in this thesis. The female menstrual cycle hormonal fluctuations may alter thermosensitivity in skin regions (Golja, Tipton, & Mekjavić, 2003) and sudomotor activity during exercise (Gagnon & Kenny, 2012), causing dissimilar thermoregulatory performance (Havenith, 1997). Consequently, females were not recruited for the experiments detailed in this thesis. Participation was conditional upon the completion of an Exercise and Health History Questionnaire (Appendix B) with no contraindications. Participants with health queries or who were aged 30 years or older were only permitted to participate after receiving approval from an independent medical officer (IMO). Contraindications for participation included:

- history of collapse on exertion
- history of heat intolerance
- anaemia (or blood donation in the week prior to the study)
- vascular disease
- ischaemic disease
- family history of early onset cardiac disease or a respiratory disease (obstructive or restrictive)
- regular or recent intake of drugs or medications which may affect thermoregulation
- musculoskeletal issues that would affect exercise performance

In addition, all of the participants were non-smokers and non-heat acclimatised, as heat acclimatisation enhances heat dissipation and therefore tolerance of exercise in the heat (Aoyagi *et al*., 1997).

3.2.3 Dietary considerations and pre-experiment guidance

Prior to all experiments, participants were asked to refrain from exercise and caffeine for 24 hours and alcohol for 48 hours before testing. Participants were also instructed to consume 500 mL of water two hours before arriving at the laboratory and to record their dietary intake in the 24 hours before the first experiment and to replicate this on the day prior to the subsequent experimental condition

3.2.4 Hydration status and maintenance throughout testing

The specific gravity of urine samples were tested in Chapters Five, Six, Seven and Eight by reagent strips for urinalysis (DUS 10 Health Mate Urinalysis Multisticks, DFI Co Ltd, South Korea), adequate hydration was assumed at values ≤1.020 (Casa *et al*., 2000). During the experiment reported in Chapter Four, participants were free to drink a 6 % carbohydrate plus electrolytes sports drink (SiS Go Electrolyte Lemon & Lime Flavour, Science In Sport PLC, Lancashire, UK) *ad libitum* throughout the exercise trial, with the mass consumed measured to enable calculation of whole body fluid losses. In chapters Five to Eight however, the participants were given 200 mL of the same 6 % carbohydrate plus electrolyte sports drink every 20 minutes at a set mean (SD) temperature of 20 (0.05) °C, with the total amount consumed recorded and any urine passed weighed to enable calculation of whole body fluid losses.

3.2.5 Anthropometry

During the familiarisation session prior to each study the participants' height and mass were measured using a stadiometer (Harpenden stadiometer, Holtain, UK) and digital electronic scales (Industrial Electronic Weight Indicator, Model I10, Ohaus Corporation, NJ, USA) respectively. Body surface area was calculated using the equation by DuBois and DuBois (1916), additionally, body fat percentage was estimated in the studies presented in Chapter Five, Six, Seven and Eight. This consisted of the seven-site skinfold method used by the American College of Sports Medicine (2000) (chest, midaxillary, triceps, subscapular, abdomen, suprailiac and thigh) with the addition of the biceps to allow for the traditional four-sites calculation (biceps, triceps, subscapular and supraílliac) (Durnin & Rahaman, 1967; Durnin & Womersley, 1974). All measurements were obtained in a private changing room using Harpenden skin fold callipers (Baty International, West Sussex UK) and all sites were measured three times and the median taken.

3.3. Control of environmental conditions

The ambient temperature and humidity within the 62 m^3 climate-controlled chamber was controlled and maintained during each experiment (with the exception of maximal exercise testing – see 3.7), either automatically or manually and monitored by the technical staff during each experiment. When automatically controlled, the environmental chamber utilised a three-mode controller (proportional, derivative and integral) to optimise stability and response. The existing control system for the environmental chamber was designed and optimised for stable conditions. As such, controlled changes of temperature and / or humidity at a prescribed rate during a test were not possible without manual intervention. For this reason, control of the temperature was undertaken manually by highly experienced technical staff who adjusted the heating and cooling as necessary to produce the temperature ramp called for by each protocol (please see 5.3.1 for the reliability of the environmental conditions).

In the chamber, air is discharged horizontally from the back wall and returned through a vent in the ceiling. Air velocity in the vicinity of the cycling participants was measured at < 0.01 $m \cdot s^{-1}$, no forced convection was provided to the participant in any of the studies within this thesis. Additionally, when low humidity conditions were required, a non-integrated standalone dehumidification system (ML1100, Munters AB, Sweden) was used. A wet bulb globe thermometer (°C) (Edale Instruments [Cambridge] Ltd, Longstanton, UK) was used to monitor the ambient temperatures (wet, dry and globe) within the chamber - located near to the participant - independently of the chamber monitoring system and the data were recorded on to a data logger (Q800 series, Grant Instruments, UK) once every 60 seconds.

3.4. Pre- instrumentation procedure

On arrival at the laboratory before an experimental condition, the participants were asked to describe their general health since their last visit or the last week and verbally confirm if they had followed the pre-experiment guidance. The participant then moved to a private changing room to void their bowels and have their naked body mass measured and any non-issued clothing (shoes and underwear) weighed (Industrial Electronic Weight Indicator, Model I10, Ohaus Corporation, NJ, USA). Prior to the arrival of the participant all issued clothing (trousers, jersey, shorts, socks) was weighed on the same scales.

3.5. Physiological measurements

3.5.1 Rectal temperature (T_{re})

Prior to all experiments, participants were asked to self-insert a rectal thermistor (REC-UU-VL4-0, Grant Instruments (Cambridge) Ltd, UK) Ltd, UK) 15 cm past the anal sphincter in a private changing room, after voiding their bowels. The rectal thermistor has a reported accuracy of 0.1 °C (Grant Instruments, n.d.). The accuracy was assessed prior to each study using a small heated water bath (Grant Instruments Ltd., Cambridge, UK) which changed temperature in 0.5 °C increments within the range expected in the experiment (36 to 40 °C). The temperature of the water bath was monitored with a thermometer (Digitron thermometer T600, RS calibration, UK) certified to British standards to an accuracy of 0.01 °C. Thermistors were not used if they deviated more than 0.1 °C from the calibrated thermometer reading. Each rectal thermistor was sterilized in an appropriate solution as per the manufacturer's instructions (Haztabs, Edenbridge, Kent, UK) between experiments, and each participant used the same thermistor for all of their conditions. Data were sampled every second to a data logger (Q2040/2020 series, Grant Instruments (Cambridge Ltd), UK) then subsequently imported into an Excel spreadsheet (Microsoft, US) and averaged for each minute.

T_{re} was selected as our criterion measure of T_c from Chapter Five onwards because Chapter Four had shown T_{au} to be influenced by ambient temperature and because the majority of previous studies investigating the upper boundary of the thermoregulatory zone (with adults) have used this measure (Belding & Kamon, 1973; Kamon *et al.*, 1978; Kenney *et al.*, 1987, 1988, 1993) and because few of the participants were able to tolerate the oesophageal probe.

3.5.2 Insulated auditory canal temperature (T_{au})

An aural thermistor (EAR-UU-VL4-0, Grant Instruments Ltd, Cambridge, UK) was placed by the participant in the auditory canal of their choosing (right side in all cases) and insulated from the outside environment by a bespoke Otoform ear mould made of silicone putty (Otoform K2, Dreve Otoplastik GmbH, Germany), non-absorbent cotton wool, Transpore tape (3M, USA) and SurgiFix netting (FRA productions, Italy), see Figure 3.1.

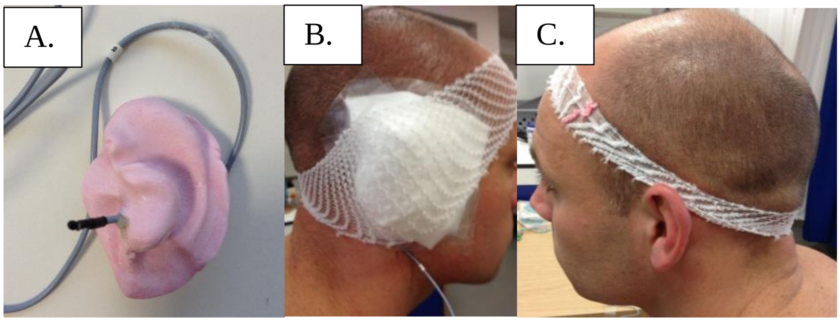


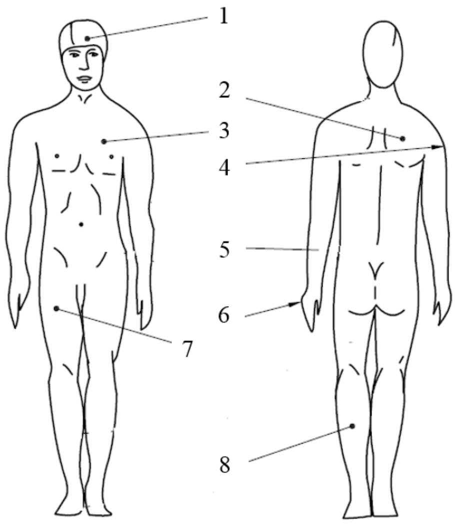
Figure 3.1. Photograph of A. the aural thermistor within the bespoke Otoform ear mould, B. the right and C. the left side of a participant's head once the aural thermistor is in place.

T_{au} was included in addition to T_{re} in all studies as it has been shown to respond more rapidly to changes in deep body temperature than T_{re} (Gagnon, Lemire, Jay, & Kenny, 2010). Accuracy of the aural thermistors was assessed alongside the rectal thermistors, and they were not used if they deviated more than 0.1 °C from the calibrated thermometer reading. Each aural thermistor was cleaned using disinfection wipes (Premi-Wipes, Premier, UK) between experiments, and each participant used the same thermistor for all of their conditions. Data were sampled every second to a data logger (Q2040/2020 series, Grant Instruments Ltd, Cambridge, UK) then subsequently imported into an Excel spreadsheet (Microsoft, US) and averaged for each minute.

3.5.3 Mean skin temperature ($\overline{T}_{sk}$) and heat flux (HF)

Participants were instrumented with eight combined skin temperature and HF transducers (FM-060-TH44033-F6, Concept Engineering, USA), secured using Tegaderm dressing (3M, USA) and Transpore tape (3M, USA) (Figure 3.2) at the anatomical locations described in BS EN ISO 9886 (2004), as shown in Figure 3.3.

Figure 3.2. Photograph of skin thermistors secured to the skin using Tegaderm dressing (over the thermistor) and Transpore tape (borders of Tegaderm).



1. Forehead ($T_{forehead}$)
2. Right scapula ($T_{scapula}$)
3. Left upper chest (T_{chest})
4. Right arm in upper location (T_{uparm})
5. Left arm in lower location (T_{lowarm})
6. Left hand (T_{hand})
7. Right anterior thigh (T_{thigh})
8. Left calf (T_{calf})

Figure 3.3. Eight skin temperature sites (BS EN ISO 9886, 2004).

The combined HF and skin thermistors have a reported accuracy of ±5 %, which was assessed in the same manner as the rectal and aural thermistors, within the temperature range of 25 °C to 42 °C, and were not used if they deviated by more than 0.2 °C from the calibrated thermometer reading. Additionally, each HF sensor has an individual calibration correction factor provided by the manufacturer which was applied using an Excel spreadsheet (Microsoft, US) at the point of data analysis. Each sensor is traceable to the US National Institute of Standards and Technology (NIST) using the reference - SRM1450. Each thermistor was sterilized using disinfection wipes (Premi-Wipes, Premier, UK) between experiments, and each participant used the same thermistor for all of their conditions in each skin location. Data were sampled every second to a data logger (Q2040/2020 series, Grant Instruments Ltd, Cambridge, UK) then subsequently imported into an Excel spreadsheet (Microsoft, US) and averaged for each minute.

3.5.4 Skin blood flow (SkBF)

SkBF was measured using laser Doppler flowmetry (LD) and using multi-fibre probes (VP1/7, MoorLab, Moor Instruments Ltd., Axminster, UK). A Doppler shift occurs when a waveform (light or sound) strikes a moving object and the wavelength (frequency) of the reflected wave changes in proportion to the velocity of the moving object (Choi & Bennett, 2003). In the context of skin blood flow measurement, the shift in frequency of laser light can be used to calculate velocity of the blood cells from the reference frequency of the laser light emitted from the probe. This laser light passes through the skin and reflects back to the probe from the red blood cells in the cutaneous circulation, the intensity of that reflected light indicates the concentration of blood cells. Combined, concentration and velocity give an estimate of flux. Flux measurements were taken at the index finger and left dorsal forearm (halfway between wrist and elbow) by attaching the probes to the skin with tape (TegadermTM Film, 3M, UK). The laser Doppler probe was calibrated before each experiment using a Probe Flux Standard solution (Moor instruments, UK). The Brownian motion of polystyrene micro-spheres of this solution provided the standard reference for calibration of the probes. Probes were submerged and clamped in the solution for 60 seconds, with expected values of approximately 200 Flux Units (FU), the calibration function was then selected through the menu on the main satellite, and once completed the probe was removed and wiped clean. The probe was then re-submerged in the fluid to check that the measured values were approximately 200-220 FU. If the reading was outside this range, the probe was checked for damage / dirt / lint and re-calibrated. If the probe had failed calibration again, it would not be used in further experimental work until successful repair.

During the experimental set up period, before each condition, a ‘biological zero’ of forearm and finger blood flow for each participant was determined by inflating a manual sphygmomanometer cuff to 250 mmHg to momentarily occlude the brachial artery and manual occlusion of the 2nd finger, respectively whilst measuring LD SkBF. Each probe was cleaned using disinfection wipes (Premi-Wipes, Premier, UK) between experiments, and each participant used the same probe for all of their conditions in each sampling location. During testing, data were sampled using an analogue to digital converter (Powerlab; AD Instruments, Ltd., UK) every second to a laptop computer. All data were imported into an Excel spreadsheet (Microsoft, US) and averaged for each minute.

3.5.5 Heart rate (HR)

HR was measured using a short range telemetry heart rate monitor (Polar RS800, Electro OY, Polar, Warwick, UK) positioned around the chest and recorded every 5 seconds. After use, the strap was washed in warm soapy water and hung to dry as suggested in care instructions. Data were downloaded using the Polar software (Version 5) and then imported into an Excel spreadsheet (Microsoft, US) and averaged for each minute.

3.5.6 Sweat rate (SR) – ventilated sweat capsule

Sweat rate was measured using ventilated sweat capsules (Q-Sweat Quantitative Sweat Measurement System, Model 1.0, WR Medical Electronics Co., Minnesota, USA) (Figure 3.4). The Q-Sweat does not require calibration by the operator as calibration is completed by the manufacturer. However maintenance involves refreshing the desiccant in the systems as necessary, checking the capsules for air leaks and cleaning the capsules. Sweat output measurement is based upon comparison of dried (by desiccant) room air and returned air from the skin sites: measured by temperature and relative humidity sensors (Honeywell International Inc, Morristown, USA) controlled by the WR TestWorks software, version 2.2. Each measurement area was 0.787 cm^2 with a dry air flow rate of 60 standard cubic centimetres per minute (SCCM*)*. Sweat volume calculations were derived from rate and time, and within the range of 0 to 1000 nanolitres per minute ($nL \cdot min^{-1}$), have an accuracy of 5 %, repeatability of 5 %, and sensitivity of 0.1 nanoliters (nL) (WR Medical Electronics Co., 2011). Measures were converted to $L \cdot m^2 \cdot min^{-1}$. The measurement location for all experiments was the right-side of the back, held in place by threading the capsule through the heart rate monitor strap (see Figure 3.4) and additionally for Chapter’s Five to Eight, the dorsal aspect of the right forearm held in place with a plastic strap. Each capsule was cleaned using disinfection wipes (Premi-Wipes, Premier, UK) between experiments, and each participant used the same probe for all of their conditions in each sampling location.

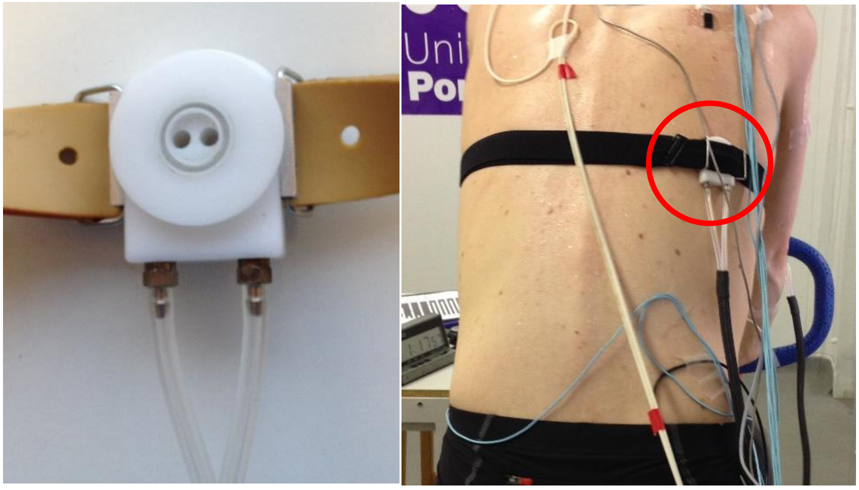


Figure 3.4. Left photo- Q-Sweat capsule. Right photo- Q-Sweat capsule fed through heart rate monitor strap on right side of body (circled).

3.5.7 Oxygen uptake ($\dot{V}O_2$)

The Douglas bag method was employed to measure pulmonary gas exchange because this is considered the gold standard measurement of oxygen uptake (Winter, Jones, Davison, Bromley, & Mercer, 2007), the environmental conditions in the experiments were outside the operational limits of the available online systems and the experiments did not require the investigation of $\dot{V}O_2$ kinetics which are available using a breath by breath system. All Douglas bags were checked for leaks and fully evacuated prior to use. Expired gas samples were obtained at set intervals during the experiments (refer to the individual chapters for details) and were typically collected for approximately 60 seconds during exercise or 300 seconds during rest though a two-way non-rebreathing valve (T-shape configuration 2700, Hans Rudolph, Inc, USA) which was connected to a 150 L capacity Douglas bag through respiratory tubing; collection duration (from inspiration to inspiration) was timed with a stopwatch.

Gas volume (Dry Gas Meter, Harvard Apparatus, UK), fractions of carbon dioxide (CO_2) and oxygen (O_2) (Rapidox 100, Sensotec Limited, Cambridge, UK), temperature (Electronic thermometer, UK) and pressure (Fortins Mercury Barometer, Russell Scientific Instruments, UK) were recorded. All $\dot{V}O_2$ data are presented as standard temperature, pressure, dry (STPD). The O_2 and CO_2 analyser (Rapidox 100, Sensotec Limited, Cambridge, UK) was calibrated with a 2-point calibration using fresh (external to the laboratory) air (20.93 % O_2 & 0.03 % CO_2) and a calibration gas with approximate values of 15 % O_2, 5 % CO_2, but certified to the actual value to within 0.01 % (BOC Special Gases, UK). These calibration gases were above and below expected values that might be encountered with exercise and rest. The analyser was calibrated and desiccant was refreshed before each use and on a few occasions, during, a test. The valves, respiratory tubing and nose clips were sterilized in an appropriate solution (Haztabs, Edenbridge, Kent, UK), in accordance with the manufacturer instructions.

3.6. Perceptual measurements

A full verbal explanation and description of the scales was given to the participants in the familiarisation session and briefly repeated prior to each experimental condition. At set intervals during each experiment (dependant on the study), participants completed three 20 cm visual analogue scales (VAS): Thermal Sensation, Thermal Comfort (Zhang, Huizenga, Arens, & Wang, 2004) and Skin Wettedness (Rissanen, Smolander, & Louhevaara, 1991) shown in Figures 3.5 to 3.7 respectively and one category- ratio scale: Rating of Perceived Exertion (RPE) (Borg, 1982) shown in Figures 3.8.

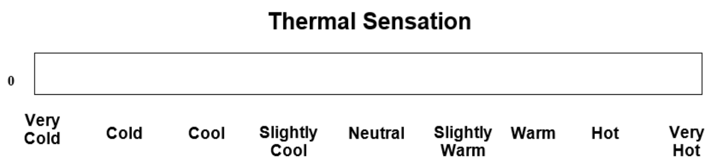


Figure 3.5. A 20cm visual analogue scale to evaluate perception of 'Thermal Sensation' (not to scale)

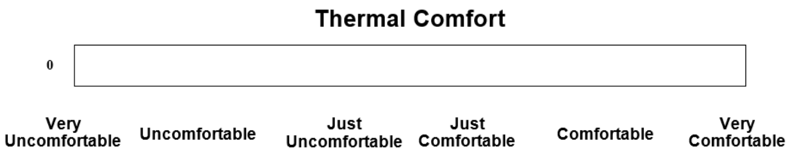


Figure 3.6. A 20cm visual analogue scale to evaluate perception of 'Thermal Comfort' (not to scale)

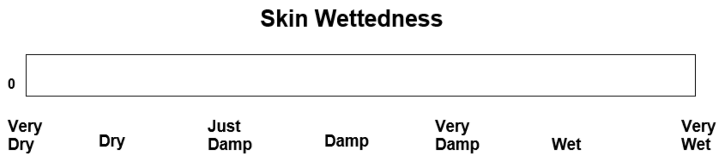


Figure 3.7. A 20cm visual analogue scale to evaluate perception of 'Skin Wettedness' (not to scale)

THE RATING OF PERCIEVED EXERTION	
6	14
7 VERY VERY LIGHT	15 HEAVY
8	16
9VERY LIGHT	17 VERY HEAVY
10	18
11 LIGHT	19 VERY VERY HEAVY
12	20
13 SOMEWHAT HEAVY	

Figure 3.8. A category-ratio scale to evaluate 'Rating of Perceived Exertion'

During the experimental set up period the participants were briefed on the VAS scales and instructed to place a clear line on the position on the scale which best described their appraisal of their whole body, for the RPE scales they were instructed to pick a whole number only. During experiments, laminated versions of scales were held in front of participants and they either marked the scale with a straight line at the location that described their perception for each scale with a washable marker or pointed to the location. On the occasions where two participants were in the chamber at the same time, care was taken to ensure neither participant could see the perceptual responses of the other and no scored were verbalised by the PI. The location of the mark was measured using a standard ruler (cm) from the left edge (0 cm), or in the case of RPE, the number chosen by the participant was noted. After recording the score on the data collection sheet, the washable mark was erased so the participants were not given any indication of their last response when next reporting their subjective status.

3.7. Exercise testing

Prior to the experiments reported in Chapters Four, Five, Six and Eight, participants performed an incremental exercise test to exhaustion on a Monark cycle ergometer (874E Exercise Ergometer, Monark Exercise AB, Sweden) at typical room temperature (~20-21 °C and ~45-50% RH) to quantify their aerobic fitness and in the case of Chapter Six, determined their suitability for inclusion in the study. The test began with a self-paced five minute warm-up, plus additional time for stretching if requested, followed by four, five minute sub-maximal stages (details of which can be found in Table 3.1). Two consecutive ~ 60 second Douglas bag collections were taken in the final two minutes of each stage.

Immediately following completion of the sub-maximal portion of the test, the external work rate was increased (by the same amount as in the submaximal stages) every 60 seconds until the participant could not maintain 60 (Chapter Four, Five, Six and Seven) or 70 (Chapter Eight) $revs.min^{-1}$ or reached volitional exhaustion. The participants were asked to indicate to the experimenter when they thought they were approximately two minutes away from exhaustion; at this point Douglas bag collections were taken until the end of the test. Maximal oxygen uptake ($\dot{V}O_{2max}$) was defined as the highest value attained during the test, analysed retrospectively from the gas collected in the Douglas bags, as described previously (3.3.7). Maximum HR was defined as the highest value attained during the test as recorded by the RS800 HR monitor (Electro OY, Polar, Warwick, UK).

Table 3.1. Details of the submaximal stages of the incremental test to exhaustion reported in different thesis chapters

Chapter	Cadence maintained ($revs.min^{-1}$)	Weight added at each submaximal stage (kg)	Work rate at each submaximal stage (W)
Four	70	0.3	70, 91, 112, 133
Five	60	0.3	60, 78, 96, 114
Six	60	0.3 / 0.5 depending on current training load	60, 78, 96, 114 / 60, 90, 120, 150
Seven	60	0.3 / 0.5 depending on current training load	60, 78, 96, 114 / 60, 90, 120, 150
Eight	60	0.3 / 0.5 depending on current training load	60, 78, 96, 114 / 60, 90, 120, 150

For the study presented in Chapter Four, linear regression of the work rate *vs*. $\dot{V}O_2$ relationship and $\dot{V}O_{2\ max}$ was used to calculate the power required to elicit an oxygen uptake equivalent to 40 %, 50 %, 60 % and 70 % of $\dot{V}O_{2\ max}$.

3.8. Exercise mode

All studies used a cycle ergometer (874E Exercise Ergometer, Monark Exercise AB, Sweden), and participants maintained cadence at either 60 (Chapter Five, Six, Seven and Eight) or 70 (Chapter Four) $revs.min^{-1}$ throughout the experiment. The experimenter monitored the cadence on the ergometer's digital display and participants were aided with the use of a metronome. Although cadence remained fixed, external work rate (W) was participant, condition and study dependent (refer to the relevant methods section for each Chapter).

3.9. Experimental end-points

Experimental end-points were broadly the same across all experiments and included:

a) on the request of the participant
b) on the decision of the principal investigator, supervisor or first aider
c) core body temperature as measured by the aural and / or rectal thermistor of >39.5 °C or, if on reaching 39.0 °C, an increase of > 0.15 °C over two consecutive five minute readings
d) participant complained of dizziness or light headedness
e) if heart rate exceeded the maximum heart rate measured at the end of the of the $\dot{V}O_{2\,max}$ test or age predicted maximum (220 minus age), whichever was greater
f) if the participant was unable to continue pedalling in a controlled manner to the required cadence
g) at the end of the protocol

For Chapters Five, Six, Seven and Eight:

h) if any skin temperature reached 42 °C
i) when the ambient dry bulb temperature reached 50 °C

3.10. Calculations

Mean skin temperature ($\overline{T}_{sk}$) in °C was calculated using the following formula from eight skin sites (BS EN ISO 9886, 2004) described in section 3.4.3:

$$\overline{T}_{sk} = (0.07 * [T_{forearm} + T_{uparm} + T_{lowarm}]) + (0.175 * [T_{scapula} + T_{chest}]) + (0.05 * T_{hand}) + (0.19 * T_{thigh}) + (0.2 * T_{calf})$$ *Equation 3.1*

The same weightings were also used to calculate mean HF.

Mean body temperature ($\overline{T}_b$) in °C was calculated using the formula by Colin, Timbal, Houdas, Boutelier, & Guieu (1971) to reflect the importance of the thermal input from the $\overline{T}_{sk}$ in the experiments.

$$\overline{T}_b = (0.79\ T_{re} \text{ or } T_{ac}) + (0.21\ \overline{T}_{sk})$$ *Equation 3.2*

Measurement of body mass loss (in kg) and estimation of sweat production and evaporation (in $L \cdot hr^{-1}$) were calculated according to Parsons (2003).

Total mass loss = $Nude_{pre}$ - $Nude_{post}$ *Equation 3.3*

Estimated sweat production = Total mass loss + Fluid consumed – Urine output

Equation 3.4

Estimated sweat evaporated = ($Nude_{pre}$ - $Nude_{post}$) – ($Clothed_{post}$ – $Nude_{post}$) – ($Clothed_{pre}$ – $Nude_{pre}$) + Fluid consumed – Urine output *Equation 3.5*

Estimated evaporation-production % = Sweat evaporated / Sweat production * 100

Equation 3.6

Body surface area (m^2) was calculated from the simplified DuBois and DuBois (1916) equation (Parsons, 2003):

$A_D = 0.202 * W^{0.425} * H^{0.725}$ *Equation 3.7*

Where A_D = DuBois surface area (m^2), W = Weight of the body (kg) and H = Height of body (m).

Relative humidity (Parsons, 2003)

Relative humidity (%) = P_a / P_{sa} *Equation 3.8*

Where P_a (kPa) is the prevailing partial pressure of water vapour and P_{sa} (kPa) is the saturated vapour pressure at a given temperature.

Saturated vapour pressure (kPa) was calculated using Antoine's equation (Parsons, 2003)

P_{sa} = exp (18.956 – (4030.18 / (temperature +235)) *Equation 3.9*

Energetic equivalent of oxygen (in Watt hours per litre of oxygen) was calculated to determine metabolic rate from oxygen consumption was calculated from ISO 8996 (Malchaire, 2004).

EE= (0.23 * RQ + 0.77) * 5.88 *Equation 3.10*

Where RQ is the respiratory quotient and is the product of $\dot{V}CO_2$ divided by $\dot{V}O_2$

Metabolic heat production (M) ($W \cdot m^{-2}$) was then calculated (Malchaire, 2004)

$M = EE * \dot{V}O_2 * (1 / A_D)$ *Equation 3.11*

3.11. Statistical analyses

Results of all studies are expressed as mean and standard deviation (SD) or median and ranges for non-normally distributed data or non-scale data. Statistical analyses were conducted using SPSS for Windows version 20-21 (IBM, USA) with an α value of 0.05. Normal distribution of the data was assessed by the Shapiro-Wilk test and specific statistical analyses performed are detailed with the individual experimental chapters.

Briefly, reliability statistics (Chapter Five) were used to establish the level of agreement between conditions by calculating the typical error of the mean and coefficient of variation (Hopkins, 2000a). Where tests of differences were required and the data met parametric assumptions, a range of ANOVAs were performed. In Chapters Six and Seven Mauchly's test of sphericity was assessed as there were more than two levels (multiple time points), where sphericity could not be assumed, the Greenhouse-Geisser corrected value is reported. In the case that parametric assumptions were not met, within participant differences were tested with a Wilcoxon or Friedman test and between participants differences were tested with a Mann Whitney U or Kruskal-Wallis test. Unless explicit, non-significant pair-wise analyses are not reported. On occasion, data were not obtained due to technical error or equipment malfunction, where this occurred, the number of participants used for analyses is reported.

4. Chapter Four – Influence of relative exercise intensity, environment and clothing on the physiological cost of thermoregulation and upper boundary of the thermoregulatory zone.

4.1. Introduction

It has long been known that over a wide range of environmental temperatures, the equilibrated deep body temperature (T_c) of an exercising human is independent of environmental temperature and is instead dependent on the external work rate performed (Nielsen, 1938); this has been termed the ‘prescriptive’ zone (PZ) (Lind, 1963a). Increases in environmental temperature beyond the PZ result in a raised rectal temperature (T_{re}) for a given work rate. Importantly, a plateau in T_{re} may still be achieved, albeit following a delay and at a higher temperature (see Figure 2.3). However, there appears to be some inconsistency in the application of this terminology as classically defined, with some authors associating moving out of the ‘prescriptive’ zone with uncompensable heat stress (Kenney *et al*., 2004). Uncompensable heat stress occurs when the rate of endogenous heat production and environmental heat gain exceeds the capacity to lose heat to the environment. Under these conditions thermal equilibrium is not achieved and T_c rises inexorably (Kraning & Gonzalez, 1991), often to the point of exhaustion (González-Alonso *et al.*, 1999). The combination of environmental conditions and work rate immediately preceding the point of uncompensable heat stress defines the upper boundary of the ‘thermoregulatory zone’ (TZ) (Mekjavić & Bligh, 1987), which characterises the upper limit of a range of environmental and exercise conditions in which individuals can achieve thermal balance. The TZ incorporates the ‘metabolic regulatory’, ‘thermoneutral’, ‘vasomotor’ (‘dead band’, ‘inter-threshold’) and ‘sudomotor regulatory’ zones (Werner *et al*., 2008), as well as the ‘prescriptive’ zone (Lind, 1963a), see Figure 2.3 in section 2.2.

The clothing worn by an individual has the potential to influence thermoregulation (Pascoe, Shanley, & Smith, 1994; Gavin, 2003). The additional layer of insulation provided by the materials themselves, as well as the trapped air layers, presents a barrier to all avenues of heat transfer (Kerslake, 1972), including radiation by altering the exposed skin surface (Gonzalez, 1987). For instance, clothing typically reduces the air velocity rates around the skin and diminishes heat transfer by convection (Pascoe, Bellingar, *et al*., 1994). Similarly, the cooling efficiency of sweating is reduced by the wicking action of clothing moving sweat away from the skin before it is evaporated (Havenith *et al*., 2009) and through heat absorption by the clothing itself (Pascoe, Shanley, *et al.*, 1994). Clothing can also reduce the potential for evaporative cooling from sweat by raising the water vapour pressure of the micro-environment next to the skin (Aoyagi *et al*., 1995). As sweat can only contribute to heat loss if it is able to evaporate (Hardy, 1949; Kerslake, 1972), the evaporative potential of the clothing is an important aspect defining the utility of a garment for exercise in the heat (Parsons, Havenith, Holmér, Nilsson, & Malchaire, 1999), particularly given that evaporation represents the primary heat loss mechanism for exercise in a hot-environment (Monteith & Mount, 1973; Sawka *et al*., 2007).

During exercise in the heat the upper limit of the TZ is often defined as with the point where the required evaporative cooling necessary to achieve thermal balance (E_{req}) exceeds the maximum evaporative potential of the environment (E_{max}) (Cheung & McLellan, 1998a; Givoni & Goldman, 1973). Because clothing typically alters vapour pressure at the skin in a manner that reduces the evaporative potential of sweat, the E_{max} of the micro-climate between the skin and garment will also become diminished (Aoyagi *et al*., 1995). Therefore, clothing may be a key factor influencing the upper boundary of the TZ. Indeed, Belding and Kamon (1973) have shown a clothing assembly consisting of work shirt and trousers, athletic shorts, cotton socks and low-cut rubber tennis shoes reduced the critical ambient water vapour pressure eliciting an inflection in T_c, compared to athletic shorts, cotton socks and rubber tennis shoes whilst exercising at a series of fixed external work rates. However, the relationship between the inflection in T_c and the upper boundary of the TZ is not entirely clear. It should also be acknowledged that under certain conditions, where the ambient temperature is in excess of the skin temperature, some clothing assemblies may also serve as a barrier to heat gain. For example, in fire fighters wearing protective clothing during a live firefighting exercise Eglin, Coles and Tipton (2004) reported a mean abdominal microclimate temperature of 32 °C between the skin and underclothing, whereas mean chest skin temperature was 38 °C, indicating that due to the insulative properties of the clothing the skin temperature was determined more by the T_c (which on average was elevated to 38.5 °C), rather than the environment.

Classically, heat strain has been defined as the inability to maintain T_c at the level prescribed by the thermoregulatory centre (Haldane, 1905). Accordingly, methods for quantifying physiological strain experienced by humans during exercise in the heat typically include some measure of T_c, these methods include the physiological strain index (Moran *et al*., 1998) or cumulative heat strain index (Frank *et al*., 2001). Whilst these approaches are likely to be able to differentiate between groups / experimental conditions when individuals are under uncompensable heat stress (Gonzalez *et al*., 1997), the value of an approach that emphasises the measurement of T_c in situations within the TZ is diminished. This may apply to many exercise scenarios, given that during prolonged exercise athletes will typically self-select work rates that allow thermal compensability in their environment (Schlader *et al*., 2011); that is, they remain within the TZ. Failure to do so would result in an inexorable rise in T_c until the attainment of critical values associated with exercise termination (González-Alonso *et al*., 1999). Indeed, both Macpherson (1962) and Budd (2008) highlight the need for careful selection of heat strain indices during experimentation, to ensure the relevance and specificity of the index to the intended use.

In a recent study in which trained cyclists exercised at a fixed ambient temperature under a variety of different external work rates and simulated wind speeds, it was shown that T_{re} was not influenced by the clothing assembly worn, which ranged from a pair of cycling shorts, to a full cycle clothing assembly consisting of a base layer, cycling shorts, cycle jersey, polyester polytetrafluoroethylene membrane trousers and jacket, gloves and skull cap (Corbett *et al*., 2014). However, differences in mean body temperature ($\overline{T}_b$) were evident between clothing conditions, due to pronounced differences in skin temperature, which resulted in increases in sweat production and heart rate as a function of the amount of clothing worn. Indeed, it is well known that under conditions where the thermal load is compensable, the thermoregulatory system functions to regulate body temperature by increasing effector output, and that this manifests primarily as an increase in skin blood flow (Charkoudian, 2003), sweat rate (Sawka, Montain, & Latzka, 2001), heart rate (Givoni & Goldman, 1973) and oxygen uptake (Arngrímsson *et al*., 2003). When this occurs, T_c may be similar between conditions and differences in the magnitude of thermal strain will solely be reflected in effector output. Therefore, approaches to the evaluation of thermal strain of clothing may lack the ability to discriminate the performance of different clothing assemblies in thermal terms if only body temperature is measured and thermoregulation is achieved in all clothing assemblies tested, *i.e.* the participant remains within the TZ. Combinations of clothing and environmental conditions inhibiting heat loss will force participants towards the higher end of the TZ, necessitating a greater effector response to be evoked in order to maintain a similar T_c, relative to conditions that are less restrictive to heat loss and are at the lower end of the TZ. Moreover, from a performance perspective, it is known that increased thermoeffector responses are associated with impaired aerobic performance (Sawka, Cheuvront, & Kenefick, 2012).

Within the TZ these effector responses can be regarded as the 'equivalent compensatory effort' (Frank *et al*., 2001) or 'physiological cost' (Corbett *et al*., 2014) of achieving thermoregulation. Comparing the magnitude of the 'physiological cost' required to achieve thermoregulation is one way of comparing different thermal and clothing conditions. Thus, when comparing clothing assemblies under a given set of conditions two questions should be considered: i) does the clothing still allow the wearer to remain within the TZ, and if so, ii) at what 'physiological cost'?

4.1.1. Aim and hypotheses

Accordingly, the aims of the present study were to: i) compare the thermoregulatory response to exercise at the same relative intensities under different ambient conditions and in different clothing assemblies; iii) develop a protocol enabling the upper limit of the TZ to be characterised for different combinations of clothing and ambient condition and to determine the highest relative work rate that could be achieved within the TZ under different ambient conditions and in different clothing assemblies.

It was hypothesised that:

H_1 Within the TZ there would be a significantly increased thermoeffector response with hotter ambient conditions and with increased clothing relative to cooler ambient conditions and less clothing

H_2 Increased ambient temperature and clothed area would lead to a plateau in T_c being achieved at a lower relative work rate

4.2. Methods

4.2.1. Participants

Twelve males volunteered to participate in this study and gave written informed consent. All were healthy, non-smoking, and exercised at least twice weekly (as determined by an exercise and health history questionnaire). Participant characteristics are shown in Table 4.1.

Table 4.1. Participant characteristics.

Variable	Mean (SD)
Age (years)	24 (5)
Height (m)	1.79 (0.07)
Body mass (kg)	72.40 (8.50)
Body surface area (m^2 [DuBois formula])	1.90 (0.14)
$\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	50.60 (6.89)

4.2.2. Experimental design

Each participant performed one preliminary test to determine maximal aerobic capacity ($\dot{V}O_{2max}$) and four experimental conditions. The preliminary exercise test was completed under ambient laboratory conditions (21 °C, 45 % relative humidity [RH]), with a fan available for convective cooling if required by the participant. The order of presentation of experimental conditions was balanced and the experimental conditions were identical with the exception of differences in the ambient conditions or clothing worn.

The four conditions were:

1. 30 °C, 50 % RH in a semi-nude condition (30N)
2. 40 °C, 29 % RH in a semi-nude condition (40N)
3. 30 °C, 50 % RH in a low clothing condition (30LC)
4. 40 °C, 29 % RH low clothing condition (40LC)

The two ambient conditions were chosen to be above and below the anticipated mean skin and body temperature, to alter the gradient for dry heat loss whilst RH was adjusted to maintain a similar partial pressure of water vapour in the ambient air (P_a) across the temperature conditions. Experiments took place in a 62 m^3 climate-controlled chamber during morning (08:00) and afternoon (13:00) sessions. Participants had at least 72 hours between tests and were always tested in either a morning or afternoon session (fixed within participant). Participants followed the dietary and pre-experiment guidance described in the General methods.

4.2.3. Experimental procedures

The experimental procedure is outlined in the General methods, briefly, all participants underwent a preliminary exercise test (section 3.7) to allow calculation of the power required to elicit an oxygen uptake ($\dot{V}O_2$) equivalent to 40 %, 50 %, 60 % and 70 % of $\dot{V}O_{2\ max}$ by linear regression and on separate days the four experimental conditions. All exercise tests were completed on a Monark cycle ergometer (874E, Sweden).

Following instrumentation (section 3.4, 3.5.1-3.5.3) the participants were re-weighed to ascertain clothed and instrumented mass (Industrial Electronic Weight Indicator, Model I10, Ohaus Corporation, NJ, USA). The participants were also weighed on a balance (UC-300, A&D co. Ltd, Japan) located within the climate-controlled chamber. Thereafter, once the aural thermistor had been in place and insulated for at least 30 minutes, the participant entered the environmental chamber and rested on the cycle ergometer, where a multichannel laser Doppler sensor (Moor instruments, VP1/7, UK) was attached to the forearm and 2nd finger pad (section 3.5.4). A heart rate monitor (RS800, Polar, Finland) was positioned around the chest for measuring heart rate (section 3.5.5) and a Q-Sweat (WR Medical Electronics, US) sensor was attached to the back (mid-right side) to provide a measure of local sweat rate (section 3.5.6). As both T_c and T_{ac} were measured, mean body temperature ($\overline{T}_b$) was calculated for both measurements using the formula by Colin, Timbal, Houdas, Boutelier, & Guieu (1971) to reflect the importance of the thermal input from the $\overline{T}_{sk}$ in the experiments.

$$\overline{T}_b = (0.79\ T_{re} \text{ or } T_{ac}) + (0.21\ \overline{T}_{sk}) \qquad \textit{Equation 4.1}$$

Giving either mean body temperature as calculated with rectal temperature ($\overline{T}_{b\ re}$) or mean body temperature as calculated with aural temperature ($\overline{T}_{b\ ac}$).

After a five minute rest period, during which baseline measures were taken, the participant cycled at the external work rate calculated to elicit 40 % $\dot{V}O_{2\ max}$ from the preliminary exercise test, at a cadence of 70 revs·min^{-1}. Our pilot work indicated that this work rate would be near to the upper limit of the TZ in the 40LC condition. The participant continued to cycle until the increase in aural temperature (T_{ac}) was $\leq \pm 0.1$ °C over a 15 minute period (monitored every 5 minutes); this was defined as a 'plateau' in deep body temperature. T_{ac} was used to define the plateau, rather than rectal temperature (T_{re}) because pilot work indicated that the slower response time of T_{re} would have prolonged the stage duration to an excessive degree, thereby increasing the likelihood of premature test termination due to fatigue. Following the attainment of a plateau in T_{ac} the participant dismounted the bike and was weighed (participants were typically off the bike for 2 minutes) to enable estimation of sweat production (UC-300, A&D co. Ltd, Japan) before being asked to re-commence cycling at the external work rate calculated to elicit 50 % $\dot{V}O_{2\,max}$ and the process was repeated. External work rate continued to be increased by an amount estimated to be equivalent to 10 % $\dot{V}O_{2max}$ until the first of the following criteria was met: i) a plateau in T_{ac} was not achieved in 60 minutes at a particular work rate, ii)the participant requested to stop; or iii) reached any of the experimental end points (section 3.9). An uncompensable thermal state was defined as either: i) an increasing T_{ac} temperature with no plateau achieved in 60 minutes or ii) if fatigue occurred before 60 minutes, confirmation of an inflection in deep body temperature by visual analysis by three independent physiologists *and* confirmation that a plateau would not have occurred by non-linear regression modelling (SPSS version 21, IBM). Participants were free to drink a 6% carbohydrate plus electrolytes sports drink (SiS Go Electrolyte Lemon & Lime Flavour, Science In Sport PLC, Lancashire, UK) *ad libitum* throughout the exercise trial, with the amount consumed measured to enable calculation of whole body fluid losses.

Rating of perceived exertion (RPE [Borg, 1982]), thermal sensation (TS), thermal comfort (TC [Zhang *et al*., 2004] and skin wettedness (SW [Rissanen, Smolander, & Louhevaara, 1991]) were measured and expired gases were collected using the Douglas bag technique (section 3.5.7) every 15 minutes from the start of the cycling period, during the final minute of a plateau in T_{ac} and during the final minute of each experimental trial. On completing the test, the participants were weighed in the chamber and then in the private changing room whilst clothed and instrumented. They were supervised while all thermistors and HF sensors were removed before being asked to remove all clothing in the private changing room, for a post exercise naked weight.

4.2.4. Clothing

Each participant was issued with two sets of clothing (Appendix C), which were fitted in accordance with the manufacturers sizing instructions. The two clothing conditions allowed for i) comparison between previous investigations which use a semi-nude condition and ii) provided additional covering of body surface area, increasing the insulation from heat loss and following previous recommendations for the use of full length garments to be used in thermoregulatory studies (Gavin *et al*., 2001). They wore their own underwear and trainers and were asked to keep these the same between conditions. The clothing worn during the N conditions consisted of: cycling shorts (74 %

Nylon, 26 % Elastane); sport socks (28 % Polamide, 26 % Wool, 26 % Acrylic, 16 % Elastane [LYCRA®], 4 % Polyamide [TACTEL®]) and the participant's own underwear and trainers (approximate Clo 0.13 for full ensemble [Parsons, 2003]). The LC clothing conditions consisted of: full length cycle jersey (86 % Nylon, 14 % Elastane [White fabric: 75 % Polyester, 25 % Elastane & Micro mesh: 100 % Polyester]); cycling leggings (85 % Nylon, 15 % Elastane, sport socks – 28 % Polamide, 26 % Wool, 26 % Acrylic, 16 % Elastan [LYCRA®], 4 % Polyamide [TACTEL®]); and the participant's own underwear and trainers (approximate Clo 0.49 for full ensemble [Parsons, 2003]). Following each trial the clothing was washed at the laboratory to ensure the same clean, dry condition each time.

4.2.5. Statistical analyses

Parametric data are presented as mean (SD), non-parametric as Median (Range). Following a Shapiro-Wilk test for normality, two-way repeated measures ANOVA (clothing × ambient temperature) were used to compare physiological responses to the four experimental conditions at discrete time points. Specifically, to investigate between-conditions differences in dependent variables within the TZ the average values of the physiological responses recorded in the final ten minutes of the plateau were examined. To examine effector responses occurring after the loss of thermoregulation *i.e.* beyond the TZ, the average values of the physiological responses of the final five minutes of each condition were compared between conditions. Mean values recorded over the four experimental conditions were examined by one-way repeated measures ANOVA. Significant F values were further analysed by paired t-tests (t). If data were non-parametric, Friedman's ANOVA (χ^2) was used and significant values further analysed using the Wilcoxon signed rank test (z). For brevity, non-significant pair-wise analyses are not reported. Non-linear regression modelling was used to ascertain the likelihood of an individual attaining a plateau in deep body within 60 minutes of their final stage if they were unable to complete the 60 minutes due to fatigue (data not presented). SPSS was used to calculate the asymptote and tau using the non-linear regression modelling function to solve the following equation:

Estimated temperature = baseline + asymptote *(1-e(-(t-delay)/tau)) *Equation 4.2*

Where: e is the exponential function, baseline is the T_c at the start of the stage in which the inflection takes place, t is the experimental time and the delay (in experimental time) was chosen visually. The resulting model was plotted against the raw T_c data to visually assess how well it fitted.

4.3. Results

4.3.1. Ambient conditions

The mean dry bulb (T_{db}), ambient water vapour pressure (P_a), and RH for each experimental condition are displayed in Table 4.2. There was a significant effect of environmental conditions on T_{db} ($\chi^2_{(3)}$ = 29.800, p= <0.001). These differences were between the 30 °C conditions and the 40 °C conditions (40N *vs.* 30N z=-3.059, p=0.002; 40N *vs.* 30LC z=-3.061, p=0.002 and 40LC *vs.* 30N z=-3.061, p=0.002; 40LC *vs.* 30LC z=-3.059, p=0.002). However, there was also a significant difference between the two clothing conditions in the 40 °C T_{db}, (z=-2.040, p=0.041), although the mean T_{db} difference of only 0.23 °C approximated the between-trials standard deviation for this measure and is likely of little practical significance. There was no significant difference between the P_a of each environmental condition.

Table 4.2. Mean (SD) environmental conditions in the experimental conditions.

Variable	30N	30LC	40N	40LC
T_{db} (°C)	30.04 (0.23)*	30.03 (0.23)*	39.09 (0.30)**	38.86 (0.22)
RH (%)	54.81 (1.13) *	54.73 (0.80) *	33.15 (1.57)	33.02 (2.16)
Pa (mb)	23.31 (0.73)	23.30 (0.99)	23.28 (0.61)	22.94 (1.65)

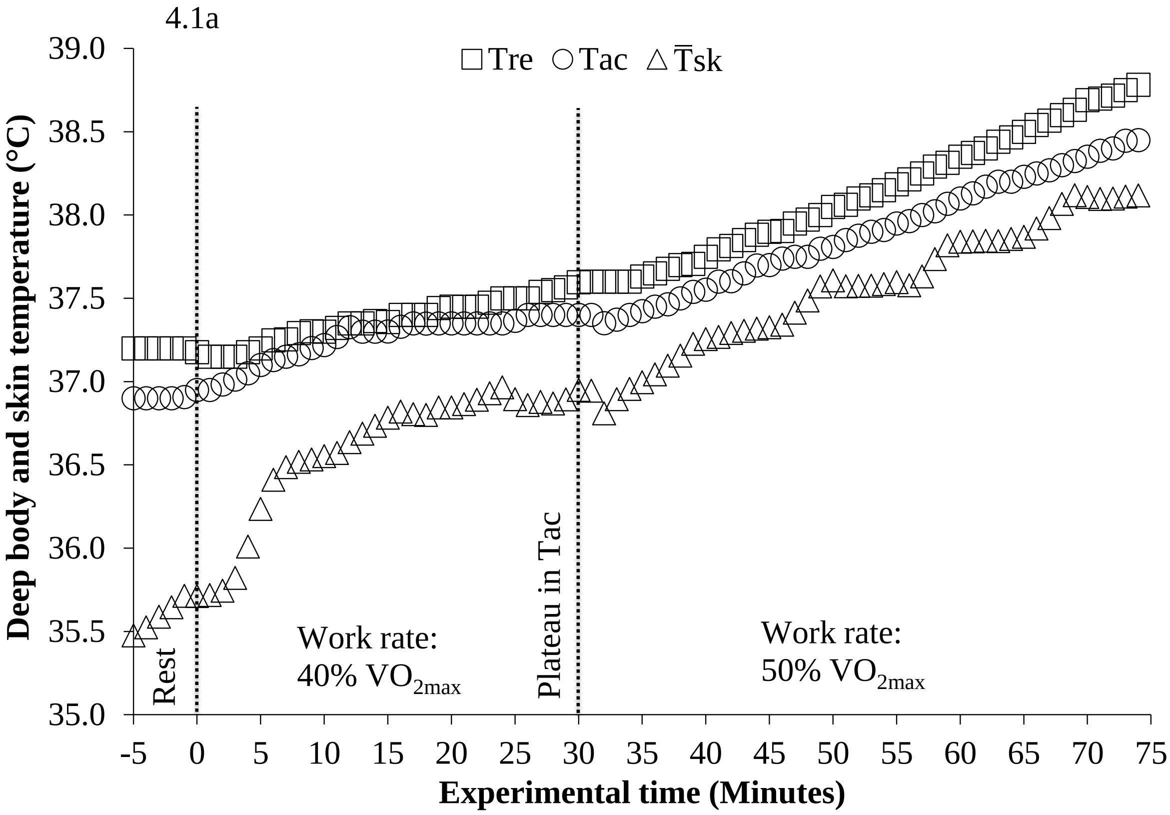
* significantly different to both 40 °C conditions, ** significantly higher than 40LC condition

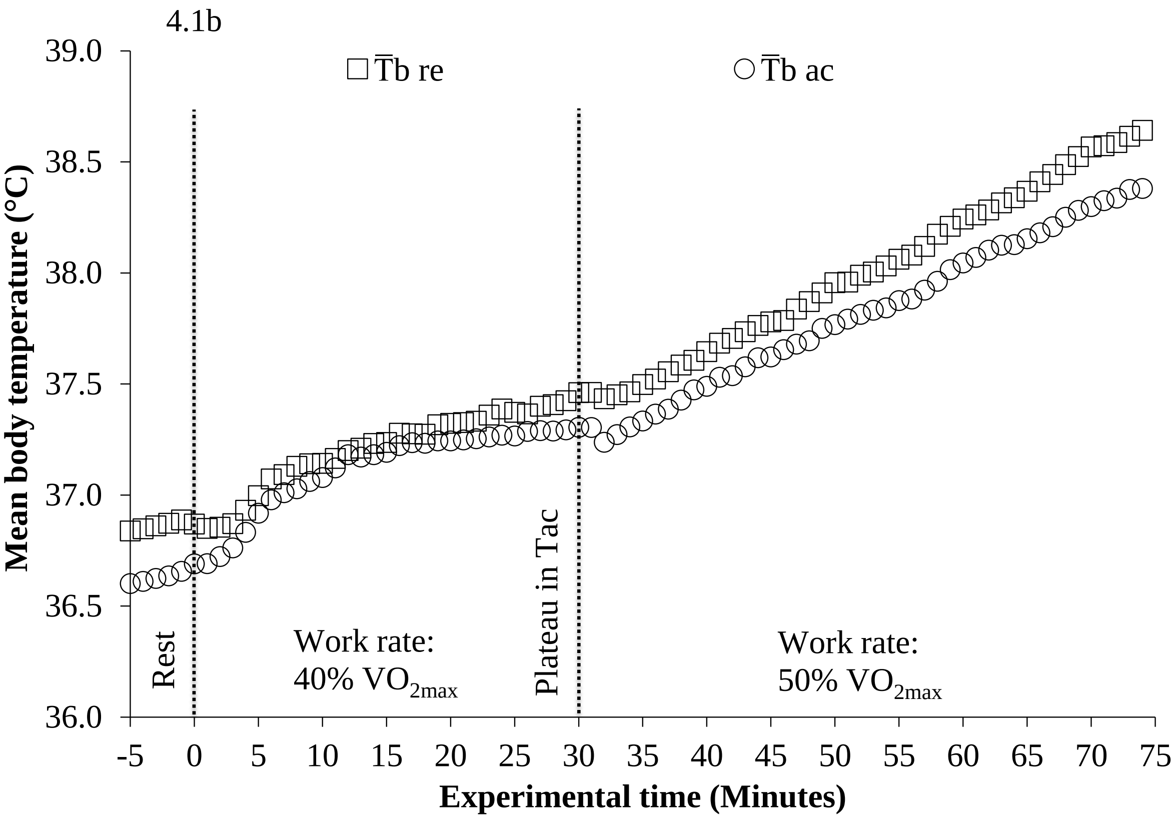
4.3.2. Example of thermal and thermo-effector profile

Because of within- and between- participant differences in: 1) the time course of the thermal and physiological responses, 2) the relative work rate at termination, 3) the stage durations and 4) experimental end points, it is inappropriate to present group mean data by time over the full duration of the experiment. Thus, an example dataset for an individual participant demonstrating a typical thermal and thermoeffector response with increasing work rate is shown in Figure 4.1(a-f).

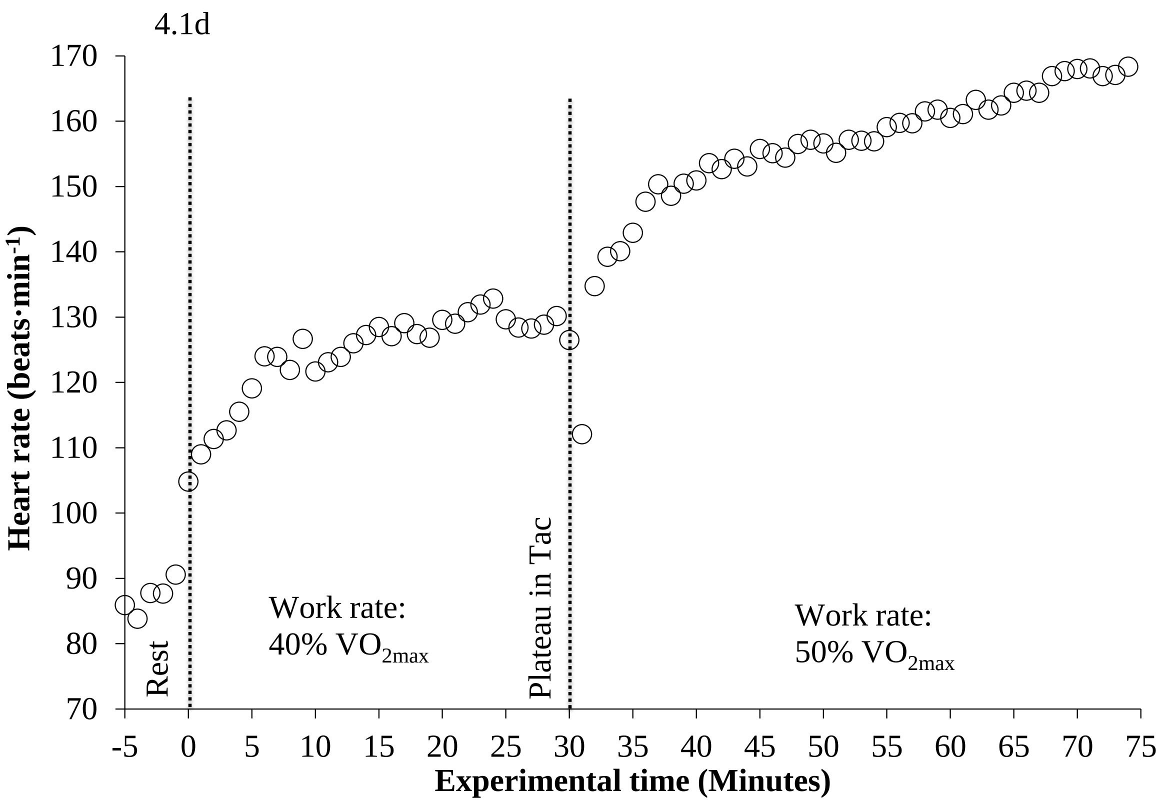
Figures 4.1a & 4.1b show the thermal response to the experimental protocol. A plateau in T_{ac} was achieved after 30 minutes at the first work rate, indicating that the participant was within the TZ at this combination of external work rate, clothing and ambient conditions. Thereafter, following the increase in external work-rate no further plateau in T_{ac} was achieved, indicating that the increased external work-rate had pushed the participant beyond their TZ and put them into in an uncompensable thermal state. Although, due to fatigue, exercise was terminated before a full 60 minute stage was complete, all three independent physiologists visually confirmed the inflection and the non-linear regression modelling estimated that there would be no plateau and T_{ac} would reach 38.80 °C by the 60th minute of the stage. T_{re} was higher than T_{ac} throughout the trial, although this is typical (Huggins, Glaviano, Negishi, Casa, & Hertel, 2012); T_{re} was, on average, 0.33 (0.16) °C higher over all conditions. Although T_{ac} did not increase more than 0.1 °C from 15 to 30 minutes (plateau), T_{re} increased by over 0.2 °C over the same time period, likely due to the slower response time for this measure of deep body temperature.

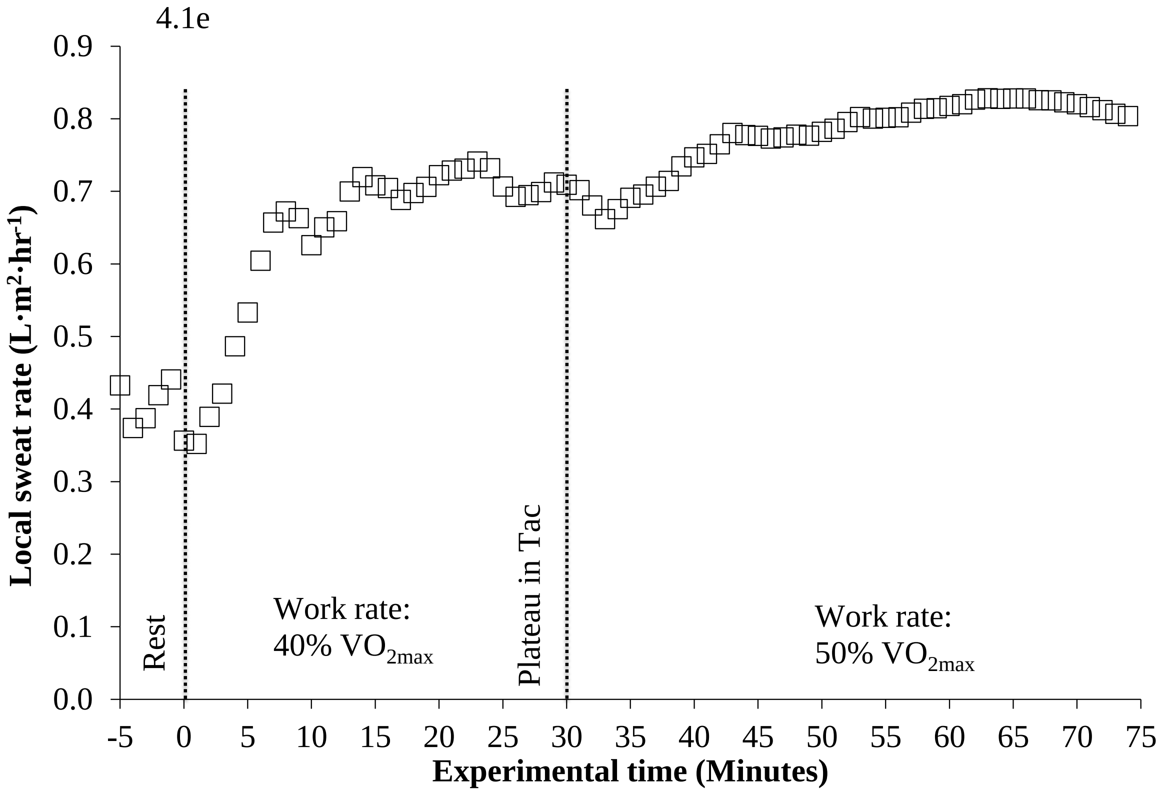
The point at which $\overline{T}_{sk}$ ceased its initial rapid increase (Figure 4.1a) corresponded with the participant approaching the peak level of peripheral vasodilatation evident for this condition (Figure 4.1c). Heart rate (Figure 4.1d) increased rapidly at the onset of exercise and paralleled the responses in $\overline{T}_b$, with a progressive increase after the change in work rate following the plateau in T_{ac}, despite work rate then remaining fixed over the same period, indicating cardiovascular drift (CV_{Drift}). Local sweat rate (Figure 4.1e) levelled off prior to the plateau, indicating that sweat production was able to match heat loss needed for thermal balance under these conditions. However, sweat rate did not increase further from the level established shortly after the increase in work rate following the plateau (~40 minutes) despite a rapid rise in T_c (Figure 4.1a & 4.1b); this suggests that this was the participant's peak sweat rate for these conditions, and was not sufficient to achieve the heat loss needed for thermal balance with increasing metabolic heat production. Figure 4.1f shows the $\dot{V}O_2$ values at rest and in each of the two stages. $\dot{V}O_2$ increased with the external work rate, but did not appear to increase during each exercise stage.


4.1a
□Tre ○Tac △T̄sk
Deep body and skin temperature (°C)
39.0
38.5
38.0
37.5
37.0
36.5
36.0
35.5
35.0
Rest
Work rate:
40% VO_{2max}
Plateau in Tac
Work rate:
50% VO_{2max}
-5 0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
Experimental time (Minutes)


4.1b
□T̄b re
○T̄b ac
Mean body temperature (°C)
39.0
38.5
38.0
37.5
37.0
36.5
36.0
Rest
Work rate:
40% VO_{2max}
Plateau in Tac
Work rate:
50% VO_{2max}
-5 0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
Experimental time (Minutes)


4.1c
Forearm skin blood flow (% of highest 5 minute period)
110
100
90
80
70
60
50
40
30
20
Rest
Work rate:
40% VO2max
Plateau in Tac
Work rate:
50% VO2max
-5 0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
Experimental time (Minutes)


4.1d
Heart rate (beats·min-1)
170
160
150
140
130
120
110
100
90
80
70
Rest
Work rate:
40% VO2max
Plateau in Tac
Work rate:
50% VO2max
-5 0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
Experimental time (Minutes)


4.1e
Local sweat rate (L·m²·hr⁻¹)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Rest
Work rate:
40% VO2max
Plateau in Tac
Work rate:
50% VO2max
-5 0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
Experimental time (Minutes)

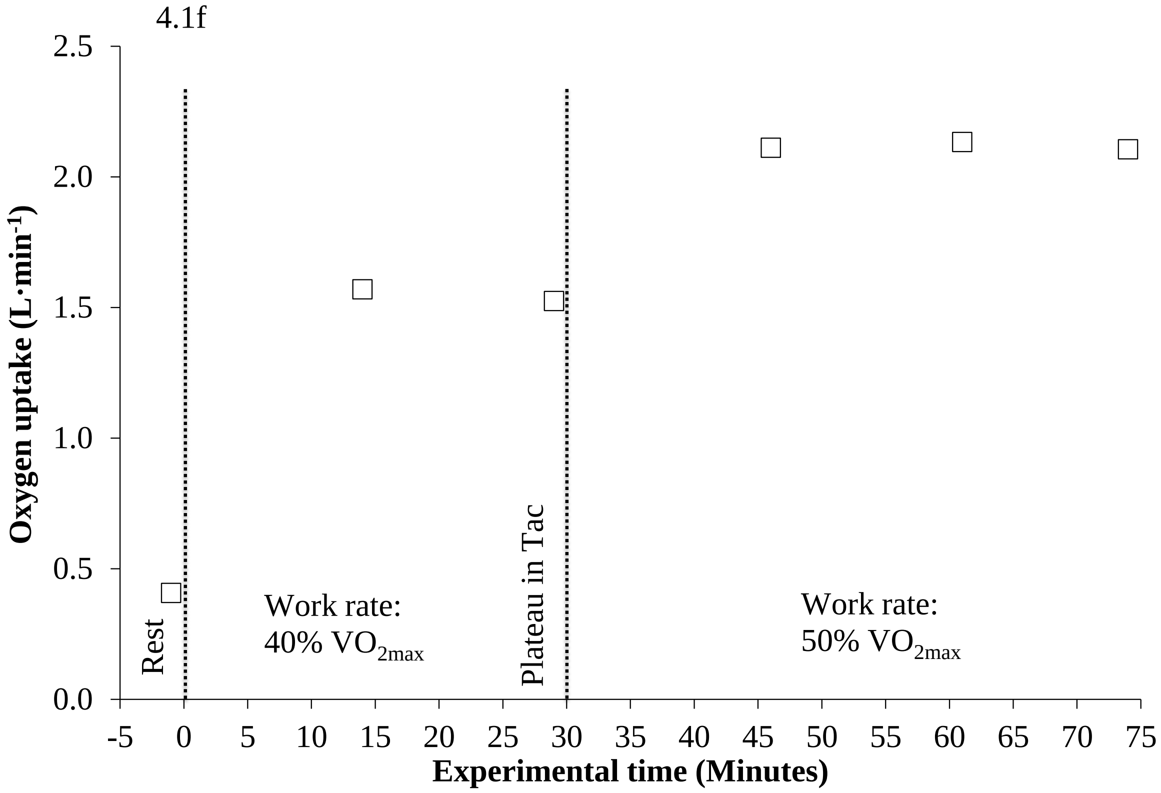


Figure 4.1. Example thermal and thermoeffector profile from a participant exercising in 40 °C with low clothing (40LC). 4.1a - T_{re}, T_{ac} and $\overline{T}_{sk}$ over the experimental time, 4.1b - $\overline{T}_{b\ ac}$ and $\overline{T}_{b\ re}$ over the experimental time, 4.1c - forearm skin blood flow as a percentage of the mean highest values over a five minute period, 4.1d - heart rate over the experimental time, 4-1e - local sweat (back) over the experimental time, 4.1f - oxygen uptake over the experimental time.

4.3.3. Influence of ambient conditions and clothing on thermal, thermoeffector and perceptual responses in the TZ

Ten of the participants were able to achieve a plateau in T_{ac}, during the initial exercise stage, in all ambient and clothing conditions, with no difference in the time taken to achieve a plateau across the conditions ($\chi^2_{(3)}$ =6.73, p=<0.081). The mean (SD) time to achieve a plateau in T_{ac} for the four conditions was: 35.50 (4.92) minutes, 33.50 (8.51) minutes, 31.00 (8.09) minutes, 30.50 (3.68) minutes, for 30N, 40N, 30LC, and 40LC respectively.

The mean thermal responses during the plateau phase are summarised in Table 4.3. There was a significant effect of the ambient conditions on T_{ac} ($F_{(1,9)}$=11.938, p=0.007). The *post-hoc* analysis showed that T_{ac} for both 40 °C conditions was significantly higher than either of the 30 °C conditions (40N *vs.* 30N $t_{(9)}$=-3.059, p=0.014; 40N *vs.* 30LC $t_{(9)}$=-3.539, p=0.006 and 40LC *vs.* 30N $t_{(9)}$=-2.822, p=0.020 and 40LC *vs.* 30LC $t_{(9)}$=-2.661, p=0.026). There was no significant effect of the clothing conditions ($F_{(1,9)}$=0.698, p=0.425), nor significant interaction effect between clothing and ambient conditions ($F_{(1,9)}$=0.000, p=1.000), on T_{ac}.

In contrast, T_{re} was not influenced by the ambient conditions ($F_{(1,9)}$=3.315, p=0.102), but there was a significant effect of clothing on T_{re} ($F_{(1,9)}$=9.445, p=0.013), with *post-hoc* analysis indicating that T_{re} for the 40N condition was significantly higher than either of the clothed conditions (40N *vs.* 30LC $t_{(9)}$=-3.119, p=0.012; 40N *vs.* 40LC $t_{(9)}$=-2.794, p=0.021). The interaction effect between clothing and ambient conditions on T_{re} was not significant ($F_{(1,9)}$=0.776, p=0.401). The between trials differences in measures of T_c were possibly the result of a baseline effect; when T_{ac} and T_{re} were normalised by analysing the change in temperature from the resting baseline to the plateau in T_{ac}, there was no significant difference in T_{ac} between the conditions ($\chi^2_{(3)}$ =1.714, p=0.634) and no significant effect of ambient conditions, clothing or an interaction on T_{re} ($F_{(1,9)}$=1.452, p=0.259, $F_{(1,9)}$=2.250, p=0.168, $F_{(1,9)}$=4.235, p=0.070, respectively).

There was a significant effect of ambient conditions ($F_{(1,7)}$=167.991, p=<0.001) and clothing ($F_{(1,7)}$=10.838, p=0.013) on $\overline{T}_{sk}$, but the interaction effect was not significant ($F_{(1,7)}$=2.751, p=0.141). The *post-hoc* analysis showed that $\overline{T}_{sk}$ in the 30N condition was significantly lower than all of the other conditions (30N *vs* 40N $t_{(7)}$=-8.305, p=<0.001; 30N *vs.* 40LC $t_{(7)}$=-12.289, p=<0.001; 30N *vs.* 30LC $t_{(7)}$=-2.826, p=0.026). Additionally, the $\overline{T}_{sk}$ in 30LC was significantly lower than both of the 40 °C conditions (30LC *vs.* 40N $t_{(7)}$=-10.743, p=<0.001; 30LC *vs.* 40LC $t_{(7)}$=-14.961, p=<0.001). There was no effect of clothing on skin temperature at 40 °C.

The ambient conditions had a significant effect on $\overline{T}_{b\ ac}$ ($F_{(1,7)}$=22.234, p=0.002), with *post-hoc* analysis showing that $\overline{T}_{b\ ac}$ for both 40 °C conditions was significantly higher than either of the 30 °C conditions. (40N *vs.* 30 N $t_{(7)}$=-4.461, p=0.003; 40N *vs* .30LC $t_{(7)}$=-4.771, p=0.002 and 40LC *vs.* 30N $t_{(7)}$=-4.084, p=0.005; 40LC *vs.* 30LC $t_{(7)}$=-3.710, p=0.008 respectively). There was no significant effect of clothing condition ($F_{(1,7)}$=0.111, p=0.749) nor significant interaction effect between clothing and ambient conditions ($F_{(1,7)}$=0.189, p=0.677) on $\overline{T}_{b\ ac}$. Similarly, a Friedman test for the $\overline{T}_{b\ re}$ data indicated a significant difference between the conditions ($\chi^2_{(8)}$=17.270, p=0.001). The *post-hoc* analysis showed that $\overline{T}_{b\ re}$ for both 40 °C conditions was significantly higher than either 30 °C condition (40N *vs.* 30N z=-2.375, p=0.018; 40N *vs.* 30LC z=-2.384, p=0.017 and 40LC *vs.* 30N z=-2.254, p=0.024; 40LC *vs.* 30LC z=-2.552, p=0.011).

Table 4.3. Comparison of thermal responses under different ambient and clothing conditions at the first plateau in T_{ac} (40% $\dot{V}O_{2max}$). Data are mean (SD).

Variable	30N	30LC	40N	40LC
T_{ac} (°C)	37.19 (0.21) **	37.16 (0.19) **	37.48 (0.24)	37.45 (0.22)
T_{re} (°C)	37.45 (0.28)	37.34 (0.25)	37.64 (0.22) ***	37.40 (0.17)
$\bar{T}_{sk}$ (°C)	35.33 (0.31) *	35.66 (0.21) **	36.84 (0.34)	36.86 (0.11)
$\bar{T}_{b\ ac}$ (°C)	36.83 (0.17) **	36.90 (0.15) **	37.32 (0.21)	37.27 (0.18)
$\bar{T}_{b\ re}$ (°C)	37.01 (0.19) **	36.95 (0.20) **	37.01 (0.21)	37.28 (0.15)

* Significantly lower than both 40 conditions and 30LC, ** Significantly lower than both 40 conditions, *** Significantly higher than both LC conditions. n= 10 for T_{ac} and $\bar{T}_b$, and n=8 for $\bar{T}_{sk}$ and $\bar{T}_b$.

The thermoeffector responses during the plateau phase of the first exercise stage are shown in Table 4.4. Friedman's test indicated differences in local SR_{Back} between the conditions ($\chi^2_{(3)}$=12.360, p=0.006). The *post-hoc* analysis showed that SR_{Back} for both 40 °C conditions was significantly higher than 30LC (40N *vs.* 30LC z=-2.499, p=0.012; 40LC *vs.* 30LC z=-2.191, p=0.028). Additionally 40LC was significantly higher than 30N (z=-2.346, p=0.019).

Mirroring the $\bar{T}_{sk}$ response, a Friedman's test indicated differences in $SkBF_{Forearm}$ between the conditions ($\chi^2_{(3)}$=15.267, p=0.002). The *post-hoc* analysis showed that $SkBF_{Forearm}$ for 30N was significantly lower than the other three conditions (30N *vs.* 30LC z=-2.547, p=0.011; 30N *vs.* 40N z=-2.666, p=0.008; 30N *vs.* 40LC z=-2.666, p=0.008).

The ambient conditions had a significant influence on HR ($F_{(1,9)}$=9.398, p=0.013), with *post-hoc* analysis showing that HR for both of the 40 °C conditions was significantly higher than 30N (40N *vs.*30N $t_{(9)}$=-3.450, p=0.007 and 40LC *vs.* 30N $t_{(9)}$=-3.320, p=0.009). Additionally, 40LC was significantly higher than 30LC ($t_{(9)}$=-2.423, p=0.038). The effect of the clothing conditions ($F_{(1,9)}$=0.734, p=0.414) and interaction between clothing and ambient conditions ($F_{(1,9)}$=2.604, p=0.141) were not significant.

There was no significant effect of the ambient conditions ($F_{(1,9)}$=0.061, p=0.810) or clothing conditions ($F_{(1,9)}$=0.603, p=0.457) on $\dot{V}O_2$. Although the ANOVA indicated that there was a significant interaction effect between clothing and ambient conditions on $\dot{V}O_2$

($F_{(1,9)}$=9.715, p=0.012), the *post-hoc* analysis did not identify statistically significant differences. On average the $\dot{V}O_2$ elicited during the plateau phase (target relative work rate = 40 % $\dot{V}O_{2max}$) was equivalent to 40.26 (6.45) % $\dot{V}O_{2max}$, indicating that the method used to determine the external work rate was appropriate.

Table 4.4. Comparison of thermoeffector responses at T_{ac} under different ambient and clothing conditions at the first plateau in T_{ac} (40% $\dot{V}O_{2max}$). Data are mean (SD)

Variable	30N	30LC	40N	40LC
SR_{Back} ($L \cdot m^2 \cdot hr^{-1}$)	0.55 (0.21) ***	0.53 (0.22) **	0.67(0.19)	0.70(0.18)
$SkBF_{Forearm}$ (% of highest 5 min period) (n=9)	49.12 (22.11) *	72.24 (16.29)	78.83 (17.99)	85.25(12.11)
HR ($b \cdot min^{-1}$)	112 (11.06) **	116 (9.64) ***	127(12.47)	127(11.27)
$\dot{V}O_2$ ($L \cdot min^{-1}$)	1.41 (0.36)	1.50 (0.30)	1.47 (0.38)	1.42 (0.34)

* Significantly lower than both 40 °C conditions and 30LC, ** Significantly lower than both 40 °C conditions, *** Significantly lower than 40LC

TC was significantly different between the conditions ($\chi^2_{(3)}$=10.918, p=0.012). *Post-hoc* analysis showed that TC was significantly higher in 30N than all other conditions (30N *vs.* 30LC z=-2.701, p=0.007; 30N *vs.* 40N z=-2.293, p=0.022 and 30N *vs.* 40LC z=-2.497, p=0.013). There was no significant effect of clothing conditions, ambient conditions, or interaction effect, on TS ($F_{(1,9)}$=1.135, p=0.314; $F_{(1,9)}$=4.923, p=0.054; $F_{(1,9)}$=0.030, p=0.866 respectively). There was a significant main effect of the ambient conditions on final SW ($F_{(1,9)}$=25.585, p=0.001). *Post-hoc* analysis showed that SW in 30N was significantly lower than all other conditions (30N *vs.* 30LC $t_{(8)}$=-4.923, p=0.001); 30N *vs.* 40N $t_{(8)}$=-5.163, p=0.001 and 30N *vs.* 40LC $t_{(8)}$=4.467, p=0.002). SW was not significantly influenced by clothing conditions ($F_{(1,9)}$=1.605, p=0.237) or the interaction of clothing and ambient conditions ($F_{(1,9)}$=2.729, p=0.133). There was no significant difference between the conditions for RPE ($\chi^2_{(3)}$=0.542, p=0.910).

During the second exercise stage, at an external work rate corresponding to 50 % $\dot{V}O_{2max}$ only three participants were able to achieve a plateau in $T_{ac,}$ as defined *a priori*, in all conditions. Accordingly, statistical analyses of thermal and thermoeffector responses were not appropriate for this work rate, and above.

4.3.4. Influence of ambient conditions and clothing on thermal, thermoeffector and perceptual responses at test termination

The total duration of the test, exercise intensity at test termination and reason for test termination for each participant, under each condition, is shown in Table 4.5. An uncompensable rise in T_{ac} resulted in test termination in 31 % of the trials. An uncompensable increase in deep body temperature was defined either by: i) no plateau in T_{ac} within 60 minutes – nine trials ii) completion of ≥ 30 minutes of a stage, independent identification of inflection by visual analysis by three individuals *and* regression modelling – six trials (two – exhaustion, two – rate of rise of T_{ac}, one – unwell and one – PI stopped). The most frequent reason for exercise termination was volitional exhaustion (54 % of trials [50 % when removing the cases which were later deemed to be uncompensable]).

There was a significant effect of ambient conditions on time to exercise termination ($F_{(1,11)}$=12.412, p=0.005) but not for the clothing conditions ($F_{(1,11)}$=1.076, p=0.322). The *post-hoc* analysis showed that time to termination in 40LC (71.75 [18.24] minutes) was significantly shorter than 30N (89.25 (13.34) minutes; $t_{(11)}$=4.272, p=0.001) and 30LC (87.25 (21.20) minutes; $t_{(11)}$=2.499, p=0.030). Additionally, the time to termination in 40N (78.08 [13.57] minutes) was significantly shorter than 30N (89.25 (13.34) min ($t_{(11)}$=4.892, p=<0.001). This resulted in a significant difference in exercise intensity at exercise termination between the conditions ($\chi^2_{(2)}$ =24.989, p=<0.001). In both 40 °C conditions the exercise intensity at the point of termination was significantly lower than in either of the 30 °C conditions (40N *vs.* 30N z=-3.051, p=0.002; 40N *vs.* 30LC z=-2.000, p=0.046; 40LC *vs.* 30N z=-3.017, p=0.003; 40LC *vs.* 30LC z=-2.714, p=0.007).

Table 4.5. Duration of test, exercise intensity at test termination and reason for test termination for each participant and average values (mean [SD] for parametric data and mode [range] for non-parametric data) for total test time and work rate for each condition.

Participant	30N			30LC			40N			40LC		
	Time (Minutes)	**Work rate (% $\dot{V}O_{2max}$)**	**Reason**	**Time (Minutes)**	**Work rate (% $\dot{V}O_{2max}$)**	**Reason**	**Time (Minutes)**	**Work rate (% $\dot{V}O_{2max}$)**	**Reason**	**Time (Minutes)**	**Work rate (% $\dot{V}O_{2max}$)**	**Reason**
1	94	70	Fatigue	65	60	Fatigue	87	60	Fatigue	97	50	***NP 60***
2	100	70	Fatigue	85	70	Fatigue	88	60	Fatigue	63	60	Fatigue
3	74	60	HR max	58	60	HR max	60	50	HR max	61	50	HR max
4	81	70	Fatigue	91	70	Fatigue	73	70	HR max	84	60	Fatigue
5	106	70	***Fatigue***	80	70	Fatigue	104	60	***NP 60***	87	50	***NP 60***
6	75	60	Fatigue	62	50	Fatigue	76	50	Fatigue	44	40	***PI stopped***
7	103	70	Fatigue	101	60	***Fatigue***	87	60	Fatigue	74	50	***Unwell***
8	68	60	Fatigue	72	60	Fatigue	68	60	Fatigue	50	60	Fatigue
9	85	60	Fatigue	95	50	***NP 60***	64	50	Fatigue	47	50	HR max
10	105	70	Fatigue	130	60	***NP 60***	88	60	Fatigue	92	50	***NP 60***
11	82	60	Unwell	101	50	***NP 60***	60	40	***NP 60***	79	50	RR T_{ac}
12	98	60	***RR T_{ac}***	107	50	***NP 60***	82	50	RR T_{ac}	83	50	***RR T_{ac}***
Mean (SD) / Median (Mode)	89.25 (13.34)	65 (10)	-	87.25 (21.20)	60 (20)	-	78.08 ** (13.57)	60 * (30)	-	71.75 * (18.24)	50 * (20)	-

Where: Fatigue – stopped due to volitional exhaustion, HR max - attainment of the heart rate exhibited at the end of the individual's $\dot{V}O_{2max}$ test, Unwell– request to stop by the participant because they were feeling unwell, RR T_{ac} - a rate of rise in T_{ac} of > 0.15 °C for two five minute readings once reaching 39.0 °C, NP60 - no plateau in T_{ac} in 60 minutes of the current exercise stage and PI stopped – principle investigator decided to stop the participant exercise due to concern for their well-being.

When reason for test termination is in ***bold italics*** the participant was judged to be in uncompensable heat stress either at the time of the test (NP 60) or *post-hoc* (any other reason). *Significantly lower than both 30 °C conditions ** significantly lower than 30N only.

The thermal responses at the point of test termination are shown in Table 4.6. There was a significant main effect of the ambient conditions on final T_{ac} ($F_{(1,11)}$=10.142, p=0.009). *Post-hoc* analysis showed that T_{ac} in both 40 °C conditions was significantly higher than the 30N condition (40N *vs.* 30N $t_{(11)}$=3.560, p=0.004 and 40LC *vs.* 30N $t_{(11)}$=2.927, p=0.014). Additionally, 40LC was significantly higher than the 30LC condition ($t_{(11)}$=0.651, p=0.028). Final T_{ac} was not significantly influenced by clothing condition ($F_{(1,11)}$=0.955, p=0.349) or the interaction of clothing and ambient conditions ($F_{(1,11)}$=0. 134, p=0.721). T_{re} was also significantly affected by the ambient conditions ($F_{(1,11)}$=7.106, p=0.022), with final T_{re} in 40N significantly higher than either 30 °C conditions (40N *vs.* 30N $t_{(11)}$=-3.633, p=0.004 and 40N *vs.* 30LC $t_{(11)}$=-2.919, p=0.014). There was no significant effect of clothing conditions ($F_{(1,11)}$=0.554, p=0.472) nor a significant interaction effect ($F_{(1,11)}$=4. 489, p=0.058) on final T_{re}.

Final $\overline{T}_{sk}$ was also affected by ambient conditions $F_{(1,11)}$=255.424, p=<0.001), being significantly higher in both 40 °C conditions than either of the 30 °C conditions (40N *vs.* 30N $t_{(11)}$=-11.130, p=<0.001; 40N *vs.* 30LC $t_{(11)}$=-8.026, p=<0.001 and 40LC *vs.* 30N $t_{(11)}$=-9.852, p=0.016; 40LC *vs.* 30LC $t_{(11)}$=- 8.104, p=<0.001). There was no significant effect of clothing conditions ($F_{(1,11)}$=2.723, p=0.127) nor a significant interaction effect ($F_{(1,11)}$=3. 303, p=0.096) on final $\overline{T}_{sk}$.

Final $\overline{T}_{b\ ac}$ was significantly different between ambient conditions ($F_{(1,11)}$=29.989, p=<0.001). *Post-hoc* analysis showed that both 40 °C conditions were significantly higher than either 30 °C conditions (40N *vs.* 30N $t_{(11)}$=-5.988, p=<0.001; 40N *vs.* 30LC $t_{(11)}$=-4.131, p=0.002 and 40LC *vs.* 30N $t_{(11)}$=-4.567, p=0.001; 40LC *vs.* 30LC $t_{(11)}$=- 4.361, p=0.001). Final $\overline{T}_{b\ ac}$ was not influenced by clothing conditions ($F_{(1,11)}$=1.350, p=0.270) and the interaction of clothing and environment was not significant ($F_{(1,11)}$=1.198, p=0.297). Likewise, final $\overline{T}_{b\ re}$ was significantly affected by the ambient conditions ($F_{(1,11)}$=29.142, p=<0.001) with final $\overline{T}_{b\ re}$ in both 40 °C conditions significantly higher than both the 30 °C conditions (40N *vs.* 30N $t_{(11)}$=-6.078, p=<0.001; 40N *vs.* 30LC $t_{(11)}$=-5.010, p=<0.001 and 40LC *vs.* 30N $t_{(11)}$=-2.854, p=0.016; 40LC *vs.* 30LC $t_{(11)}$=-3.520, p=0.005). There was no significant effect of clothing conditions ($F_{(1,11)}$=0.118, p=0.738) nor a significant interaction effect ($F_{(1,11)}$=0. 134, p=0.721) on final $\overline{T}_{b\ re}$.

Table 4.6. Comparison of mean (SD) thermal responses during the final 5 minutes of exercise under different ambient and clothing conditions.

Variable	30N	30LC	40N	40LC
T_{ac} (°C)	37.72 (0.72) **	37.82 (0.39) *	38.10 (0.29)	38.16 (0.39)
T_{re} (°C)	38.23 (0.48)	38.27 (0.47)	38.62 (0.35)	38.42 (0.46)
$\bar{T}_{sk}$ (°C)	35.97 (0.53) **	36.37 (0.31) **	37.50 (0.43)	37.50 (0.32)
$\bar{T}_{b\ ac}$ (°C)	37.35 (0.40) **	37.49 (0.35) **	37.97 (0.34)	38.02 (0.35)
$\bar{T}_{b\ re}$ (°C)	37.76 (0.48) **	37.84 (0.42) **	38.39 (0.33)	38.23 (0.38)

*Significantly lower than 40LC, ** Significantly lower than both 40 °C conditions

The thermoeffector responses at the point of test termination are shown in Table 4.7. There was no significant effect of clothing conditions, ambient conditions or an interaction effect on final local sweat rate ($F_{(1,11)}$=0.023, p=0.881; $F_{(1,11)}$=2.913, p=0.116; $F_{(1,11)}$=2.913, p=0.116 respectively). Despite the differences in external work rate, there was no significant difference in final SkBF or final HR between the conditions ($\chi^2_{(3)}$=1.500, p=0.682; $\chi^2_{(3)}$=2.673, p=0.445). Unlike the thermal responses at exercise termination or the thermoeffector responses at the initial plateau, the only statistical differences between the conditions at the point of exercise termination were in $\dot{V}O_2$ ($\chi^2_{(3)}$=8.500, p=0.037). The post-hoc analysis showed that $\dot{V}O_2$ for both 40 °C conditions was significantly lower than 30N (40N *vs.* 30LC z=-2.510, p=0.012 and 40LC *vs.* 30LC z=-2.824, p=0.005), with 40LC also significantly lower than 30LC (z=-2.040, p=0.041). This probably reflects the lower external work rate at test termination in the 40 °C conditions compared to the 30 °C conditions, due to them stopping earlier.

Table 4.7 also shows there was a significant main effect of ambient temperature on whole body SR calculated over the entire duration of the condition ($F_{(1,7)}$=9.625, p=0.017), which *post-hoc* analysis indicated was higher in both 40 °C conditions than the 30N condition (40N *vs.* 30N $t_{(7)}$=-2.956, p=0.021 and 40LC *vs.* 30N $t_{(7)}$=-2.924, p=0.022). The main effect of clothing and interaction effect between clothing and ambient conditions were non-significant ($F_{(1,7)}$=0.229, p=0.647, $F_{(1,7)}$=1.172, p=0.315, respectively. There was a significant effect of the clothing conditions ($F_{(1,6)}$=8.825, p=0.025) and ambient conditions ($F_{(1,6)}$=6.637, p=0.042), however no interaction affect ($F_{(1,6)}$=0.206, p=0.666) on sweat trapped in the participants' clothing. The *post-hoc* analysis showed that sweat trapped at the 30N condition was significantly lower than both the 40 °C conditions (30N *vs.* 40N $t_{(6)}$=-3.284, p=0.017 and 30N - 40LC $t_{(6)}$=-3.787, p=0.009).

Table 4.7. Comparison of mean (SD) thermoeffector responses during the final 5 minutes of exercise and whole body sweat rate and sweat trapped in clothing calculated over the whole trial under different ambient and clothing conditions

Variable	30N	30LC	40N	40LC
SR_{Back} ($L \cdot m^2 \cdot hr^{-1}$) (n=10)	0.72 (0.17)	0.73 (0.21)	0.78 (0.18)	0.76 (0.18)
$SkBF_{Forearm}$ (% of highest 5 min period) (n=11)	90.95 (13.58)	91.22 (8.79)	94.55 (5.52)	94.42 (3.83)
Heart rate ($b \cdot min^{-1}$)	167 (14.23)	166 (12.73)	170 (8.21)	169 (9.73)
$\dot{V}O_2$ ($L \cdot min^{-1}$)	2.59 (0.72)	2.35 (0.51)	2.15 (0.29) *	2.07 (0.39) **
Whole body sweat rate ($L \cdot m^2 \cdot hr^{-1}$) (n=8)	0.48 (0.08)	0.52(0.09)	0.62(0.12) *	0.61(0.14) *
Sweat in clothing ($L \cdot m^2 \cdot hr^{-1}$) (n=7)	0.11 (0.05)	0.15(0.04)	0.15(0.06) *	0.20(0.08) *

*Significantly higher than 30N ** Significantly higher than both 30 °C conditions

There was no significant difference between the conditions for TC ($\chi^2_{(3)}$=2.054, p=0.561), TS ($\chi^2_{(3)}$=0.921, p=0.820) or for SW($\chi^2_{(3)}$=3.167, p=0.367) at the point of test termination RPE was significantly different between the conditions ($\chi^2_{(3)}$=13.663, p=0.003). *Post-hoc* analysis showed that 40LC was significantly lower than both 30 °C conditions (40LC *vs.* 30N z=-2.687, p=0.007 and 40LC *vs.* 30LC z=-2.323, p=0.020).

4.4. Discussion

The aim of the present study was to develop a protocol to compare the thermoregulatory responses to exercise at the same relative intensities, and to determine the highest relative work rate that could be achieved within the 'thermoregulatory zone' (TZ), under different ambient conditions and in different clothing assemblies. It was hypothesised that within the TZ, under steady-state deep body temperature conditions (*i.e.* plateau in T_{ac}) and at the same work rate, there would be an increased thermoeffector response with increased ambient temperature and / or clothing, reflecting an increased 'physiological cost' of thermoregulation.

Consistent with our hypothesis, there was an increased thermoeffector response as evidenced by an increase in HR, SR and SkBF when exercising under steady state T_c conditions in 40 °C air compared to 30 °C air. There was also some limited evidence of increased thermoregulatory cost of clothing under the cooler ambient conditions, as indicated by an increased skin blood flow response in the LC clothing ensemble compared to N clothing ensemble in 30 °C air, as well as an elevated but not statistically significant heart rate. An augmented thermoeffector response is consistent with the increased skin temperature in LC relative to N conditions in these ambient conditions. However, there was no increased thermoeffector response as a consequence of wearing the N clothing ensemble compared to LC under the hotter ambient conditions.

It was also hypothesised that increased ambient temperature and clothing would reduce the highest relative work rate that could be achieved within the TZ. This was the case; only one participant was able to thermoregulate above 50 % $\dot{V}O_{2max}$ in the 40N condition with none able in the 40LC (in fact two participants were unable to thermoregulate at 40 % $\dot{V}O_{2max}$ in this condition), whereas nine participants were able to achieve a plateau at this work rate in 30 °C conditions (six – 30N and three – 30LC). Thus, as the environmental temperature increased and the clothing levels increased, the relative work rate (%$\dot{V}O_{2max}$) at which a plateau in T_{ac} occurred reduced.

There was no difference in SkBF and SR_{Back} response between the N and LC condition in either environmental temperature. This is in contrast to work by Corbett *et al.* (2014), who reported differences in local sweat rate (forehead) and heart rate between clothing conditions when exercising at the same external work rate. However, differences in experimental design, likely account for these findings. The ambient conditions employed by Corbett *et al.* (2014) were much cooler than the present study (14.5 [0.2] °C air temperature, 46.8 [2.9] % RH) and while the N clothing conditions are comparable between studies, the HI clothing condition employed by Corbett *et al.* (2014) consisted of a base layer, cycling shorts, cycle jersey, polyester polytetrafluoroethylene membrane trousers and jacket, gloves, skull cap and helmet and therefore presented a far greater barrier to heat loss than the LC clothing conditions employed in the present study. This resulted in larger mean differences in $\overline{T}_{sk}$, (~10 °C) than those seen in the present study (0.33 °C [p=0.026] and 0.02 °C [p=0.844]) at the first plateau and (0.4 °C [p=0.052] and 0.00 °C [p=0.993]) at the end of the test (varying work rates), between clothing conditions at 30°C and 40°C respectively. It may be the case that the magnitude of increase in $\overline{T}_{sk}$ caused by clothing in the 30 °C condition was not of a physiologically meaningful size, sufficient to evoke measurable differences in local SR and HR, although it was sufficient to elicit a difference in SkBF. Perhaps of more relevance to the present study, Corbett *et al.* (2014) also reported no significant difference in thermoeffector responses between the two 'intermediate' clothing conditions (socks, shorts, jersey, gloves, and helmet), where T_c and $\overline{T}_{sk}$ were also unchanged. 'Physiological cost' does not appear to discriminate between garment performance during exercise within the TZ when garments have similar insulative properties and T_c and $\overline{T}_{sk}$ are not different. However, conclusions drawn from the present results are confounded by the fact that the T_{re} was different between the 40 °C conditions (Table 4.3). This is a clear limitation, but when both measures of $\overline{T}_b$ are compared there was no difference between clothing conditions, only between the two environmental conditions. It may therefore be argued that the thermoafferent input was similar between clothing conditions.

The two ambient air temperatures chosen for the present study were selected to be either below (30 °C) or above (40 °C) the anticipated $\overline{T}_{sk}$ for all conditions; inspection of our data indicates that this was the case. Additionally, ambient water vapour pressure was controlled to be constant across the ambient temperature conditions as this affects the capacity for sweat to evaporate and so cool the participant (Kerslake, 1972). It is acknowledged that the gradient for sweat evaporation could not have been controlled between conditions, as every individual would have a slightly different $\overline{T}_{sk}$ (and therefore gradient with the environment). However, as the difference in $\overline{T}_{sk}$ was minimal the effect of this discrepancy may also be minimal. Although the additional clothing in the LC condition will have provided increased insulation, it might be that this acted as a barrier to heat *gain* in the 40 °C air, but as a barrier to heat *loss* in the 30 °C air condition, which may account for the lack of observable effect on $\overline{T}_{sk}$ and thermoeffector function in the 40 °C condition.

Thermal insulation of clothing can be reduced if the garment is wet (Kenney *et al*., 1993) as water is much more conductive to heat than air (Watkins, 1984). Garments will take up moisture by wicking sweat from the skin (Havenith, Richards, *et al*., 2008) and this will become distributed around the garment. Depending on the amount of perspiration that accumulates in the clothing, the reduction in insulation of the clothing can be 2 to 8 %

(Chen *et al*., 2003). Once the clothing became saturated, it would offer less protection from dry heat gain (from the environment and surrounding objects), but would promote heat loss through evaporation of sweat, which occurs from within the garment and beneath and above the surface of the material. It is known that efficiency of evaporation through clothing will never equal or exceed that directly from the skin (Kerslake, 1972), however a potential benefit of the clothing condition is that sweat is distributed and held against the skin, rather than dripping from it, increasing sweating efficiency (Candas *et al*., 1979). Consequently, the combination of these factors may have caused the similar $\overline{T}_{sk}$ between the two clothing conditions in 40 °C. Whereas in 30 °C, where $\overline{T}_{sk}$ and SkBF were significantly different between clothing conditions, the LC clothing may have reduced the potential for evaporative cooling from sweat by raising the water vapour pressure of the micro-environment next to the skin (Aoyagi *et al*., 1995) and also diminished heat transfer by convection (Pascoe, Bellingar, *et al*., 1994).

Unlike clothing, the ambient temperature had a clear influence on the thermoeffector response, as shown by an elevated HR, local SR and SkBF in the 40 °C condition, compared to the 30 °C condition (Table 4.7). Presumably this occurred due to larger differences in $\overline{T}_{sk}$ as a consequence of the ambient conditions, which was on average 1.55 (0.29) °C higher in 40 °C compared to 30 °C, or approximately 4.7 times greater than the effect of clothing on $\overline{T}_{sk}$ in the 30 °C condition. Indeed, Sawka *et al.* (2012) argue that hot skin will impair performance and induce earlier exhaustion due to the associated high SkBF requirements, which increases HR and relative exercise intensity and decreases muscle blood flow (Rowell, Marx, Bruce, Conn, & Kusumi, 1966). Interestingly, despite differences in $\overline{T}_{sk}$ which were of a sufficient magnitude to elicit clear physiological differences between conditions, there was no difference in TS (between 'Slightly warm' and 'Warm') between the different temperature conditions, although the participants felt their skin was wetter in 40 °C compared to 30 °C. Confirming the observation that SW is linked to decreased TC (Havenith, Holmér, & Parsons, 2002), significantly greater levels of TC were noted in 30N compared to all other conditions.

The time to test termination was significantly shorter in the 40 °C conditions compared to the 30 °C conditions. It is well known that exercise in a hot (Nybo & Nielsen, 2001; Saltin, Gagge, Bergh, & Stolwijk, 1972) or humid environment (Maughan *et al*., 2012) markedly reduces exercise performance (Hettinga *et al*., 2007). In this study, exercise performance was indicated by the work rate at exhaustion / test termination, which was significantly lower in 40 °C than 30 °C. Volitional exhaustion was the most common reason for exercise termination for all conditions, indeed, final RPE of both 30 °C conditions was rated as equivalent to ‘Very very heavy’ and both 40 °C were rated as equivalent to ‘Very heavy’. This is despite the participants exercising between 40 – 70 % $\dot{V}O_{2max}$. The lower RPE for the 40 °C conditions compared to the 30 °C conditions may be due to the broader range of reasons for exercise termination in the 40 °C conditions. Similar RPEs (18.1 [SE ± 0.7]) and heart rates (164 [SE ± 5] $b{\cdot}min^{-1}$) to those seen in the present experiment have been exhibited by untrained males at exhaustion after walking in 40 °C 30 % RH whilst wearing protective clothing (Wright, Selkirk, Rhind, & McLellan, 2012). Despite substantial differences in the clothing worn and exercise mode, it is clear individuals reach similar levels of perceived exertion at the end of exercise in the heat, albeit with varying levels of thermal and thermoeffector responses to the specific conditions.

The present study was not without limitations. It was expected that there would be no significant difference in T_c between the two clothing conditions at either environmental temperature when the participants were within the TZ. However, this was not the case in the present study, for either measure of deep body temperature employed. This raises the possibility that there were confounding issues with the insulation of the auditory canal temperature measurement and variation in pre-exercise T_{re} (within participant, between conditions) which may have influenced the results. The T_{ac} at the start of the exercise period were significantly higher in both the 40 °C compared to both the 30 °C conditions. While higher brain temperature at rest cannot be discounted, it seems unlikely. On the other hand, there were no significant differences in rate of rise (delta) in deep body temperature (T_{ac} and T_{re}) between the four conditions from end of the rest period to the first plateau in T_{ac} and effects of ambient temperature on T_{au} will have been constant within ambient temperature condition. Also, volitional exhaustion was the dominant reason for exercise termination rather than an uncompensable rise in T_{ac}. Future experiments in this series of work investigating the upper limits of the TZ must consider developing protocols where participants are able to work at a lower work rate. Although this will necessitate more strenuous environmental conditions and may compromise the ecological validity of such approaches, it will likely increase the number of individuals able to transition into uncompensable heat stress before fatigue causes test termination.

In summary, there was some effect of clothing on thermoeffector response at 30 °C (increased T_{sk} and evidence of increased thermoeffector response), but not at 40 °C. This indicates that under the specific conditions of the experiment, the clothing used may have provided a barrier to heat loss at 30 °C and a barrier to heat gain or more efficient sweat distribution at 40 °C. Therefore, the first hypothesis that *within the TZ there would be a significantly increased thermoeffector response with hotter ambient conditions and with increased clothing relative to cooler ambient conditions and less clothing* is supported at 30 °C only. The second hypothesis tested was that *with increased ambient temperature and clothing, a plateau in T_c would occur at a lower relative work rate*. The data supports the second hypothesis and it was also noted that there was a general trend for participants to stop exercising due to fatigue at the lower temperature. However, the study was not successful in its aim to develop a protocol which enabled all participants to become uncompensable. Therefore a different methodology had to be developed for use in the studies described in the rest of this thesis.

5. Chapter Five – Development of a protocol for defining the inflection point in deep body temperature during cycling exercise in a high and low humidity environment.

5.1. Introduction

In the previous Chapter, a protocol was employed for examining the upper limits of the thermoregulatory zone (TZ), whereby the participants exercise at a fixed external work rate, under a given set of environmental conditions, until a plateau in deep body temperature (T_c) was achieved. At this point the work rate was increased and the process repeated until the participant was not able to achieve a steady-state in T_c over a 60 minute period or reached the withdrawal criteria (see 3.9). However, although a number of individuals were able to achieve uncompensability, in the main, participants terminated the exercise test due to volitional exhaustion as a consequence of the high work rates and prolonged work duration. Thus, an alternative protocol for eliciting thermal uncompensability was required. The next two chapters document the processes used in the development of this protocol with the specific focus of investigating the reliability and validity of this approach.

A variety of different approaches have previously been employed for determining the environmental conditions eliciting an inflection in T_c when exercising at a given work rate. Typically, some aspect of exogenous thermal load such as dry bulb temperature (T_{db}) (Kenney *et al*., 1993; Kenney & Zeman, 2002), corrected effective temperature (Lind, 1963a, 1970), or water vapour pressure is progressively increased until an inflection in T_c is evident. Broadly, these approaches can be categorised as either: i) continuous protocols, in which the inflection point can be determined in a single laboratory visit and exogenous thermal load is progressively increased after an initial baseline period of ~ 1 hour (Dougherty *et al*., 2010; Kamon *et al*., 1978; Kamon & Avellini, 1976; Kenney & Zeman, 2002; Kenney *et al*., 1987, 1993); or, ii) discontinuous protocols in which multiple visits are required and participants exercise under a given set of environmental conditions for up to 1 hour on each occasion to allow sufficient time to enable a plateau in T_c to become evident (Belding & Kamon, 1973; Lind, 1963a, 1970). The more recent research in this area has favoured the former approach, presumably because of the substantial logistical benefits which afford increased opportunity for studying multiple combinations of work-rate and environment in a larger number of participants, as well as reducing the effects of inter-day within-participant biological variability. However, this type of approach necessitates a sophisticated environmental facility with the capability of incrementing temperature and / or humidity in a reliable manner. To determine the environmental conditions eliciting an inflection in T_c it is necessary to develop a reliable method for identifying this point. Reliability refers to the consistency of a test or measurement, or the reproducibility of values of a measurement in repeated tests conducted on the same individuals (Hopkins, 2000a); a reliable protocol will provide similar results from day to day when no intervention is used (also see Appendix D). Good test-retest reliability of any measure is essential because it affects the precision of estimates of change in the variable of an experimental study, and because a test, protocol, or measure, cannot be considered valid if it is not reliable (George, Batterham, & Sullivan, 2000). However, it is important to note that measurement error, which includes technical errors, systematic bias, and biological variance, can never be completely absent (Batterham & George, 2000).

A variety of approaches have previously been used to identify an inflection in T_c, including: i) visual assessment during the experiment (Kamon & Avellini, 1976), ii) drawing a line through the steady state T_c, which is either at a set experimental time (Dougherty *et al*., 2010; Kamon *et al*., 1978; Kenney & Zeman, 2002), or determined by visual assessment (Kenney *et al*., 1987), and visually identifying the point of departure from the T_c versus time curve, or iii) D_{max} method, which uses linear and quadratic equations to mathematically identify the inflection point within a visually assessed 'window' of data (Kenney *et al*., 1993) with confirmation of point identified by: a) analysis of variance between the two lines (Kamon *et al*., 1978) and b) redrawing line with different time axes, including logarithmic scales (Kenney & Zeman, 2002). At present there is no agreement as to which of these approaches is most appropriate or if other approaches may be superior.

Although a number of studies have attempted to define the upper boundary of the theremoregulatory zone (TZ) (Belding & Kamon, 1973; Kamon *et al*., 1978; Kamon & Avellini, 1976; Kenney *et al*., 1987, 1993), measures of reliability have seldom been reported for these protocols, the associated physiological responses, or the methods of determining the T_c inflection point. There is only limited information regarding the test-retest reliability of the T_c inflection point (Dougherty *et al*., 2010; Kenney & Zeman, 2002) and physiological responses to the heat in general (Brokenshire, Armstrong, & Williams, 2009; Hayden, Milne, Patterson, & Nimmo, 2004; Kenefick, Cheuvront, Elliott, Ely, & Sawka, 2012). For example, Kenney and Zeman (2002) examined the reliability of T_{crit} (critical T_{db} forcing an inflection of T_c) and P_{crit} (critical water vapour pressure forcing an inflection of T_c) in unacclimated men and women by asking four of their participants to repeat the test on a different day to the original test. A correlation coefficient (r) of 0.97 was reported for the time points at which each critical point was achieved, but no specific detail is given as to the selected conditions (other than they ranged from the driest to the most humid environments tested in the study) or the gender of the individuals. Similarly, Dougherty *et al*. (2010) assessed the reliability of their P_{crit} data in lean and obese children with two participants completing a repeat of each of the T_{db} conditions examined (34 °C [1 lean and 1 obese participant]; 36 °C [1 lean and 1 obese participant]; 38 °C [2 obese participants]; 42 °C [1 lean and 1 obese participant]); they reported a correlation coefficient (r) of 0.99 for the time points at which each critical point was achieved. However, whilst correlation coefficients of above 0.9 have been suggested to be indicative of acceptable reliability (Atkinson & Nevill, 1998), the validity of using correlation coefficients as a measure of reliability has been questioned (Ball & Scurr, 2010; Batterham & George, 2000; Hopkins, 2000a), this is explained further in Appendix D. In addition to selecting the correct approach for assessing reliability, determining acceptable levels of reliability is also a challenge to the researcher, particularly for multiple measures employing different units. In some instances there are published thresholds for specific tests or measures, but these are often arbitrary and not agreed upon within the literature (Atkinson & Nevill, 1998). For instance, a CV of 10 % has been arbitrarily selected as the threshold for some studies in sports medicine and science (Hopkins, 2000a), but protocol design can affect the CV (Currell & Jeukendrup, 2008) and certain variables, such as skin blood flow (SkBF) have accepted levels of reliability greater than 10 % (Farkas, Kolossváry, Járai, Nemcsik, & Farsang, 2004; Roustit *et al*., 2010).

5.1.1 Experimental aims

Accordingly, the aims of this investigation were threefold. First, to develop a reliable laboratory protocol enabling T_{db} to be incremented in a reliable manner after an initial, stable, baseline period and under conditions of high and low relative humidity. Second, to assess the test- retest reliability of the associated human thermoregulatory responses during such a protocol. Third, to develop a reliable method of determining the inflection point in T_c with an increasing T_{db} under conditions of high and low relative humidity.

5.2 Methods

5.2.1 Participants

Eight males volunteered to participate in this study and gave written informed consent. Eight males have been used in similar research previously (Billat, Renoux, Pinoteau, Petit, & Koralsztein, 1994; Brokenshire *et al*., 2009; Todd, Taylor, & Gandevia, 2004). All were healthy (as determined by an Exercise and Health History questionnaire [Appendix B]), non-smokers, and exercised on two occasions per week. A favourable ethical opinion was given by SFEC prior to recruiting volunteers for this study (Appendix A). Participant characteristics are shown in Table 5.1.

Table 5.1. Participant characteristics (n=8).

	Mean (SD)
Age (years)	21.75 (3.96)
Height (m)	1.78 (0.06)
Body mass (kg)	74.17 (11.93)
Body surface (m^2)	1.91 (0.16)
Body fat (%) – Seven sites (American College of Sports Medicine, 2000)	10.27 (4.61)
Body fat (%) – Four sites (Durnin & Rahaman, 1967)	15.05 (4.24)
$\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1})	46.53 (6.73)
$\dot{V}O_{2max}$ (L·min^{-1})	3.44 (0.68)

5.2.2 Experimental design

Participants were required to visit the Extreme Environments Laboratory on five occasions. On the first visit each participant performed a maximal aerobic capacity test ($\dot{V}O_{2max}$) and had their body fat estimated by measurement of skinfolds, details of which can be found in General methods (section 3.7 and 3.2.5 respectively). The remaining four visits were the test and retest of two experimental protocols designed to determine the T_{re} inflection point under conditions of high (‘H’ – 80 % RH) and low (‘L’ – 20 % RH) relative humidity, with Tests 1 and 2 referred to as H1, H2, L1, and L2 respectively. The tests were undertaken in a balanced order. T_{re} was selected as our criterion measure of T_c because the previous experiment had shown T_{au} to be influenced by ambient temperature and because the majority previous work in this area (with adults) has used this measure (Belding & Kamon, 1973; Kamon *et al*., 1978; Kenney *et al*., 1987, 1988, 1993) and because none of the participants were able to consistently tolerate the oesophageal probe or refused it. Participants had at least 48 hours between tests and were always tested in either a morning or afternoon session (fixed within participant) in order to minimise diurnal variation and heat acclimation.

5.2.3 Experimental procedures

Details of the pre-experiment guidance and preliminary measures can be found in the General Methods section 3.2.3 - 3.2.4. On arrival at the laboratory for the experimental conditions the participants entered a private changing room to produce a urine sample, void their bowels and have their naked body mass measured (I10 Industrial Electronic Weight Indicator, Ohaus Corporation, NJ, USA). The specific gravity of the urine sample was tested by reagent strips for urinalysis (DUS 10 Health Mate Urinalysis Multisticks, DFI Co Ltd, Korea), adequate hydration was assumed at values ≤1.020 (Casa *et al.*, 2000). Participants were provided with the same cycling shorts and socks on each occasion (see section 4.2.4 and Appendix C), but wore their own shoes and underwear. Details of physiological and perceptual measurements are detailed in section 3.5 and 3.6 respectively. Following instrumentation the participants were re-weighed to ascertain clothed (all items separately) and instrumented mass. Thereafter, once the aural thermistor had been in place for at least 30 minutes, the participant entered a 62 m^3 climate-controlled chamber and rested on the cycle ergometer for a further 20 minute period while being instrumented for the measurements of SkBF (3.5.4), HR (3.5.5) and SR (3.5.6). Dependent upon the experimental condition the chamber was set to achieve an initial target temperature of either 28 °C, 80 % RH (H) or 34 °C, 20 % RH (L).

Following a five minute resting baseline measures period, including an expired gas sample (3.3.7), the participant commenced cycling at 60 $revs.min^{-1}$, eliciting an external work rate of 60 W. Pilot work and consultation of psychometric charts (Kenney, DeGroot, and Holowatz, 2004) indicated that this combination of work rate, T_{db} and RH would be tolerated by our moderately fit participants for a sufficient length of time to see an inflection in T_{re} during the second hour of the experiment. Expired gases were measured every 20 minutes from the start of the cycling period for one hour, then at 10 minute intervals and during the final minute of each experiment. After 60 minutes of cycling the T_{db} was increased by a target rate of 1 °C every 5 minutes until exhaustion, or attainment of any of the experimental end point criteria (see 3.7). To reduce the confounding effects of dehydration and carbohydrate depletion over the course of the test, the participants were given 200 mL of a 6 % carbohydrate plus electrolyte sports drink (SiS Go Electrolyte Lemon & Lime Flavour, Science In Sport PLC, Lancashire, UK) every 20 minutes at a set temperature of 20 °C (±0.05), with the total amount consumed recorded and any urine passed weighed to enable calculation of whole body fluid losses (Baker, Lang, & Kenney, 2009).

On completing the test the participants were re-weighed in the private changing room, whilst clothed and instrumented. They were supervised while all thermistors and heat flux sensors were removed before being asked to remove all clothing in the private changing room, weigh each item separately and then have their post exercise naked body weight measured.

5.2.4 Determination of inflection in T_c

Three methods were used to determine the inflection point of T_c.

5.2.4.1 Method 1 - Visual assessment

The visual assessment method has been used previously for the identification of the inflection in T_{re} (Kamon & Avellini, 1976; Kenney *et al*., 1987) and is a widely used threshold analysis technique (Beaver, Wasserman, & Whipp, 1986; McGehee, Tanner, & Houmard, 2005). Two experienced thermal physiologists were asked to visually determine the inflection point in T_c on printed graphs (identical size and scale) of each participant's T_{re} plotted against time. If the values selected by the first and second assessor were within three minutes of each other, the mean of the two values was used as the inflection point. If the values differed by more than three minutes a third assessor was consulted and if their value was within three minutes of either assessor the mean of the two closest values was used. If all three assessors differed by more than three minutes, they met together and came to a group decision. The order of presentation of graphs was randomized and anonymized, and each data set was presented twice to gain an index of intra-, and inter-assessor reliability.

5.2.4.2 Method 2 - D_{max} assessment

This method has also been previously used to locate the inflection point in T_{re} during a similar protocol (Kenney *et al*., 1993), as well as for determining the lactate threshold (McGehee *et al*., 2005) where it has been shown to be more reproducible than other threshold measures (Morton, Stannard, & Kay, 2012). An initial estimate of the inflection point was visually determined (by the principle investigator only), and a large window of data (up to 60 minutes) was selected with the visually determined inflection point near its centre. The window was chosen to exclude data from the early and late stages of the test in the analysis, where T_{re} may have not reached a plateau, or been increasing rapidly or erratically. Using Microsoft Excel 2013, a quadratic equation was fitted to the data curve and a linear equation was obtained from the first to last data points of the data in the window. The inflection point was defined by the maximal perpendicular distance between the two line lines determined by subtracting the linear equation from the quadratic $((mx + c) – (ax^2 + bx+ c))$ for each minute.

5.2.4.3 *Method 3 – Increase of 0.1 °C above the value at 60 minutes (set value)*

The values were compared at the point at which T_{re} increased by 0.1 °C above the value measured at 60 minutes cycling time. This method and resulting value was selected because it was assumed that after 60 minutes the participants would be in thermal steady state or near-steady state, and a positive increase of 0.1 °C would be an objective method to identify the inflection point (the manufacturer reported accuracy of the rectal thermistor is ± 0.1 °C).

5.2.5 Data analysis and statistical methods

All data are presented as mean (SD), with the exception of change in the mean and CV which are in the units of the variable and as a percentage, respectively. Statistical significance was accepted at $p<0.05$. Test retest reliability was calculated using the typical error of the mean (TEM) (Hopkins, 2000a) and CV calculated using the method described by Atkinson and Nevill (1998), which is appropriate for use with small sample sizes (Hopkins, 2000a). Systematic bias was calculated using change in the mean (Batterham & George, 2000) which is simply the change in the mean value between two trials of a test (Hopkins, 2000a). Differences between the mean values were analysed with 2-way repeated measures ANOVA (test number × humidity condition) with *post-hoc* analysis by paired samples t-test if necessary. If the data were not normally distributed (as determined by the Shaprio-Wilk test) differences were analysed by the Friedman test with *post-hoc* analysis by the Wilcoxon test if necessary. The test-retest reliability of the environmental conditions was determined for each participant during each condition from rest to the 100th minute of experimental time, to ensure comparison took place with equal participant numbers (n=8) in each condition. Likewise, the test-retest reliability of the physiological and perceptual responses to the protocol were determined for each participant during each condition from rest to the 100th minute of experimental time. The sampling periods were 5 minute average for baseline period and then 5 minute averages from 55 minutes onwards (*i.e.* after the initial increase in T_{re} towards a steady-state) until the 100th minute. For brevity, the average of the thermal and physiological responses over the 5 minute blocks from 60th to the 100th minute are presented, with the exception of $\dot{V}O_2$ which, as with the perceptual measures, were only collected at 10 minute intervals. Perceptual values and $\dot{V}O_2$ were compared at the discreet time points at which they were collected. The test-retest reliability of the environmental temperature at which an inflection in T_c occurred, and the coincident thermoeffector responses (or in the case of $\dot{V}O_2$ and perceptual measures, the nearest data collection point), were determined for each environmental condition. To compare the three different methods of determining the inflection point in T_{re} across the four different conditions, a mixed factorial ANOVA was used, with method as the between subjects factor, and condition as the repeated measures within subjects factor. Assessor intra- and inter- test-retest reliability of the visual assessment method was determined from their individual assessment of each participant's separate tests, determined on two occasions.

5.3 Results

5.3.1 Reliability of the environmental conditions

The target T_{db} and the actual T_{db} for both of the high humidity and low humidity conditions are shown in Figure 5.1, with the target RH and the actual RH for both of the high humidity and low humidity conditions shown in Figure 5.2. The T_{db} was on average (SD) within 0.13 (0.20) °C of the target T_{db} throughout the high humidity conditions and on average within 0.23 (0.48) °C of the target T_{db} during the low humidity conditions. In the first 60 minutes RH was on average 3.80 (0.67) % RH above the target throughout the high humidity conditions and on average 4.78 (0.89) % RH above the target during the low humidity conditions (Figure 5.2.). However, during the temperature ramp (from 60 minutes onwards) in the high humidity condition the RH fluctuated around the target RH, whereas in the low humidity condition the RH continued to increase above the target RH during the same time period. Of course, the distance from the target T_{db} and RH is not an issue if it is consistent, *i.e.* all participants were exposed to similar temperatures and relative humidity each time. Reliability statistics (TEM and CV) were calculated for average T_{db} (Table 5.2) and RH (Table 5.3) in each trial, for three distinct time periods: i) the rest period and the first hour of cycling (stable air temperature [n=8]), ii) the entire period that n=8 (start of rest period to the 100th minute), and iii) from the start of the temperature ramp until 100th

minute (n=8). There was no significant difference between the mean for Test 1 *vs*. Test 2 in T_{db} or RH for any time period. The CV during the rest to 60th minute period (where the target air temperature was stable) was lower than the other analysed periods. The reliability of the RH during the low humidity condition was lower than the high humidity condition when expressed as CV, although they were similar in absolute terms (TEM).

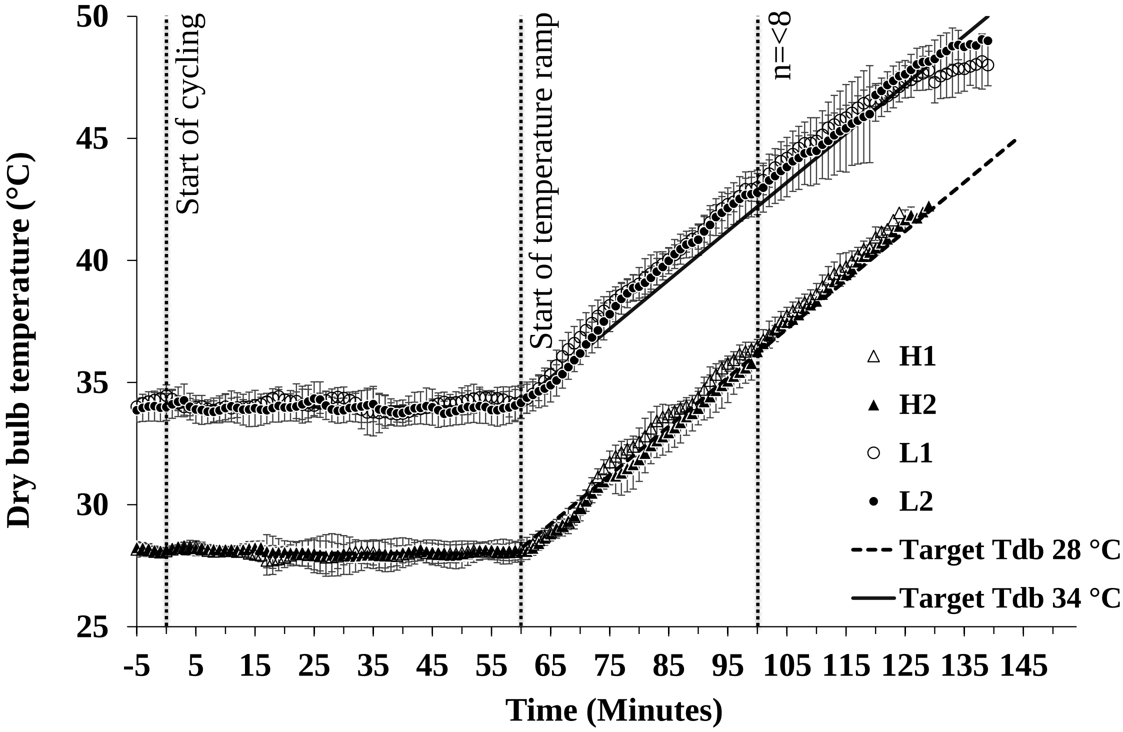


Figure 5.1. Mean (SD) T_{db} (°C) for test and retest of high (initial T_{db} of 28 °C) and low humidity (initial T_{db} of 34 °C) conditions, relative to target T_{db}. From the 100th minute, n reduces in each condition as individuals stop exercising at different points. Where H1 is the first test completed in the high humidity condition, H2 is the second test completed in the high humidity condition, L1 is the first test completed in the low humidity condition, and L2 is the second test completed in the low humidity condition.

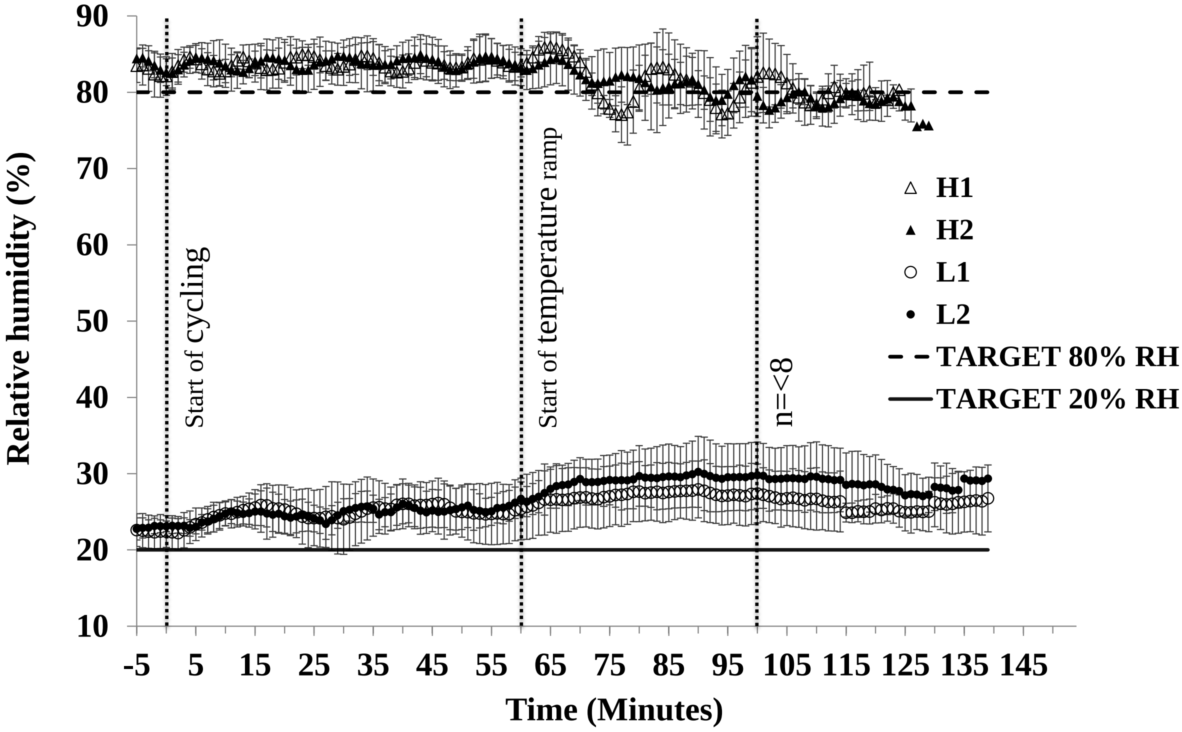


Figure 5.2. Mean (SD) RH for test and retest of high and low humidity conditions, relative to target RH. From the 100th minute, n reduces in each condition as individuals stop exercising at different points. Where H1 is the first test completed in the high humidity condition, H2 is the second test completed in the high humidity condition, L1 is the first test completed in the low humidity condition, and L2 is the second test completed in the low humidity condition.

Table 5.2. Mean (SD) and test-retest reliability (TEM in °C and CV as a percentage) of T_{db} over three time periods for high and low humidity conditions (n=8).

Time period	Mean (SD) Test 1 (% RH)	Mean (SD) Test 2 (% RH)	Change in the mean (°C)	TEM (°C)	CV (%)
High humidity condition					
Rest to 60th minute	28.03 (0.26)	28.06 (0.39)	0.04	0.36	1.27
Rest to 100th minute	29.66 (0.26)	29.51 (0.44)	-0.15	0.39	1.33
60th to 100th minute	32.21 (0.29)	31.76 (0.57)	-0.44	0.50	1.55
Low humidity condition					
Rest to 60th minute	34.11 (0.13)	33.96 (0.54)	-0.15	0.40	1.16
Rest to 100th minute	35.91 (0.23)	35.73 (0.56)	-0.18	0.42	1.16
60th to 100th minute	38.72 (0.47)	38.48 (0.59)	-0.24	0.51	1.32

Table 5.3. Mean (SD) and test-retest reliability (TEM in % RH and CV as a percentage) of RH over three time periods for high and low humidity conditions (n=8).

Time period	Mean (SD) Test 1 (% RH)	Mean (SD) Test 2 (% RH)	Change in the mean (% RH)	TEM (% RH)	CV (%)
High humidity condition					
Rest to 60th minute	83.68 (0.96)	83.87 (1.73)	0.19	1.61	1.92
Rest to 100th minute	82.84 (0.90)	83.06 (2.23)	0.22	1.81	2.18
60th to 100th minute	81.53 (1.19)	81.80 (3.08)	0.27	2.19	2.68
Low humidity condition					
Rest to 60th minute	24.63 (2.77)	24.62 (1.85)	-0.01	2.34	9.51
Rest to 100th minute	25.56 (2.84)	26.29 (2.38)	0.73	2.81	10.85
60th to 100th minute	27.03 (3.65)	28.93 (3.40)	1.90	3.92	14.01

5.3.2 Reliability of physiological and perceptual responses

Tables 5.4 to 5.6 show that the reliability for T_{re}, $\overline{T}_{sk}$ and HR is consistently below 10 % CV at the three selected time points. However, the CV was higher for SR and SkBF and more variable for $\dot{V}O_2$. In general, the test-retest reliability for perceptual responses appears to be better later in the protocol. The range of responses (the maximum and minimum response by any individual) are also given in Table 5.6 to show the spread of the data over the period where T_{db} was increasing.

Table 5.4. Mean (SD) and test-retest reliability (TEM in the units specified for each variable and CV as a percentage) of selected physiological and perceptual responses during the rest period (-5 to 0 minutes experimental time) (n=8). Target temperatures were: 28 °C in the High humidity condition and 34 °C in the Low humidity condition.

Variable (Units)	**Humidity condition**	**Mean (SD) Test 1**	**Mean (SD) Test 2**	**Change in the mean**	**TEM**	**CV** (%)
Thermal profile						
T_{re} (°C)	High	36.98 (0.28)	36.92 (0.35)	-0.06	0.14	0.39
	Low	36.86 (0.32)	36.81 (0.23)	-0.04	0.14	0.38
$\bar{T}_{sk}$ (°C)	High	33.33 (0.22)	33.37 (0.35)	0.04	0.24	0.71
	Low	34.86 (0.24)	34.87 (0.33)	0.02	0.22	0.62
Thermoeffector responses						
SR_{Back} ($L \cdot m^2 \cdot hr^{-1}$)	High	0.09 (0.02)	0.09 (0.02)	0.00	0.01	16.14
	Low	0.07 (0.03)	0.07 (0.04)	0.00	0.02	23.14
$SR_{Forearm}$ ($L \cdot m^2 \cdot hr^{-1}$)	High	0.10 (0.01)	0.09 (0.02)	0.00	0.01	14.51
	Low	0.06 (0.02)	0.06 (0.02)	0.00	0.01	17.52
$SkBF_{Forearm}$	High	10.37 (4.13)	13.05 (5.94)	2.68	3.40	29.01
(% of highest 5 mins)	Low	15.12 (4.68)	15.88 (8.07)	0.76	5.69	36.68
$SkBF_{Finger}$	High	47.52 (22.35)	65.73 (26.25)	18.22	14.87	26.25
(% of highest 5 mins)	Low	64.06 (26.53)	82.30 (14.70)	18.24	19.75	26.98
HR ($b \cdot min^{-1}$)	High	76.55 (8.03)	78.93 (7.90)	2.37	3.34	4.30
	Low	70.85 (22.63)	71.88 (22.76)	1.02	3.88	5.44
$\dot{V}O_2$ ($L \cdot min^{-1}$)	High	0.37 (0.05)	0.37 (0.09)	0.01	0.03	9.32
	Low	0.36 (0.08)	0.34 (0.06)	-0.02	0.04	12.52
Perceptual measures						
TS	High	10.98 (2.15)	9.99 (3.87)	-0.99	1.64	15.65
(VAS- 20cm scale)	Low	12.10 (1.44)	11.35 (1.70)	-0.75	1.01	8.60
TC	High	16.79 (1.68)	16.45 (2.32)	-0.34	1.68	10.11
(VAS- 20 cm scale)	Low	14.99 (2.39)	16.34 (2.11)	1.35	1.02	6.48
SW	High	4.09 (2.94)	2.89 (1.79)	-1.20	1.59	45.65
(VAS- 20 cm scale)	Low	2.36 (1.81)	2.85 (1.71)	0.49	1.29	49.49
RPE	High	8.50 (1.41)	7.88 (1.64)	-0.63	0.84	10.26
(Category ratio scale)	Low	8.25 (1.16)	8.00 (1.41)	-0.25	0.63	7.71

Table 5.5. Mean (SD) and test-retest reliability (TEM in the units specified for each variable and CV as a percentage) of selected physiological and perceptual responses over 55-60 minutes experimental time (representing the period of assumed steady state or near-steady state in T_{re}) in the high and low humidity condition when cycling at 60 W (n=8). Target temperatures were: 28 °C in the High humidity condition and 34 °C in the Low humidity condition.

Variable (Units)	Humidity condition	Mean (SD) Test 1	Mean (SD) Test 2	Change in the mean	TEM	CV (%)
Thermal profile						
T_{re} (°C)	High	37.49 (0.37)	37.39 (0.39)	-0.10	0.14	0.37
	Low	37.35 (0.38)	37.35 (0.28)	-0.01	0.16	0.44
$\bar{T}_{sk}$ (°C)	High	35.23 (0.32)	34.91 (0.28)	-0.32	0.25	0.72
	Low	35.98 (0.25)	35.98 (0.29)	0.00	0.17	0.47
Thermoeffector response						
SR_{Back} ($L·m^2·hr^{-1}$)	High	0.28 (0.07)	0.25 (0.05)	-0.03	0.03	12.81
	Low	0.28 (0.07)	0.27 (0.05)	-0.01	0.05	18.75
$SR_{Forearm}$ ($L·m^2·hr^{-1}$)	High	0.43 (0.10)	0.42 (0.20)	-0.01	0.14	32.51
	Low	0.47 (0.11)	0.44 (0.15)	-0.03	0.09	20.63
$SkBF_{Forearm}$	High	76.59 (23.02)	68.26 (18.41)	-8.34	15.64	21.60
(% of highest 5 mins)	Low	74.90 (15.68)	67.39 (25.52)	-7.51	18.41	25.88
$SkBF_{Finger}$	High	70.91 (24.71)	80.51 (18.04)	9.60	13.77	18.19
(% of highest 5 mins)	Low	73.75 (22.77)	80.77 (14.91)	7.03	10.67	13.81
HR ($b·min^{-1}$)	High	123.44 (14.10)	115.35 (17.22)	-8.09	6.36	5.33
	Low	117.00 (11.26)	118.49 (15.45)	1.49	4.90	4.16
$\dot{V}O_2$ ($L·min^{-1}$)	High	1.46 (0.24)	1.33 (0.18)	-0.13	0.27	19.50
	Low	1.36 (0.20)	1.29 (0.07)	-0.06	0.10	7.79
Perceptual measures						
TS	High	15.33 (1.33)	14.41 (1.29)	-0.91	0.81	5.43
(VAS- 20cm scale)	Low	15.28 (0.68)	14.65 (1.24)	-0.63	0.83	5.54
TC	High	8.40 (3.27)	11.29 (2.31)	2.89	3.22	32.69
(VAS- 20 cm scale)	Low	9.79 (3.21)	11.03 (2.44)	1.24	1.97	18.93
SW	High	12.99 (4.13)	11.34 (2.99)	-1.65	1.97	16.19
(VAS- 20 cm scale)	Low	9.51 (3.29)	9.53 (3.09)	0.01	1.89	19.91
RPE	High	12.38 (1.60)	11.13 (1.73)	-1.25	1.40	11.93
(Category ratio scale)	Low	12.00 (0.93)	11.13 (1.55)	-0.88	1.03	8.91

Table 5.6. Mean (SD) and test-retest reliability (TEM in the units specified for each variable and CV as a percentage**)** of selected physiological and perceptual responses during period of increasing T_{db} (60-100 minutes experimental time) in the high and low humidity condition when cycling at 60 W (n=8). Target temperatures were: 28 °C increasing to 36 °C in the High humidity condition and 34 °C increasing to 42 °C in the Low humidity condition.

Variable (Units)	Humidity condition	Mean (SD) Test 1	Mean (SD) Test 2	Range Test 1	Range Test 2	Change in the mean	TEMs	CV (%)
Thermal profile								
T_{re} (°C)	High	37.64 (0.38)	37.50 (0.38)	37.00 – 38.39	37.01 – 38.52	-0.14	0.16	0.42
	Low	37.48 (0.35)	37.49 (0.28)	36.86 – 38.12	37.04 – 38.10	0.01	0.16	0.43
$\bar{T}_{sk}$ (°C)	High	36.19 (0.63)	35.83 (0.66)	34.88 – 37.51	34.33 – 37.51	-0.36	0.20	0.55
	Low	36.73 (0.54)	36.78 (0.52)	35.59 – 37.81	35.60 – 37.87	0.05	0.22	0.59
Thermoeffector response								
SR_{Back} ($L·m^2·hr^{-1}$)	High	0.39 (0.12)	0.35 (0.10)	0.17 – 0.76	0.17 – 0.72	-0.04	0.05	14.77
	Low	0.33 (0.08)	0.33 (0.07)	0.15 – 0.47	0.21 – 0.52	0.00	0.05	16.81
$SR_{Forearm}$ ($L·m^2·hr^{-1}$)	High	0.63 (0.17)	0.58 (0.18)	0.35 – 0.95	0.21 – 0.89	-0.04	0.14	23.77
	Low	0.58 (0.12)	0.54 (0.16)	0.34 – 0.83	0.21 – 0.87	-0.04	0.09	17.13
$SkBF_{Forearm}$	High	87.18 (12.34)	83.69 (12.30)	48.85 – 118.76	46.90 – 101.41	-3.48	9.37	11.39
(% of highest 5 mins)	Low	86.29 (13.51)	75.61 (22.53)	55.91 – 122.69	37.11 – 122.64	-10.68	17.35	21.52
$SkBF_{Finger}$	High	78.71 (18.22)	85.15 (11.96)	11.60 – 97.34	43.94 – 100.30	6.44	9.96	12.26
(% of highest 5 mins)	Low	81.85 (12.64)	84.52 (9.81)	41.77 – 104.71	52.95 – 100.00	2.67	9.49	11.43
HR ($b·min^{-1}$)	High	138.09 (16.00)	128.44 (21.37)	98.33 – 170.02	94.22 – 181.10	-9.65	9.09	6.79
	Low	128.01 (14.00)	127.26 (14.22)	104.15 –156.58	101.92 – 160.03	-0.75	5.53	4.31
$\dot{V}O_2$ ($L·min^{-1}$)	High	1.52 (0.24)	1.40 (0.20)	1.19 – 2.07	1.05 – 1.73	-0.11	0.26	17.50
	Low	1.46 (0.18)	1.38 (0.09)	1.13 – 1.84	1.10 – 1.54	-0.08	0.11	7.72

Perceptual measures								
TS	High	16.61 (1.17)	16.14 (1.43)	13.80 – 18.50	12.40 – 18.30	-0.47	0.65	4.01
(VAS- 20cm scale)	Low	16.11 (0.81)	15.67 (1.57)	14.70 – 17.50	12.20 – 18.70	-0.45	0.80	5.06
TC	High	6.30 (3.17)	8.43 (3.07)	1.50 – 11.50	3.20 – 13.50	2.13	3.23	44.76
(VAS- 20 cm scale)	Low	7.34 (3.73)	8.76 (3.67)	2.30 – 14.10	1.30 – 13.70	1.42	2.04	25.08
SW	High	15.56 (3.00)	14.65 (3.65)	10.90 – 20.00	6.60 – 20.00	-0.91	2.01	13.42
(VAS- 20 cm scale)	Low	12.73 (3.88)	12.22 (2.94)	5.30 – 19.20	7.30 – 17.90	-0.51	1.71	13.81
RPE	High	13.79 (1.50)	12.83 (1.79)	11.00 – 17.00	10.00 – 17.00	-0.96	1.30	9.76
(Category ratio scale)	Low	13.25 (1.15)	12.63 (1.56)	12.00 – 16.00	10.00 – 15.00	-0.63	1.03	8.01

5.3.3 Reliability of the identification of the inflection point in T_{re}

Three methods were used for identification of the T_{re} inflection, Figure 5.3 shows an example data set indicating the T_{re} inflection point as identified by each of the three methods. There was no significant main effect of the method used to identify the inflection in T_{re} or significant effect of the method used between the conditions on mean T_{re} $F_{(2,21)}$= 0.032, p=0.969, mean T_{db} $F_{(2,21)}$=2.068, p=0.151 and experimental time at T_{re} inflection $F_{(2,21)}$=1.837, p=0.184. Table 5.7 shows the reliability of the three methods to determine the experimental time at T_{re} inflection in each Test.

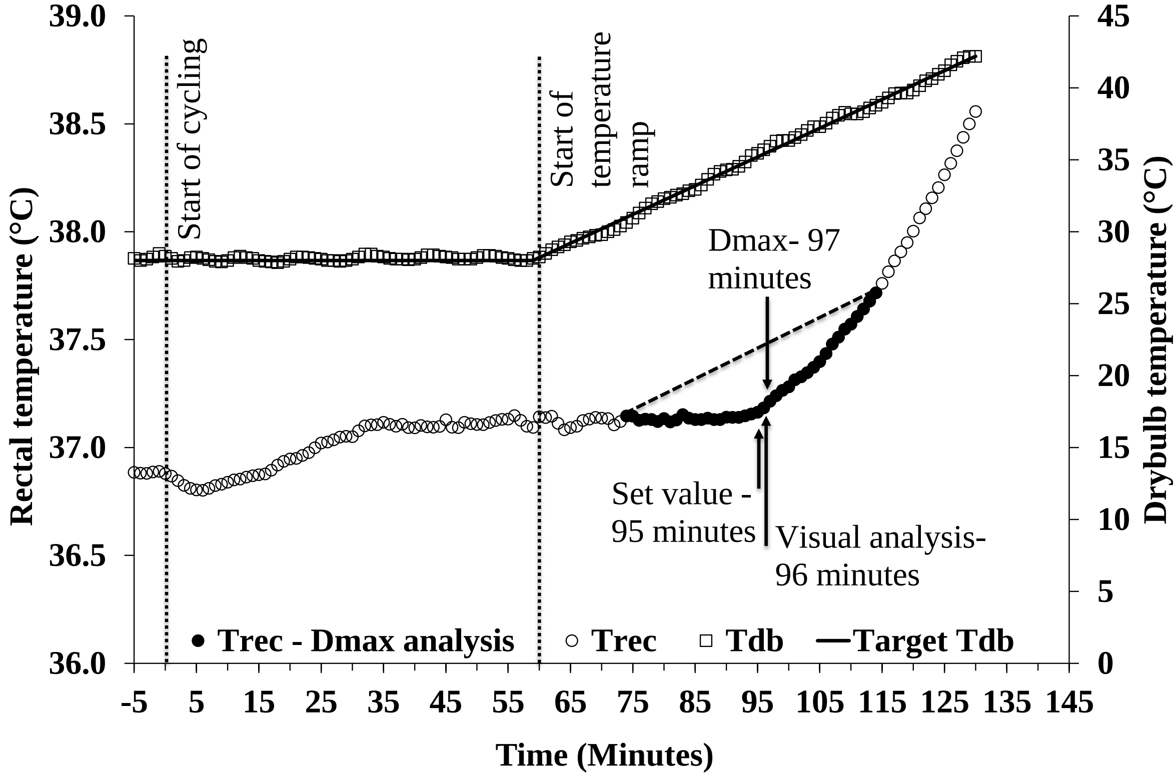


Figure 5.3. Example data set from a single participant comparing the three methods of identifying the inflection point in T_{re}. Closed circles = visually assessed window for D_{max} method, open circles = full data set, Open squares = measured T_{db}, Solid line = target T_{db}.

Table 5.7. Mean (SD) and test-retest reliability (TEM in minutes and CV as a percentage) of the experimental time at the inflection in rectal temperature (°C) as defined by each of the three chosen methods (n=8).

Method	Humidity condition	Mean (SD)Test 1 (Minutes)	Mean (SD)Test 2 (Minutes)	Change in the mean (Minutes)	TEMs (Minutes)	CV (%)
Visual	High	86.50 (5.63)	88.75 (10.47)	2.25	6.50	7.42
	Low	87.63 (13.52)	90.63 (17.77)	3.00	10.12	11.36
D_{max}	High	87.50 (4.66)	90.63 (9.91)	3.13	6.58	7.39
	Low	89.75 (12.28)	90.75 (16.32)	1.00	7.47	8.28
Set-point	High	80.13 (7.77)	87.88 (12.96)	7.75	9.14	10.88
	Low	80.50 (10.23)	80.63 (8.90)	0.13	6.26	7.77

5.3.3.1 *Method 1 – visual assessment*

At the point of T_{re} inflection, mean T_{db} values in H2 were not normally distributed, Friedman's test indicated differences between the conditions ($\chi^2_{(3)}$=19.350, p=<0.001). *Post-hoc* analysis indicated that T_{db} was not significantly different within the two humidity conditions (H1 *vs*. H2 z=-0.280 p=0.779; L1 *vs*. L2 z=<0.001 p=1.000) but, as expected, the T_{db} in the low humidity conditions was significantly higher than in the high humidity conditions (L1 *vs*. H1 z=-2.521 p=0.012; L2 *vs*. H1 z=-2.521 p=0.012; L1 *vs*. H2 z=-2.521 p=0.012; L2 *vs*. H2 z=-2.521 p=0.012). At the same comparison point there was no significant effect of humidity, test or an interaction effect between the conditions on mean T_{re} ($F_{(1,7)}$=0.830 p=0.392, $F_{(1,7)}$=1.067 p=0.336 and $F_{(1,7)}$=4.097 p=0.083 respectively), or a significant difference between the two humidity conditions in mean experimental time at the T_{re} inflection ($\chi^2_{(3)}$=1.350 p=<0.717) (see Table 5.9).

Assessor A and B chose an inflection point within + / - 3 minutes of one another for 37 out of 64 thermal profiles, therefore the opinion of Assessor C was required for 27 thermal profiles. On those occasions, Assessor C and either A or B chose an inflection point within ±3 minutes of one another for 17 thermal profiles, meaning that a group discussion was needed for 10 out of the thermal profiles where agreement was made in all cases. Additionally, Table 5.8 shows the reliability of each independent assessor.

Table 5.8. Mean (SD) and test-retest reliability (TEM in minutes and CV as a percentage) for three independent assessors judging the point of inflection of rectal temperature in eight participants (64 profiles in total) exercising in the heat with either a low and high humidity (n=8).

Assessor	Mean T1 (SD) Time (minutes)	Mean T2 (SD) Time (minutes)	Change in the mean Time (minutes)	TEMs Time (minutes)	CV (%)
A	84.03 (11.84)	86.06 (11.09)	2.03	7.98	9.39
B	84.00 (20.99)	85.81 (21.46)	1.81	3.62	4.26
C	84.41 (19.93)	85.50 (19.84)	0.09	2.91	3.41

5.3.3.2 *Method 2 – D_{max} assessment*

At the point of T_{re} inflection, mean T_{db} values in H2 were not normally distributed, a non-parametric Friedman's test indicated differences between the conditions ($\chi^2_{(3)}$=19.350 p=<0.001). *Post-hoc* analysis indicated that T_{db} was not significantly different within the two humidity conditions (H1 *vs*. H2 z=<0.000 p=1.000; L1 *vs*. L2 z=-0.560 p=0.575) but, as expected, the T_{db} in the low humidity conditions were significantly higher than the high humidity conditions (L1 *vs*. H1 z=-2.521 p=0.012; L2 *vs*. H1 z=-2.524 p=0.012; L1 *vs*. H2 z=-2.521 p=0.012; L2 *vs*. H2 z=-2.521 p=0.012). At the same comparison point, there was no significant effect of humidity, test or an interaction effect between the conditions on mean T_{re} ($F_{(1,7)}$=0.499 p=0.503, $F_{(1,7)}$=0.718 p=0.425 and $F_{(1,7)}$=3.618 p=0.099 respectively), nor mean time of inflection ($F_{(1,7)}$=0.073 p=0.795, $F_{(1,7)}$=0.966 p=0.359 and $F_{(1,7)}$=0.141 p=0.718 respectively) (see Table 5.9).

5.3.3.3 *Method 3 – Increase of 0.1 °C above the T_{re} value at 60 minutes (set value)*

At the point of T_{re} inflection, there was a significant effect of humidity but not test or an interaction effect on T_{re}, at the point of T_{re} inflection between the conditions ($F_{(1,7)}$=49.661 p=<0.001, $F_{(1,7)}$=0.244 p=0.636 and $F_{(1,7)}$=0.355 p=0.570 respectively). At the same comparison point, there was no significant effect of humidity, test or an interaction effect between the conditions on mean T_{re} ($F_{(1,7)}$=3.367 p=0.109, $F_{(1,7)}$=0.379 p=0.558 and $F_{(1,7)}$=0.975 p=0.402 respectively) nor mean time of inflection ($F_{(1,7)}$=0.929 p=0.367, $F_{(1,7)}$=1.868 p=0.214 and $F_{(1,7)}$=2.063 p=0.194 respectively) (see Table 5.9).

Table 5.9. Mean (SD) T_{db} and T_{re} (°C) at the point of T_{re} inflection (as defined by the three chosen methods) (n=8).

Method	H1	H2	L1	L2
T_{db} (°C)				
Visual	34.13 (0.85)	33.68 (2.52)	40.69 (2.93)*	40.63 (3.35)*
D_{max}	34.21 (0.9)	34.29 (2.25)	41.24 (2.61)*	40.70 (2.73)*
Set-value	32.70 (1.97)	33.45 (3.07)	39.03 (2.42)*	38.96 (2.16)*
T_{re} (°C)				
Visual	37.66 (0.43)	37.51 (0.40)	37.51 (0.40)	37.53 (0.29)
D_{max}	37.66 (0.43)	37.52 (0.39)	37.53 (0.40)	37.55 (0.30)
Set-value	37.61 (0.37)	37.53 (0.39)	37.47 (0.38)	37.47 (0.27)

* significantly higher than H1 & H2 (P<0.05)

5.3.4 Retest reliability of the protocol to induce similar physiological and perceptual responses

The reliability of the thermoregulatory and perceptual variables at the point of inflection of T_{re} (or nearest value in the case of $\dot{V}O_2$ and all perceptual ratings) was influenced by the inflection determination method, as presented in Tables 5.10 to 5.12. Briefly, the visual and D_{max} methods gave similar results to one another, but in the main the set value method resulted in lower mean values in the thermal profile and thermoeffector response, therefore higher levels of TC and RPE.

Table 5.10. Mean (SD) and test-retest reliability (TEM in the units specified for each variable and CV as a percentage for the measured variables at the point (or nearest value – $\dot{V}O_2$ and perceptual ratings) of inflection of rectal temperature, using visual assessment method for participants exercising in the heat with either a low and high humidity (n=8).

Variable	Humidity condition	Mean (SD) Test 1	Mean (SD) Test 2	Change in the mean	TEMs	CV (%)
Thermal profile						
T_{re} (°C)	High	37.66 (0.43)	37.51 (0.40)	-0.15	0.13	0.35
	Low	37.51 (0.40)	37.53 (0.29)	0.02	0.17	0.45
T_{db} (°C)	High	34.13 (0.85)	33.68 (2.52)	-0.46	1.94	5.72
	Low	40.69 (2.93)	40.63 (3.35)	-0.06	2.38	5.86
$\bar{T}_{sk}$ (°C)	High	36.55 (0.25)	36.32 (0.43)	-0.23	0.29	0.80
	Low	37.04 (0.57)	37.17 (0.42)	0.13	0.44	1.20
Thermoeffector response						
SR_{Back} ($L \cdot m^2 \cdot hr^{-1}$)	High	0.41 (0.12)	0.40 (0.13)	-0.01	0.05	11.60
	Low	0.35 (0.09)	0.40 (0.18)	0.05	0.13	33.99
$SR_{Forearm}$ ($L \cdot m^2 \cdot hr^{-1}$)	High	0.68 (0.16)	0.69 (0.18)	0.01	0.10	14.86
	Low	0.64 (0.16)	0.62 (0.19)	-0.02	0.11	17.55
$SkBF_{Forearm}$	High	91.44 (8.78)	86.53 (6.71)	-4.91	8.22	9.23
(% of highest 5 mins)	Low	104.97 (28.52)	88.00 (18.38)	-16.97	25.00	25.92
$SkBF_{Finger}$	High	81.96 (11.74)	89.39 (15.31)	7.43	11.32	13.22
(% of highest 5 mins)	Low	85.41 (12.13)	86.26 (14.20)	0.85	12.99	15.13
HR ($b \cdot min^{-1}$)	High	145.03 (17.67)	135.47 (18.31)	-9.56	5.95	4.24
	Low	133.35 (16.40)	133.06 (17.45)	-0.29	8.23	6.18
$\dot{V}O_2$ ($L \cdot min^{-1}$)	High	1.52 (0.20)	1.38 (0.18)	-0.14	0.22	15.33
	Low	1.52 (0.20)	1.40 (0.11)	-0.12	0.16	10.93
Perceptual measures						
TS	High	16.78 (0.77)	16.40 (1.27)	-0.31	1.00	6.05
(VAS-20cm scale)	Low	16.31 (0.51)	16.66 (1.03)	0.35	0.70	4.25
TC	High	5.94 (2.29)	7.94 (2.23)	2.64	2.45	36.96
(VAS-20 cm scale)	Low	7.15 (3.30)	7.58 (2.85)	0.42	3.63	49.36
SW	High	16.04 (2.50)	15.28 (3.73)	-1.14	1.44	9.07
(VAS-20 cm scale)	Low	13.21 (3.38)	12.68 (3.60)	-0.54	2.85	22.05
RPE	High	14.00 (1.07)	13.13 (1.46)	-1.00	1.00	7.34
(Category ratio scale)	Low	13.50 (1.20)	12.88 (2.95)	-0.50	2.07	15.62

Table 5.11. Mean (SD) and test-retest reliability (TEM in the units specified for each variable and CV as a percentage for the measured variables at the point or nearest value – $\dot{V}O_2$ and perceptual ratings) of inflection of rectal temperature, using the D_{max} method for participants exercising in the heat with either a low and high humidity (n=8).

Variable	Humidity condition	Mean (SD) Test 1	Mean (SD) Test 2	Change in the mean	TEMs	CV (%)
Thermal profile						
T_{re} (°C)	High	37.66 (0.40)	37.52 (0.39)	-0.14	0.15	0.39
	Low	37.53 (0.40)	37.55 (0.30)	0.02	0.17	0.46
T_{db} (°C)	High	34.21 (0.90)	34.24 (2.25)	0.08	1.71	5.01
	Low	41.24 (2.61)	40.70 (2.73)	-0.54	1.56	3.80
$\bar{T}_{sk}$ (°C)	High	36.59 (0.26)	36.43 (0.42)	-0.16	0.31	0.85
	Low	37.13 (0.48)	37.19 (0.43)	0.06	0.34	0.90
Thermoeffector responses						
SR_{Back} ($L \cdot m^2 \cdot hr^{-1}$)	High	0.43 (0.13)	0.44 (0.20)	0.01	0.08	18.35
	Low	0.35 (0.09)	0.38 (0.13)	0.03	0.10	26.16
$SR_{Forearm}$ ($L \cdot m^2 \cdot hr^{-1}$)	High	0.70 (0.16)	0.71 (0.16)	0.00	0.11	15.33
	Low	0.65 (0.15)	0.62 (0.18)	-0.03	0.11	17.98
$SkBF_{Forearm}$	High	80.47 (33.17)	92.28 (6.99)	-8.53	17.63	18.26
(% of highest 5 mins)	Low	91.53 (5.57)	92.63 (30.52)	1.10	19.61	21.30
$SkBF_{Finger}$	High	81.99 (11.91)	81.13 (21.63)	-0.85	11.92	14.61
(% of highest 5 mins)	Low	90.16 (11.82)	82.48 (14.15)	-7.68	10.01	11.59
HR ($b \cdot min^{-1}$)	High	143.24 (14.97)	133.54 (16.64)	-9.70	5.99	4.33
	Low	134.95 (14.56)	132.73 (16.80)	-2.22	7.98	5.96
$\dot{V}O_2$ ($L \cdot min^{-1}$)	High	1.53 (0.21)	1.38 (0.16)	-0.15	0.21	14.10
	Low	1.51 (0.25)	1.42 (0.09)	-0.09	0.17	11.33
Perceptual measures						
TS	High	16.81 (0.79)	16.79 (1.05)	0.04	0.60	3.58
(VAS-20cm scale)	Low	16.35 (0.58)	16.75 (0.99)	0.40	0.66	3.97
TC	High	5.81 (2.48)	7.51 (2.41)	2.34	2.52	39.76
(VAS-20 cm scale)	Low	6.84 (3.32)	7.13 (3.02)	0.29	3.21	45.93
SW	High	16.11 (2.54)	15.73 (3.59)	-0.70	1.41	8.73
(VAS-20 cm scale)	Low	13.34 (3.52)	12.93 (3.69)	-0.41	2.84	21.64
RPE	High	14.13 (1.13)	13.25 (1.39)	-1.00	1.00	7.27
(Category ratio scale)	Low	13.63 (1.19)	13.00 (2.78)	-0.50	1.81	13.55

Table 5.12. Mean (SD) and test-retest reliability (TEM in the units specified for each variable and CV as a percentage for the measured variables at the point or nearest value – $\dot{V}O_2$ and perceptual ratings) of inflection of rectal temperature, using arise in 0.1° C from the value of rectal temperature after 60 minutes of cycling for participants exercising in the heat with either a low and high humidity (n=8).

Variable	Humidity condition	Mean (SD) Test 1	Mean (SD) Test 2	Change in the mean	TEMs	CV (%)
Thermal profile						
T_{re} (°C)	High	37.61 (0.37)	37.53 (0.39)	-0.08	0.20	0.42
	Low	37.47 (0.38)	37.47 (0.27)	0.00	0.20	0.41
T_{db} (°C)	High	32.70 (1.97)	33.45 (3.07)	0.75	2.10	6.45
	Low	39.03 (2.42)	38.96 (2.16)	-0.06	1.70	4.47
$\bar{T}_{sk}$ (°C)	High	36.22 (0.43)	36.25 (0.73)	0.03	0.50	1.46
	Low	36.74 (0.69	36.85 (0.43)	0.10	0.30	0.79
Thermoeffector response						
SR_{Back} ($L \cdot m^2 \cdot hr^{-1}$)	High	0.40 (0.14)	0.40 (0.13)	0.00	0.10	13.70
	Low	0.34 (0.08)	0.32 (0.05)	-0.02	0.00	14.10
$SR_{Forearm}$ ($L \cdot m^2 \cdot hr^{-1}$)	High	0.65 (0.18)	0.69 (0.18)	0.04	0.20	24.10
	Low	0.59 (0.10)	0.56 (0.12)	-0.03	0.10	11.74
$SkBF_{Forearm}$	High	85.04 (10.94)	89.63 (9.25)	4.6	9.0	10.25
(% of highest 5 mins)	Low	86.72 (7.98)	79.85 (19.37)	-6.88	13.62	16.35
$SkBF_{Finger}$	High	84.70 (12.21)	93.78 (10.75)	9.08	9.28	10.40
(% of highest 5 mins)	Low	77.41 (24.02)	86.57 (15.00)	9.16	22.52	27.46
HR ($b \cdot min^{-1}$)	High	138.61 (14.52)	138.40 (22.55)	-0.22	10.10	7.30
	Low	127.65 (15.55)	127.27 (13.52)	-0.38	5.80	4.58
$\dot{V}O_2$ ($L \cdot min^{-1}$)	High	1.51 (0.21)	1.44 (0.17)	-0.1	0.2	11.71
	Low	1.45 (0.19)	1.32 (0.10)	-0.11	0.11	7.71
Perceptual measures						
TS	High	16.53 (1.16)	16.36 (1.41)	-0.25	1.10	6.68
(VAS 20cm scale)	Low	16.00 (0.74)	15.39 (1.79)	-0.61	1.11	7.07
TC	High	6.84 (3.34)	8.56 (3.35)	2.58	3.05	41.95
(VAS 20cm scale)	Low	7.49 (3.70)	9.80 (3.30)	2.31	2.31	26.77
SW	High	41.98 (3.38)	14.43 (3.44)	-0.91	2.13	14.31
(VAS 20cm scale)	Low	11.88 (3.36)	11.28 (2.66)	-0.60	1.52	13.13
RPE	High	13.50 (1.51)	13.13 (1.36)	-0.50	1.07	7.99
(Category ratio scale)	Low	13.13 (1.36)	12.25 (1.67)	-0.88	0.80	6.28

5.4 Discussion

The aims of this chapter were to: i) develop a reliable laboratory protocol enabling T_{db} to be incremented in a reliable manner after an initial, stable, baseline period and under conditions of high and low relative humidity, ii) determine the reliability of the associated human thermoregulatory and perceptual responses during such a protocol and iii) to develop a reliable a method of determining the inflection point in T_c with an increasing T_{db} under conditions of high and low relative humidity.

The reliability (expressed as CV) of the T_{db} ranged between 1.16 to 1.55 % with a TEM of 0.36 to 0.51 °C. In similar previous studies where T_{db} was progressively increased until an inflection in T_c was evident, the authors did not report reliability data for their ambient conditions so no comparisons can be made (Kenney *et al*., 1993; Kenney & Zeman, 2002). Nevertheless, given the high repeatability of T_{db} that was evident, it would appear that the present protocol is reproducible and can be deemed acceptable for the purposes of the studies reported in this thesis. Compared to T_{db}, RH was less reliable in terms of CV (see Table 5.3), particularly in the low humidity condition. However, the TEM ranged from 1.61 to 3.92 % RH which is acceptable given the constraints of the environmental chamber, and so this approach can be considered reliable. It was not possible to control the influence of the evaporation of sweat from the participants and experimenters into the environmental chamber in the second hour of the test in the low humidity condition as it was necessary to switch-off the non-integrated standalone dehumidification system. This was to prevent the cooler air introduced into the chamber by the dehumidification system from increasing the difficulty of manually controlling the progressive temperature increase. This can be seen in Figure 5.2, but this phenomenon occurred consistently. It was of primary concern was to ensure that the high and low humidity conditions were clearly distinct from one another. Maughan, Otani, and Watson (2012) found that when exercising in the heat (30.2 [0.2] °C) there was no significant decrement in time to exhaustion between 24 % RH and 40 % RH, but a significantly reduced time to exhaustion when comparing 24 % with 60% RH and 80 % RH, highlighting the need for clear differences between the high and low humidity conditions, specifically to have two environments with distinctly different P_a, which was achieved in this study (see Figure 5.2).

It is extremely difficult to separate biological variability from technical and measurement error. Despite controlled conditions and appropriate pre-test instructions (see 3.2.3 and

3.2.4) in an effort to enhance reliability, some amount of error will always be present with continuous measurements (Atkinson & Nevill, 1998). The CV of T_{re} in the present study was between 0.37 to 0.51 % over the various time points assessed, which is consistent with the mean CV of 0.3 % reported for T_{re} in adults exercising at 29.5 % of peak work rate for 60 minutes in 36 °C 60 % RH on three occasions (Hayden *et al*., 2004) and the 0.6 % reported for T_{au} in adolescents exercising for three bouts of 20 minutes at 45 % $\dot{V}O_{2peak}$ in 35.1 °C 46.6 % RH on two occasions (Brokenshire *et al*., 2009). Unfortunately, a study by Barnett and Maughan (1993) which looked at the T_{re}, $\overline{T}_{sk}$, sweat loss and $\dot{V}O_2$ for five males exercising in 34.6 °C, 60 % RH for 60 minutes at 55 % of $\dot{V}O_{2max}$ on three occasions, did not report any reliability statistics, only that there was no significant differences between the trials with a RM ANOVA. Similarly, the CV of $\overline{T}_{sk}$ in the present study ranged from 0.37 to 0.71 % across the various time points which is in general agreement with Hayden *et al*. (2004) who report a CV of 0.7 % using three sites and Brokenshire *et al*. (2009) who report a CV of 0.5 to 1.5 % using four sites with the Ramanathan (1964) weighting. This indicates that the choice to use eight sites used in this study (BS EN ISO 9886, 2004) did not noticeably influence the reliability of the $\overline{T}_{sk}$ relative to other studies, but of course allows for greater precision when wishing to compare the variability in skin temperature across the body, for example comparing active with non-active muscles, and skin areas exposed to radiant heat load or forced convective cooling.

The CV of HR in the present study at various time points varied from 3.77 to 7.21 % which in general is slightly higher than the previous reports of 3.9 % (Hayden *et al*., 2004) and 4 % (Brokenshire *et al*., 2009). Clearly, differences between the studies in terms of participants, and exercise protocol (duration, work rate, ambient conditions *etc*.) would affect HR. As T_c rises, HR increases even with fixed work rates and ambient conditions, (Wright, Selkirk, Rhind, and McLellan 2012). This is due to competition for blood flow for thermoregulation and exercise (Arngrímsson *et al*., 2004; González-Alonso, 2012; Nybo *et al*., 2001), leading to cardiovascular drift which is accompanied by a decrease in $\dot{V}O_{2max}$ (Wingo *et al*., 2005) and, in extremes, can result in a reduction in cardiac output (González-Alonso & Calbet, 2003). It may be the case that the CV for HR in this study (3.77 – 7.21 %) was affected by this phenomenon, although the reliability values reported under these hot conditions are similar to those reported for exercise in cooler conditions (4.4 and 5.1 % depending on work rate [Wilmore *et al*., 1998]).

SkBF as measured by laser Doppler flowmetry, has been shown to have varying levels of reliability. Abbink, Wollersheim, Netten, and Smits (2001) reported short-term (1.5 h) and long-term (7 days) test retest reliability of 25.4 % and 37.3 % respectively for SkBF at the nail fold of five healthy, normotensive, non-smoking volunteers (three males and two females), aged 25 to 48 years. They concluded that the short-term CV is high but acceptable for the physiological variable SkBF and that laser Doppler flowmetry is better suited for measurements of acute changes in SkBF rather than investigating long-term effects. Previous research has shown that movement of a laser-Doppler probe over distances of 2-6 mm caused 100 % variation in SkBF (Braverman, Keh, & Goldminz, 1990). The participants in the present study had at least 48 hours between testing, with a maximum of 10 weeks between their first and last session, which may in part explain why CV ranged from 3.89 to 49.01 %. Future studies measuring SkBF should consider trying to balance the potential improvement in reliability afforded through reducing the time between tests with the possibility of acclimation effects induced by repeated heat exposures with short recovery periods.

There are many ways of measuring SR, and the limited data available shows varying levels of reliability. Using a different method, closed-pouch sweat collection, local SR mean (SD) CV for the scapula, forearm and thigh were 10.3 (5.2) %, 6.3 (5.6) %, 16.5 (9.1) % respectively, for seven exercising males over three repeated trials (Hayden *et al*., 2004). In the present study, the ventilated sweat capsule appears to have a higher CV (10.5 to 33.49 %), which is similar to previous reports also using this method of within-subject reliability of 22.3 % (Kenefick *et al*., 2012). A repeated measures design with at least 48 hours between visits was necessary for this protocol in order to compare the response of each individual to the same condition on two occasions, reduce the negative impact of fatigue, and minimise heat acclimation. However, this meant that there may have been variability in the placement of thermistors, sensors, ventilated sweat capsules and laser Doppler probes, despite careful placement being used in order to reduce this effect. Nevertheless, Machado-Moreira, Smith, van den Heuvel, Mekjavić and Taylor (2008) have shown that the torso does not have a uniform distribution of thermally-induced sweating, therefore any variability in capsule placement (either at the start of testing, or as a consequence of movement during testing) may increase the measurement error and therefore the test-retest reliability.

In the present study, physiological and perceptual variables were divided into 5 minute averages for analysis, whereas comparative studies tend to calculate their CV over the whole experimental trial *e.g.* 60 minutes of exercise. It was not appropriate to calculate CV over the full time period in this case for two reasons: i) it was not a fixed duration protocol meaning that the participants may become exhausted and finish at different time points; and ii) there are three distinct phases in the protocol which may affect physiological or perceptual responses. This will influence the calculated CV, because the mean and SD will vary depending on how many data points are included in its calculation.

The methods used to identify the inflection were subjective (visual assessment), part-subjective / objective (D_{max}) and objective (set point). Values for T_{re} and T_{db} at the inflection point were in close agreement across the three Methods (Table 5.9). Limited comparison can be drawn between the current studies' retest reliability and that of Kenney and Zeman (2002) and Dougherty *et al*. (2010) given that their study used an inappropriate method of calculating reliability (see Appendix D).

As a fully subjective method, for the visual assessment method to be deemed satisfactory, it must be reproducible, therefore the intra-reliability of the visual assessment method was assessed. Table 5.8 shows that CV was 9.39, 4.26, 3.41 % for assessors A, B, C respectively, which is similar to the intra-observer CVs of 6.1 and 8.9 %, reported for two independent assessors who visually reassessed (blinded) 179 tests for ventilatory threshold (Myers *et al*., 2010). Clearly it is difficult to draw comparisons as every individual will have a different CV and is likely to improve with experience. The typical errors for the present study's assessors ranged between 7.98, 3.62, 2.91 minutes which would equate to a change in ambient T_{db} of approximately 1.6, 0.8, 0.6°C. Moreover, it appeared that despite written and verbal instructions being provided, there were subtle differences in interpretations of these instructions by each assessor. In the future, increased instruction clarity and example plots for assessors prior to the independent assessment could potentially reduce intra-assessor variability.

The D_{max} method was chosen by Kenney *et al*. (1993) because it was claimed that the mathematical identification of the precise point within the subjectively chosen window prevents investigator bias in the identification of the breakpoint. However, the analysis window is subjectively chosen, and manipulation of the size and position of the window can result in the movement of the mathematically derived inflection point. Another limitation of this method, is that in some circumstances the inflection point occurred close to the end of the test, which only allowed a small window of data to be selected for analysis. However, it was the method with the lowest CV (Table 5.7) and has reduced subjectivity in comparison with the visual method.

Using the set point method produced statistically non-significantly different results to the other methods, with the advantage of being less time consuming and entirely objective. However, the face validity (whether a test actually measures what it intends) of the chosen inflection points was questionable in a number of instances. In particular, some individuals exhibited a pseudo-steady state in T_{re} after the initial 60 minute steady-state period, whereby a progressive drift in T_{re} was evident before a clear breakpoint occurred. Previous authors (Kenney & Zeman, 2002) have also observed that some individuals, and in particular those with low aerobic fitness, do not achieve a clear plateau in T_{re} over a 60 minute period of exercise at a fixed external work rate. For these individuals the identified inflection point was clearly before the 'true' breakpoint.

There is no clear-cut definition or threshold with respect to an acceptable CV or TEM (Atkinson & Nevill, 1998); the researchers should, therefore, be guided by previous literature and common sense as to what is acceptable for that particular measure. The present study has shown that T_{db} can be increased in a reliable manner and that RH can be repeatability controlled within acceptable levels. The protocol that was employed caused an inflection in T_c which could be reliably detected using visual analysis, D_{max} method and an increase of 0.1 °C above the value at 60 minutes, although the face validity of this latter method is questionable. Importantly, a clear inflection in T_{re}, which was taken to represent the upper limit of the TZ, was identified in all of participants in each condition; this was not the case in the previous experimental Chapter. Physiological variables such as the SkBF and SR in the current study have relatively large CVs, but it has been argued that a CV of approximately 20 % is still acceptable for parameters that show a considerable biological variation (Abbink *et al.*, 2001). Given the paucity of available papers, there remains a clear need for further reliability data examining key thermo-physiological and perceptual measures. Many thermoregulatory studies employ repeated measures designs, but compare experimental conditions rather than using a test-retest protocol. This leaves current knowledge limited on the variability of the measurements used, especially SkBF and SR during exercise in the heat.

In conclusion, this study has been successful in its three aims:1) to develop a reliable laboratory protocol enabling T_{db} to be incremented in a reliable manner after an initial, stable, baseline period and under conditions of high and low relative humidity, 2) to determine the reliability of the associated human thermoregulatory responses during such a protocol and 3) to develop a reliable a method of determining the inflection point in T_c with an increasing T_{db} under conditions of high and low relative humidity. It has been shown that the high and low humidity conditions chosen can be reliably reproduced with a clear distinction between the initial stable 60 minutes and the incremental rise in T_{db} for the remainder of the protocol. In the main, the test-retest reliability for the physiological responses was acceptable with T_{re}, $\overline{T}_{sk}$ and HR consistently below 10 % CV. The perceptual responses were more variable and so had higher CV, but this reduced over the course of the protocol. It is recommended that the D_{max} method is used in future studies in order to determine T_{re} inflection. The D_{max} method has a low CV, good face validity, and is less subjective than a purely visual approach; on a practical level it is unlikely that experimenters will have access to three independent thermal physiologists to visually assess the thermal profiles of each participant. It remains to be established whether the T_{re} inflection, as established using a continuous protocol, provides a valid representation of the upper limit of the TZ, *i.e.* the transition from compensable to uncompensable conditions or, if the T_{db} were clamped just beyond the T_{re} inflection, if thermal equilibrium would be established as classically proposed (Lind, 1963a), albeit at an elevated T_{re}.

6. Chapter Six – Defining the upper limits of thermal balance: the validity of a continuous protocol for defining the critical environmental limits in high and low humidity environments.

6.1. Introduction

Chapter Five highlighted the variety of approaches that have been employed for defining the critical environmental limits of human thermoregulation, and detailed the first study where dry bulb temperature (T_{db}) was continuously increased after a 60 minute equilibrium period with simultaneous measurement of thermoeffector responses. The protocol was effective as it enabled a stable rectal temperature (T_{re}) to be established within the initial 60 minute period, before eliciting an inflection in T_{re} in the second part of the study, under conditions of high and low humidity. Chapter Five also demonstrated that this protocol, and the methods of identifying the inflection point in T_{re}, were reliable, but it is yet to be established if the T_{re} inflection truly represents the point of thermal uncompensability *i.e.* the validity of the protocol.

Validity is one of the fundamental aspects of scientific research alongside reliability and generalizability (George *et al.*, 2000). There are three types of validity which are relevant for thermophysiological studies: logical or face validity, criterion validity, and construct validity (Currell & Jeukendrup, 2008). Face validity assesses whether a test actually measures what it intends, criterion validity is established in a new technique or measurement when it's compared with a 'Gold standard', and construct validity relates to the ability of a measurement to detect differences between groups who are expected to differ in a given construct (Williams & Wragg, 2004). As there is no established 'Gold standard' for comparison, and given that Chapter Five demonstrated construct validity, because the T_{db} at the T_{re} inflection differed between low and high humidity conditions, this chapter is concerned with examining the face validity of the T_{re} inflection point.

When classically defined, the critical environmental limit of the prescriptive zone (PZ) represents the ambient conditions at the point which deep body temperature (T_c) is driven to a higher *equilibrium* value by the environmental conditions (Lind, 1963, 1970) *i.e.* thermal compensability can be re-established, but at an elevated T_c. More recently, the critical environmental limit has been described as representing the upper-limit for thermal balance, delimiting conditions above which heat balance cannot be maintained and T_c will rise continuously (Belding & Kamon, 1973; Dougherty *et al*., 2010; Kamon *et al*., 1978; Kamon & Avellini, 1976; Kenney & Zeman, 2002; Kenney *et al*., 1987, 1993), *i.e.* the transition to an uncompensable environment or the upper limit of the thermoregulatory zone (TZ). Although the latter definition may be more relevant for characterizing safe limits for prolonged heat exposure, it is unclear whether the T_c inflection, as determined by a continuous protocol rather than the discontinuous method originally favoured (Belding & Kamon, 1973; Lind, 1963a) truly represents the critical environmental limit for thermal compensability. Put another way, is the inexorable rise in T_c beyond the initial T_c inflection an inevitable consequence of the continually increasing thermal stress of continuous protocols, or, if the increments in thermal stress were stopped shortly after the T_c inflection could thermal equilibrium be re-established beyond the critical loci, as 'classically' described?

The response of most peripheral thermoreceptors consist of an initial dynamic (phasic) component in which the cells are very active when the ambient temperature is changing, followed by a reduced activation as they quickly adapt to a stable temperature (Bligh, 1998); this allows a rapid reaction to environmental changes (Romanovsky, 2007b). Therefore, if the ambient thermal load is clamped shortly after the T_c inflection, the activity of the thermoreceptors should diminish when the new ambient temperature is maintained. However, as peripheral deep-body sensors (located in the oesophagus, stomach, large intra-abdominal veins, and other organs) respond to T_c (Romanovsky, 2007b), there will be a continued thermoafferent drive from theses sensors beyond the inflection in $T_{c.}$ It remains to be seen how these two aspects of the thermoregulatory system interact and integrate in the specific conditions of the proposed protocol.

Accordingly, the aim of the present study was to investigate the face validity of the T_{re} inflection point and to establish whether the T_{re} inflection point as defined during a continuous temperature ramp protocol provides a valid index of the upper limit of the TZ *i.e.* the transition from thermal compensability to uncompensability, or if it would be possible to re-establish thermal balance at temperatures in excess of the T_{re} inflection point.

6.1.1 *The hypotheses tested were:*

H_1 A plateau in T_{re} will be re-established (albeit at a higher value) if ambient air temperature (T_{db}) is held stable shortly after the inflection point in T_{re}

H_2 Once the ambient air temperature (T_{db}) is held stable after a period of incremental increase, the rate of rise in thermoeffector responses will be less and a plateau will be established in sweat rate (SR) and/or skin blood flow (SkBF)

6.2 Methods

6.2.1 *Participant characteristics*

Nine males volunteered to participate in this study and gave written informed consent. All were healthy (as determined by an Exercise and Health History questionnaire [Appendix B]), non-heat acclimated and non-smokers. Participant characteristics are shown in Table 6.1.

Table 6.1. Participant characteristics (n=9).

	Mean (SD)
Age (years)	27.56 (5.00)
Height (m)	1.81 (0.05)
Body mass (kg)	74.85 (9.41)
Body surface (m^2 [DuBois formula])	1.94 (0.13)
Body fat (%) – Seven sites (ACSM, 2000)	9.08 (3.46)
Body fat (%) – Four sites (Durnin & Rahaman, 1967)	14.09 (3.26)
$\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	55.39 (6.33)
$\dot{V}O_{2max}$ ($L \cdot min^{-1}$)	4.87 (0.86)

6.2.2 *Experimental design*

Participants were required to visit the Extreme Environments Laboratory on three occasions. Participants had at least 48 hours between tests and environmental chamber tests were always scheduled in either a morning or afternoon session (fixed within participant) to minimise diurnal variation. Every participant performed an incremental exercise test (see 3.7) to determine maximal aerobic capacity ($\dot{V}O_{2max}$) and had their body fat percentage estimated by skinfold measurements (see 3.2.5), the remaining two visits were conducted in the environmental chamber where the participants exercised in a hot-dry

(34 °C, 20 % RH) environment at an external work rate of 60 W. Participants were fully briefed before each session and reminded that they could terminate the exercise at any point.

6.2.3 Experimental procedures

Details of measurements are described in the General Methods (Chapter Three), the experimental procedures are identical to those reported in Chapter Five, with the exception of the protocol employed in Test 2 (T2). Following Test 1 (T1), the D_{max} method was used to identify the inflection point in T_{re} for all participants as this was shown to be reliable and more objective than the visual method with good face validity (see 5.3.3). To determine the time point at which the T_{db} should be held stable in T2, 8 minutes was added to the D_{max} determined time of inflection in T1 as the TEM of the T_{re} inflection point determined using this method was 7.5 minutes. As described in section 3.3, the chamber was controlled manually in the second hour of the protocol, this involved the technical staff adjusting the heat input manually in 5 minute sections, therefore four of the nine target times were amended (reduced by no more than two minutes) to fit in with this schedule. It was anticipated that this small time difference would lie within the variability of the chamber's performance (see 5.3.1). In order to allow ample time for thermal and thermoeffector responses to respond to the new ambient conditions once the T_{db} was held, participants were asked to cycle for a further 60 minutes.

6.2.4 Data analyses and statistical methods

Differences between tests in pre-exercise measures, and the difference between the mean T_{db} and RH at the point of inflection in T_{re} that occurred in T1 and the average of the temperature maintained over the second stable T_{db} period of T2, were analysed with paired sample t-tests or Wilcoxon test for data which violated assumptions of parametric data (according to the Shapiro-Wilk). To test the hypotheses that thermal and thermoeffector responses re-established a plateau in T2 once the T_{db} was held stable, the responses at three distinct time points were compared (see Figure 6.1):

1) Increase over the time period from inflection in T_{re} in T1 to the end of exercise
2) Increase over the same time period in T2
3) Increase over the time period in T2 from the start of the stable T_{db} to the end of exercise in T2

Additionally, paired sample t-tests were used to compare thermal and thermoeffector responses in T2 only at discreet five minute time points from the point of inflection in T_{re} to 40 minutes post inflection (when all nine participants are still exercising). This was used as a method to determine if a plateau had occurred in T2.

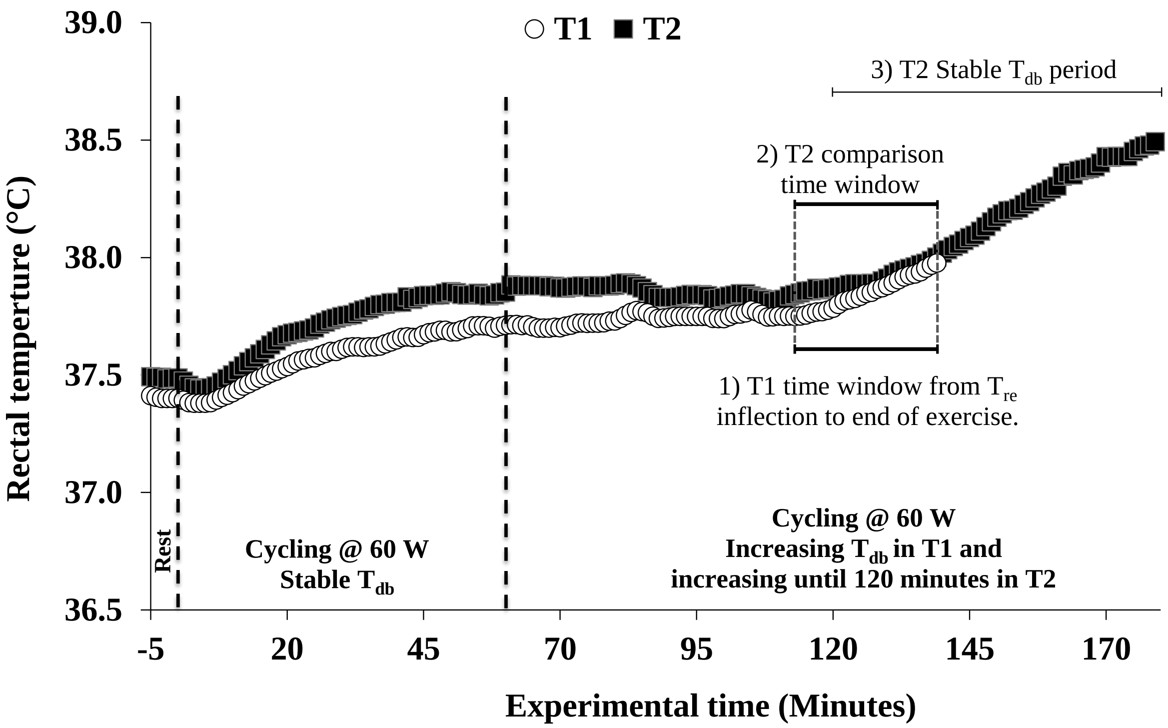


Figure 6.1. Example T_{re} profile (Participant 4) to demonstrate the data analysis sections during cycling exercise. T_{db} is stable for the first 60 minutes and then either increases at a rate of 1 °C every five minutes for a further 80 minutes (T1) or increases at a rate of 1 °C every five minutes for a further 60 minutes and then is held stable once more (T2). Analysis sections are 1) the time window from the T_{re} inflection to end of exercise in T1 and 2) its matched time comparison in T2 and 3) from the start of the stable T_{db} to the end of exercise in T2.

6.3 Results

6.3.1 Participant hydration, ambient temperature and relative humidity control

All participants verbally confirmed that they had followed the pre-test instructions, and presented with a urine specific gravity equal to or below 1.020. There were no significant difference between trials in pre-exercise body mass ($t_{(8)}$=1.191, p=0.268) or resting T_{re} ($t_{(8)}$=-0.051, p=0.960). During experimental trials the participants lost on average 0.87 (0.59) %, with a maximum of 1.73 %, of their starting nude body weight, suggesting that each subject began each trial in a similar physiological state and did not become excessively dehydrated during the trials. The average (SD) T_{db} over the rest period and first hour of exercise was 34.28 °C (0.52) and RH was 23.09 % (1.78) in T1 and 34.25 °C (0.47) and RH was 22.99 % (1.99) in T2. These values were not significantly different (z=-

1.244, $p=0.214$; $t_{(8)}=0.312$, $p=0.763$) and compared favourably to the target conditions of 34 °C and 20 % RH respectively.

Importantly, there was no significant difference in the mean RH ($t=1.507$, $_{(8)}$ $p=0.170$) but a significantly lower T_{db} ($z=-2.668$, $p=0.008$) at the point of inflection in T_{re} in T1 (26.98 [3.00] %; 44.02 [2.88] °C) than the mean temperature maintained over the second stable T_{db} period of T2 (25.63 [3.22] %; 45.26 [2.59] °C), thus indicating that the T_{db} was successfully held at the desired higher T_{db} in T2. Of the nine participants, seven were able to continue cycling for a further 60 minutes once the T_{db} was held stable beyond the initial T_{re} inflection in T2, while one participant had to stop at 40 minutes due to muscular discomfort and another terminated the test due to feeling faint at 55 minutes.

6.3.2 Comparison of thermal and thermoeffector responses in T1 and T2.

The rate of change over the time period from inflection in T_{re} to the end of exercise in T1 was compared to the matched time period in T2. There was a significant difference between environmental conditions, with lower rate of rise in T_{db} ($t_{(8)}=6.663$, $p<0.001$) but with a higher rate of rise in RH ($t_{(8)}=-3.624.663$, $p=0.007$) in the matched time period in T2. This caused a lower rise in $\overline{T}_{sk}$ ($z-2.192$ $p=0.028$) and increased HF ($t_{(8)}=-3.334$, $p=0.010$), but no difference between T_{re}. Thermoeffector responses were similar ($SkBF_{Forearm}$, $SkBF_{Finger}$, SR_{Back} and HR) with the exception being $SR_{Forearm}$ which had a significantly lower rate of rise ($t_{(8)}=-2.565$, $p=0.033$) in the matched time period in T2. Perceptually, only TS ($z-2.075$, $p=0.038$) was significantly different, indicating lower rate of rise in the matched time period in T2.

The rate of change over the time period from inflection in T_{re} to the end of exercise in T1 was compared to the stable T_{db} period in T2. There was a significant difference between in environmental conditions, with lower rate of rise in T_{db} ($t_{(8)}=18.312$, $p<0.001$) but with a higher rate of rise in RH ($t_{(8)}=-7.462$, $p<0.001$) in the matched time period in T2. This caused a lower rise in $\overline{T}_{sk}$ ($t_{(8)}=-5.102$, $p=0.001$) and increased HF ($t_{(8)}=-5.105$, $p=0.003$), but no difference between T_{re}. Thermoeffector responses were similar ($SkBF_{Forearm}$, $SkBF_{Finger}$ and HR) with the exception being $SR_{Forearm}$ and SR_{Back} which both had a significantly lower rate of rise in the matched time period in T2 ($t_{(8)}=-4.397$, $p=0.002$; $t=-3.612$, (8) $p=0.007$). Perceptually, TS and SW were significantly different ($z-2.668$, $p=0.008$; $z=-2.073$, $p=0.038$), indicating lower rate of rise in the matched time period in T2.

Table 6.2. Mean (SD) change over time in thermal, thermoeffector and perception measures (Δ·unit·hr^{-1}) in select time periods in Test 1 (T1) and Test 2 (T2), n=9. * Significantly different from T1 values.

Variable	1) Inflection period to end of exercise, in T1	2) Matched time window to 1), in T2	3) Stable T_{db} period to end of exercise, in T2
T_{db} (Δ °C·hr^{-1})	8.91 (1.33)	2.88 (2.39)*	-0.12 (0.70)*
RH (Δ %·hr^{-1})	-3.25 (1.45)	0.75 (2.94)*	2.36 (1.76)*
T_{re} (Δ °C·hr^{-1})	0.67 (0.43)	0.48 (0.36)	0.63 (0.38)
$\bar{T}_{sk}$ (Δ °C·hr^{-1})	1.37 (0.49)	0.71 (0.61)*	0.27 (0.51)*
HF (Δ W·m^{-2}·hr^{-1})	-30.41 (45.64)	3.27 (44.44)*	27.42 (25.88)*
SR_{Back} (Δ L·m^2·hr^{-1}·hr^{-1})	0.25 (0.17)	0.16 (0.19)	0.07 (0.13)*
$SR_{Forearm}$ (Δ L·m^2·hr^{-1}·hr^{-1})	0.45 (0.21)	0.24 (0.18)*	0.10 (0.13)*
$SkBF_{Forearm}$ (Δ FluxUnits·hr^{-1})	13.94 (75.98)	-73.10 (96.19)	-22.76 (79.43)
$SkBF_{Finger}$ (Δ Flux units·hr^{-1})	42.60 (49.28)	20.81 (68.44)	0.74 (37.74)
HR (Δ b hr^{-1})	25 (8)	2 (11)	19 (15)
$\dot{V}O_2$ (Δ L·hr^{-1})	0.04 (0.20)	0.20 (0.27)	0.08 (0.15)
TS (Δ cm·hr^{-1})	3.60 (5.45)	1.20 (5.05) *	1.30 (3.00)*
TC (Δ cm·hr^{-1})	-4.21 (18.50)	-4.50 (9.77)	-2.10 (4.40)
SW (Δ cm·hr^{-1})	5.60 (11.04)	4.37 (8.45)	1.90 (6.84)*
RPE (Δ VAS·hr^{-1})	2 (7)	2 (5)	2 (3)

Taken together, the data in Table 6.2 indicates that T_{db} was held successfully in T2, causing a reduction in the rate of increase in $\overline{T}_{sk}$, SR, TS, SW and a switch from heat gain to heat loss as measured by HF. Of note, is that there was no difference in the rate of increase in T_{re} in T2 compared to T1 (over the selected timeframes). Figure 6.2 and 6.3 demonstrate that T_{re} continues rise post inflection, in both conditions.

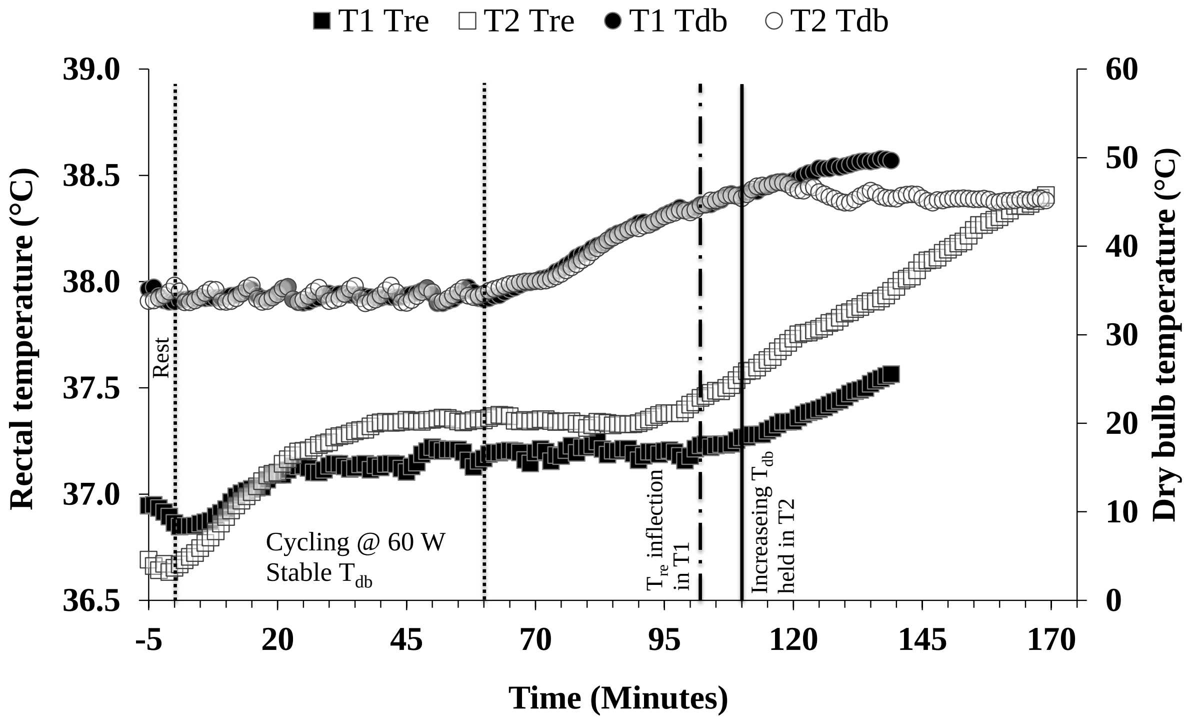


Figure 6.2. Example T_{re} and T_{db} profile (Participant 6) during cycling exercise where T_{db} is stable for the 60 minutes and then either increases at a rate of 1 °C every five minutes for a further 80 minutes (T1) or increases at a rate of 1 °C every five minutes for a pre-set time (dependent on participant) and then is held stable once more (T2).

6.3.3 Re-establishment of a plateau in thermal and thermoeffector responses past the point of stable T_{db} in T2

Figure 6.3 shows that in T2 there was no apparent re-establishment of a plateau in T_{re}, this is confirmed by the absolute T_{re} at each time point from five minutes post inflection to forty minutes post inflection in T2 being significantly greater than the previous (all $p \leq 0.027$).

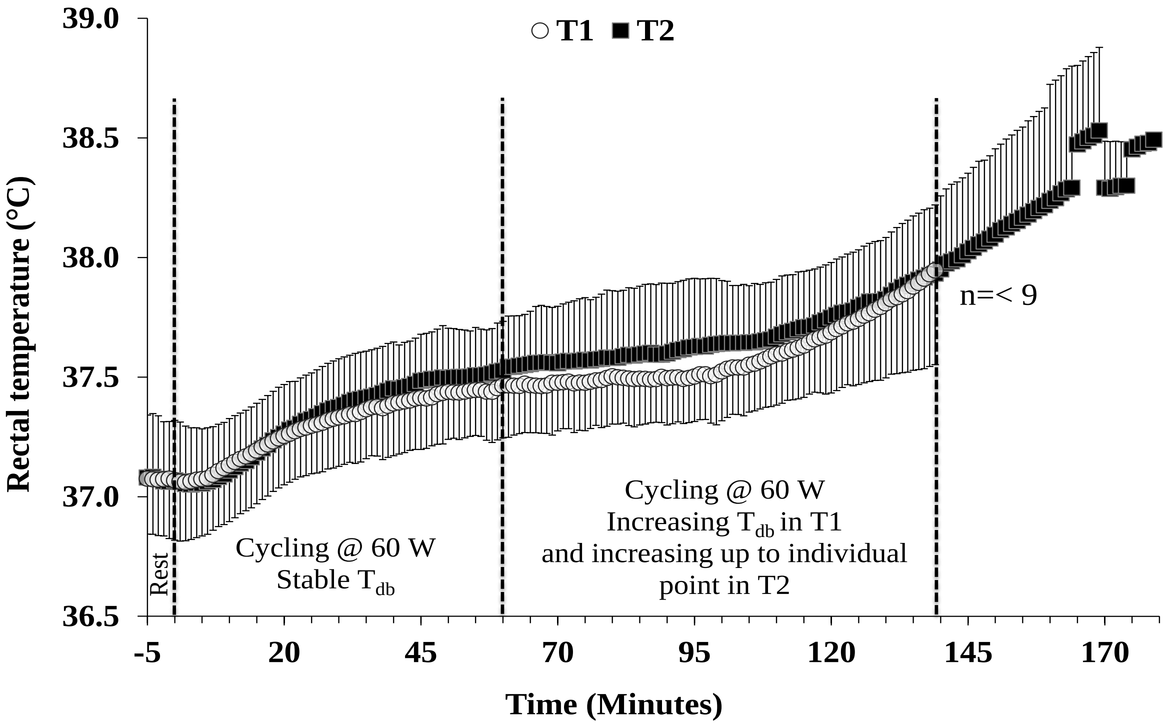


Figure 6.3. Mean (SD) of T_{re} response to cycling exercise where T_{db} is stable for the 60 minutes and then either increases at a rate of 1 °C every five minutes for a further 80 minutes (T1) or increases at a rate of 1 °C every five minutes for a pre-set time (dependent on participant) and then is held stable once more (T2). n=9 up to the third dashed line.

Figure 6.4 shows that in T2 the rate of increase in SR_{Back} reduces but a clear plateau is not established. There was no significant difference between the absolute SR_{Back} at each time point from five minutes post inflection to forty minutes post inflection in T2.

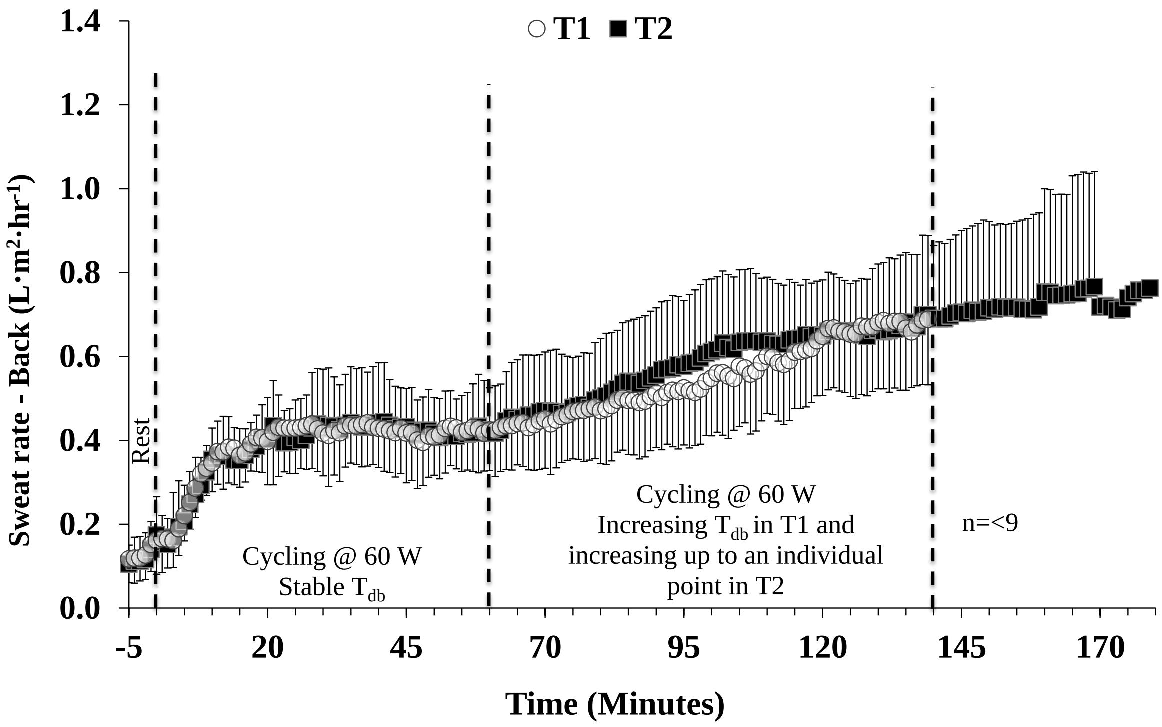


Figure 6.4. Mean (SD) of SR_{Back} response to cycling exercise where T_{db} is stable for the 60 minutes and then either increases at a rate of 1 °C every five minutes for a further 80 minutes (T1) or increases at a rate of 1 °C every five minutes for a pre-set time (dependent on participant) and then is held stable once more (T2). n=9 up to the third dashed line.

Figure 6.5 shows that the absolute rate of sweating in T2 is higher than in T1. The rate of increase in $SR_{Forearm}$ reduces, but a clear plateau is not established (possibly due to reducing numbers of participants, n=8 at 40 minutes, n=7 at 55 minutes and n=6 at 60 minutes). There was no significant difference between the absolute $SR_{Forearm}$ at each time point from five minutes post inflection to forty minutes post inflection in T2.

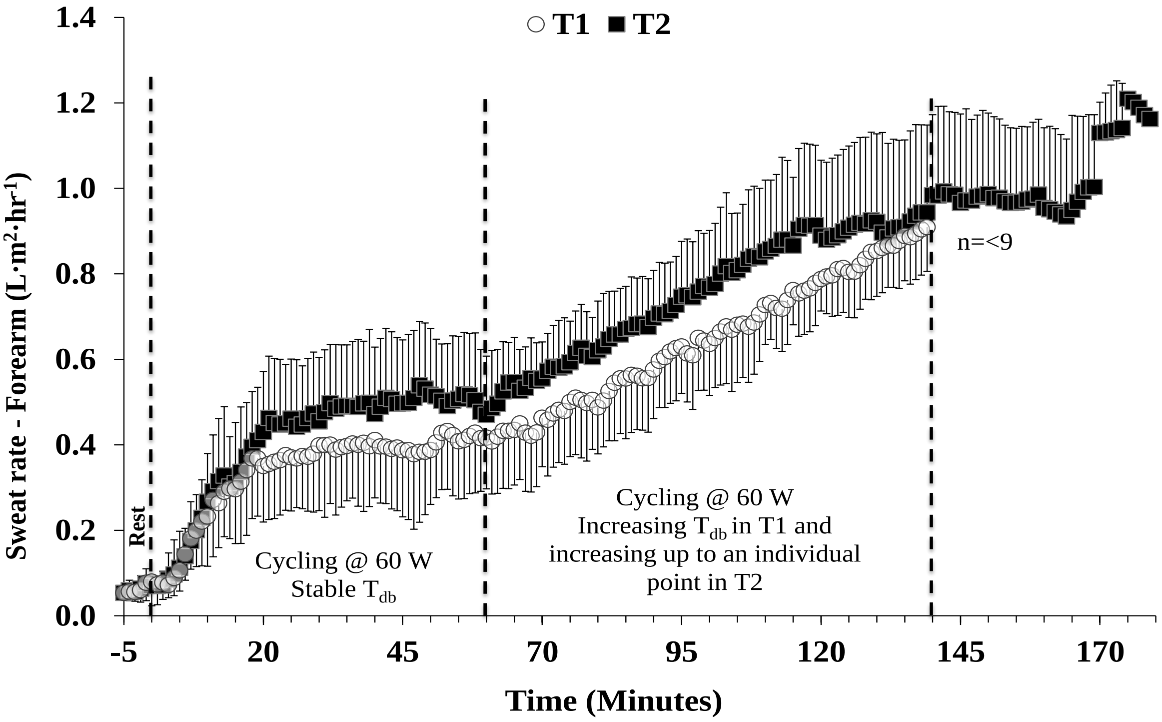


Figure 6.5. Mean (SD) of $SR_{Forearm}$ response to cycling exercise where T_{db} is stable for the 60 minutes and then either increases at a rate of 1 °C every five minutes for a further 80 minutes (T1) or increases at a rate of 1 °C every five minutes for a pre-set time (dependent on participant) and then is held stable once more (T2). n=9 up to the third dashed line.

Figure 6.6 shows that the absolute rate of $SkBF_{Forearm}$ is initially higher in T2 than T1. However, in both conditions a plateau is established (possibly due to reducing numbers of participants) which is likely to be peak vasodilation. There was no significant difference between the absolute $SkBF_{Forearm}$ at each time point from five minutes post inflection to forty minutes post inflection in T2.

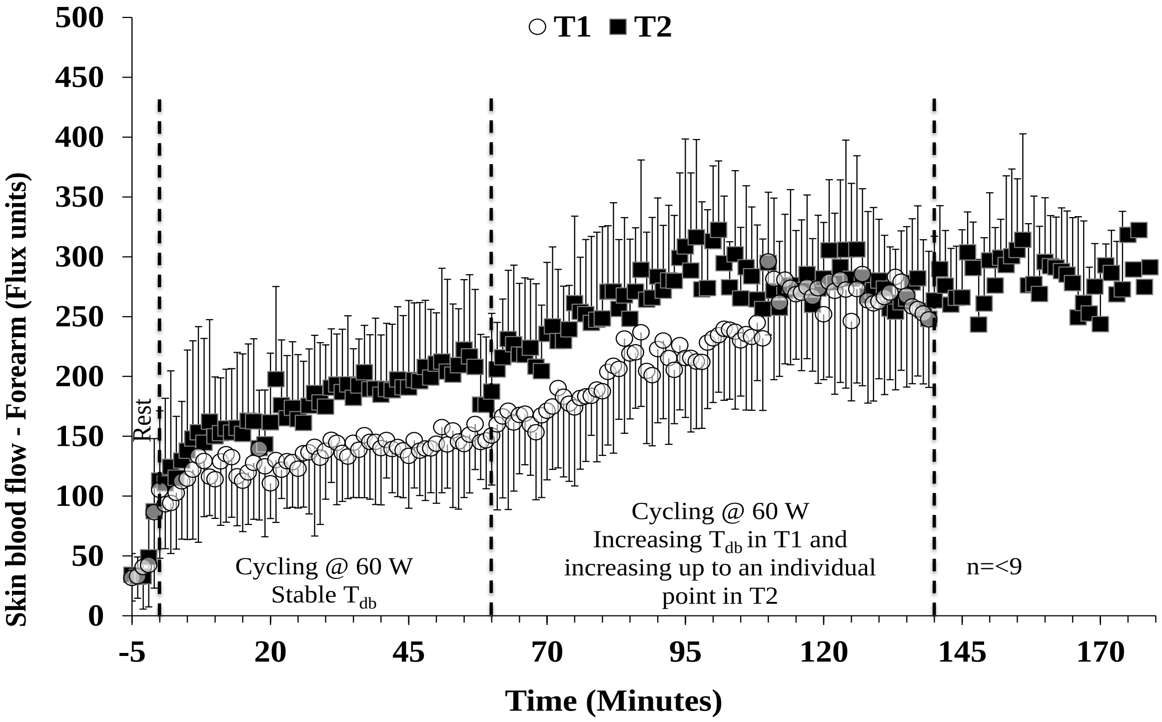


Figure 6.6. Mean (SD) of $SkBF_{Forearm}$ response to cycling exercise where T_{db} is stable for the 60 minutes and then either increases at a rate of 1 °C every five minutes for a further 80 minutes (T1) or increases at a rate of 1 °C every five minutes for a pre-set time (dependent on participant) and then is held stable once more (T2). n=9 up to the third dashed line.

Figure 6.7 shows that in both T1 and T2, $SkBF_{Finger}$ reach established peak vasodilation after 10 minutes, following a temporary reduction in SkBF on the commencement of exercise. There was no significant difference between the absolute $SkBF_{Finger}$ at each time point from five minutes post inflection to forty minutes post inflection in T2.

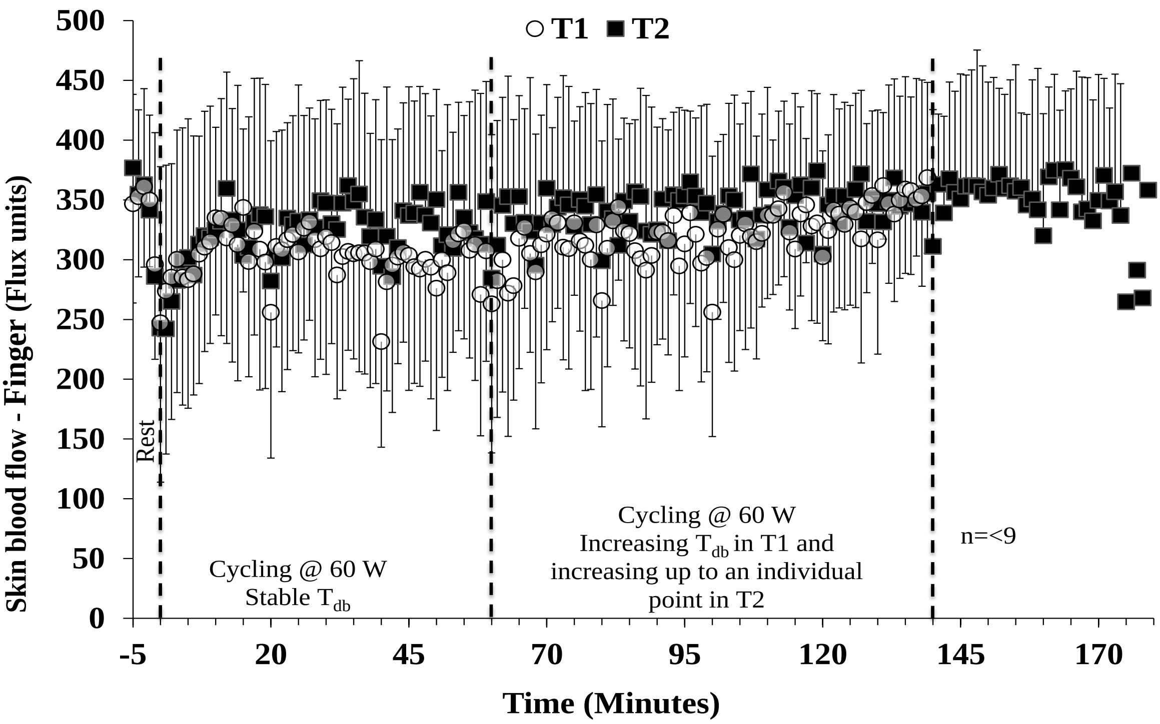


Figure 6.7. Mean (SD) of $SkBF_{Finger}$ response to cycling exercise where T_{db} is stable for the 60 minutes and then either increases at a rate of 1 °C every five minutes for a further 80 minutes (T1) or increases at a rate of 1 °C every five minutes for a pre-set time (dependent on participant) and then is held stable once more (T2). n=9 up to the third dashed line.

6.4 Discussion

In the previous experimental study it was unclear whether the inflection in T_{re} observed with increasing T_{db} represented the transition to a 'true' uncompensable heat stress, *i.e.* the upper bound of the 'thermoregulatory' zone, or was a consequence of the continually increasing thermal stress of the protocol. This study sought to investigate the face validity of the continuous temperature ramp protocol for defining the upper bound of the thermoregulatory zone. The hypotheses tested in this chapter were: 1) if the ambient air temperature was held constant after a period of incremental increase and just beyond the inflection point in T_{re}, a plateau in T_{re} would be re-established; and 2) if the ambient air temperature was held constant after a period of incremental increase and just beyond the inflection point in T_{re}, a plateau in thermoeffector responses (sweat rate and/or skin blood flow) will be re-established.

The data from this study indicated that when T_{db} was clamped in T2 (at a temperature just beyond the T_{re} inflection point in T1) T_{re} continued to increase for a further 40-60 minutes. Thus, the first hypothesis is rejected as it appears that the participants had achieved an uncompensable thermal state when the T_{re} inflection occurred. Therefore, previous authors (Belding & Kamon, 1973; Dougherty *et al.*, 2010; Kamon *et al.*, 1978; Kamon & Avellini, 1976; Kenney & Zeman, 2002; Kenney *et al.*, 1987, 1993) are correct to conclude that their protocols provide insight into factors which affect the point of uncompensability in adults and children. However, it is important to note that these authors write that the T_{re} inflection is synonymous with the edge of the prescriptive zone (Dougherty *et al.*, 2010; Kenney & Zeman, 2002; Kenney *et al.*, 1987), which is incorrect as the original definition from Lind (1963a) states that thermoregulation is possible beyond this point, but will cause strain to the circulatory system (Lind, 1963a).

It has previously been suggested that the function of a rising T_{re} is to maintain a favourable gradient and reduce SkBF requirements for heat loss (Cheuvront, Kenefick, Montain, & Sawka, 2010). Similar to the T_{re} response, HR also continued to rise beyond the initial T_{re} inflection point in T2, despite T_{db} being held constant just beyond this point. A number of studies which report high T_c reached at exhaustion in laboratory settings also exhibit considerable cardiovascular strain ([95 % HR_{max}] González-Alonso *et al.*, 1999; Périard, Caillaud, & Thompson, 2012; Périard, Cramer, Chapman, Caillaud, & Thompson, 2011; Rowell, Marx, Bruce, Conn, & Kusumi, 1966) causing Périard *et al.* (2012) to suggest that impairments in aerobic exercise performance are caused by a thermal strain induced increase in cardiovascular strain. The data from the present study shows that as T_{re} rises uncontrollably, HR also increases despite no increase in external work rate or SkBF and so points towards a cardiovascular drift (progressive change over time in heart rate, stroke volume, mean arterial and pulmonary pressures with a relatively constant cardiac output) (Wingo *et al.*, 2005) and / or a reduction in efficiency (Hettinga *et al.*, 2007) as a possible mechanisms for the viewpoint of Périard *et al.* (2012).

During the period where T_{db} was held stable in T2, the $\overline{T}_{sk}$ rate of rise reduced significantly and HF indicated that the previous rate of heat gain from the environment had reversed to a heat loss, this would presumably have reduced the drive for sweating. While an increase in (glabrous) skin temperature has been shown to increase sweating without a concurrent increasing T_c (Nadel, Mitchell, & Stolwijk, 1973), the majority of the body is covered in non-glabrous skin and its main thermoregulatory role is to provide negative and positive auxiliary feedback to the thermoregulatory system, and so reduces the system's response time and increases the stability of body temperature (Romanovsky, 2014). It may be the case that as thermoreceptor cell activity diminished, as they adapt to the new stable temperature in T2 (Bligh, 1998), the sweating and skin blood flow response reduces. In the present study, SR and SkBF had a reduced rate of rise in T2. Therefore, the second hypothesis can be partially accepted, as there was no clear plateau. The implication of SR not increasing over this period is a reduction in evaporative cooling, and therefore a reduction in the likelihood that a T_{re} plateau could be established. This has important implications for those exercising in the heat in an environment where evaporation is not limited, because the peak sweat rate under a given set of ambient conditions is determined by the thermoafferent input and may not be sufficient to re-establish thermal equilibrium.

Previous studies that have used continuous temperature ramp protocols to elicit an inflection in T_c while participants exercised at a fixed work rate were primarily concerned with the critical environmental limits at which an inflection in T_c occurred (Kenney *et al*., 1993; Kenney & Zeman, 2002) and did not, therefore, measure thermoeffector responses. The data from the present study indicates that while the rate of increase in SR reduces in T2 once T_{db} is held stable, a clear plateau is not established (Figures 6.4 and 6.5). Generally, the forearm has a higher sweat rate, reflecting the greater density of activated sweat glands than the back (Kondo *et al*., 1998). Also, sweat rate has been shown to be influenced by local skin temperature, through local heating increasing neurotransmitters for a given sudomotor signal arriving at the eccrine sweat gland (Nadel *et al*., 1971). In the present study $T_{Scapula}$ was 0.8 °C higher compared to T_{LowArm} although this did not result in a greater sweat rate on the back, however it should be noted that the measurement sites of T_{sk} and SR were different. In the main, the local SR responses appeared to mirror the $\overline{T}_{sk}$ increase, rate of increase was slowed during the stable T_{db} in T2 (Table 6.2). In T1 there was raised and increasing $\overline{T}_{sk}$ *and* T_{re} driving SR, however in T2 there was raised and increasing T_{re} but *only* raised $\overline{T}_{sk}$. So, the reduction in SR may be seen as a measure of the dynamic (changing) $\overline{T}_{sk}$ input into sweating.

The present study was not without limitation. The point at which T_{db} temperature was held in T2 was based on T1 and the TEM reliability data from the previous chapter, however the participants were not identical between studies, although their characteristics were very similar. It may be argued that as some individuals clearly inflected before the temperature was held, the reduced thermal burden occurred too late to enable a re-establishment of thermoregulation. Conversely the opposite could be true, with the stable T_{db} occurring in advance of a T_{re} inflection. However, given that an inflection in T_{re} was observed in T2 for all participants, this would appear unlikely. Pilot testing prior to the data collection showed that it was extremely difficult to define an inflection in 'real-time' and the consequence was that the ambient temperature was held after a considerable delay in order to be sure that an inflection had occurred. Regulation of body temperature is achieved primarily by the initiation of thermoeffector mechanisms by the thermoafferent drive from peripheral temperature, T_c, and central temperature sensors (Mekjavić & Eiken, 2006). There are, of course, limitations with using thermometry as a surrogate measure for thermoafferent input. It is acknowledged that T_{re} is associated with slower responses to thermal change because the rectum is surrounded by a large mass of abdominal tissue with low thermal conductivity (Taylor *et al.*, 2014). It is also possible that an inflection in T_{re} may have actually occurred after uncompensability as determined by another measure of T_c, because of the slow response time in T_c. However, the participants continued to cycle for 40-60 minutes beyond the T_{re} inflection point determined in T1 (which for some was to the limit of tolerance) so it could be argued ample time was allowed for T_{re} to respond and for accumulated heat storage to be dissipated.

Taken together the data from the present study suggest that, when exercising in uncompensable conditions, it is not possible to re-establish thermal equilibrium, even if the ambient thermal load is clamped just above the inflection point of uncompensability. This may be a function of a diminished thermoafferent drive and thermoeffector output as a consequence of the phasic behaviour of the thermoreceptors (Bligh, 1998). Of course, if an athlete or worker has the opportunity to behaviourally thermoregulate, such as reducing work rate or move themselves to a cooler environment then control may not be lost at all or could possibly be re-established as behavioural thermoregulation is a highly powerful tool (Parsons, 2014; Schlader *et al.*, 2011), but this is not always possible and these present data show that even with a stable temperature, T_c continued to increase. Kraning and Gonzalez (1991) have shown that intermittent exercise can, in fact, cause greater levels of heat strain (due to interruptions in heat transfer by changes in cutaneous circulation and posture), those who are employed or employ others to work in the heat need to be mindful that periodic resting or moving to cooler ambient temperatures may not be sufficient to prevent cumulative uncompensable heat stress, particularly when protective clothing is worn and exercise, even intermittent exercise, is performed.

To our knowledge this is the first study to look at the reliability (Chapter Five) and the face validity (present Chapter) of a protocol to determine the point of uncompensable heat stress, and therefore answer questions regarding thermoeffector response and thermal profile at this critical point. The work has shown that once an inflection in T_{re} is induced by the progressively increasing T_{db}, clamping the ambient T_{db} does not halt the rate increase in T_{re}. Moreover, a reduction in thermoeffector response, as evidence by a reduction of the rate of rise in sudomotor response, despite a continually increasing core temperature. This observation highlights the dynamic, phasic nature of the thermoafferent input in static and dynamic environments. Researchers using experimental protocols that have changing environmental conditions should be aware of these findings as the thermoafferent feedback for a given thermal profile will be influenced by the rate of change in that profile, as well as the absolute profile.

In summary, this protocol is reliable and valid and will therefore be used in the thesis to compare the effect of humidity, aerobic fitness and work rate on the inflection point in T_{re} in young healthy males.

7. Chapter Seven – Thermophysiological Responses and Biophysical Characteristics at the Inflection Point in Deep Body Temperature during Cycling Exercise in High and Low Humidity Environments.

7.1. Introduction

The preceding two experiments (Chapters Five and Six) have shown that both the continuous protocol, where dry bulb temperature (T_{db}) is incrementally increased after an initial stabilisation period, and the D_{max} analysis approach, are reliable methods for producing an identifiable inflection in rectal temperature (T_{re}) in exercising males, and that this is a valid index of the upper limit of the thermoregulatory zone (TZ). The next two Chapters explore the effect of specific thermal (humidity) and non-thermal (aerobic fitness) factors on the upper limit of the TZ to provide insight into their effect on thermoregulatory function and control, and to improve our understanding of the physiological responses precipitating the transition from compensable to uncompensable heat stress.

It is known that to maintain a constant deep body temperature (T_c) the rate of heat dissipation must match the rate of metabolic heat production (minus the external work) and thus the heat balance equation must be satisfied, *i.e.* S = 0.

$$S = M - (\pm W) - E \pm R \pm K \pm C \; [W \cdot m^{-2}] \qquad \textit{Equation 7.1}$$

Where S is storage of body heat, M is metabolic energy transformation (referred to as metabolic heat production in this thesis), W is mechanical work, E is evaporative heat transfer, C is convective heat transfer, K is conductive heat transfer and R is radiant heat exchange (IUPS Thermal Commission, 2001; Parsons, 2014).

During exercise at a fixed external work rate, metabolic heat production will be constant (assuming no changes in efficiency over time) and the ability to dissipate heat is influenced by the environment and the physiological responses of the individual, primarily in the form of the thermoeffector (sudomotor and vasomotor) responses which affect the pathways for dry and evaporative heat exchange. With increasing environmental stress, the temperature gradient between the environment, the mean skin temperature ($\overline{T}_{sk}$) and the T_c reduces and will eventually reach a point where the level of heat production is greater than heat dissipation possible through heat loss pathways (E, R, K, C), leading to an increase in T_c

(Kerslake, 1972; Parsons, 2014). In the situation when ambient temperature (T_a) is greater than skin temperature, dry heat exchange pathways (R, K, C) will become pathways for heat gain and so evaporation is the only route available because it is primarily determined by a water vapour pressure gradient and not a temperature gradient (Kerslake, 1972).

Rates of evaporation are driven by ambient water vapour pressure gradients (Kerslake, 1972; Parsons, 2014). With increasing RH, the water vapour pressure gradient between the saturated skin and the environment falls, thereby reducing the maximal evaporative capacity of the environment (E_{max}) which can, in turn, impair an individual's evaporative heat loss (Berglund & Gonzalez, 1977; Givoni & Goldman, 1973). Indeed, it has been shown that exercising under high RH conditions increases the absolute and rate of rise in deep body temperature, reduces exercise performance (Maughan *et al*., 2012; Moyen, Ellis, *et al*., 2014) and increases the risk of heat illness (Coris *et al*., 2004). At a low RH an individual's ability to achieve heat balance is often not limited by the environment because the evaporative requirement for heat balance (E_{req}) may be less than $E_{max,}$ and the upper limit of the TZ may, therefore, be determined by individual sweating capacity (Gagnon, 2012; Kamon & Avellini, 1976), which is in turn driven by the thermal drive to sweat as determined by the absolute and rate of change in body temperatures (Nadel *et al*., 1971; Taylor *et al*., 2008), as well as some non-thermal factors (Reilly & Waterhouse, 2009; Sawka *et al*., 2001; Taylor, 2006b).

However, the neurophysiological (thermoafferent) aspect of the thermoregulatory response is sometimes ignored. Indeed, it has been suggested that the level of sudomotor activity achieved during exercise is determined by E_{req}, when not limited by E_{max} (Cramer *et al*., 2012; Jay *et al*., 2011). However, the body is unable to sense humidity (Newton, 2011) and by extension E_{req}. It can only evoke an effector response based on the thermal profile of the individual, which determines the thermoafferent input to the thermoregulatory centres. Therefore, it is proposed that E_{max} could alternatively be defined by two terms, '$E_{max\text{-}physical}$' and '$E_{max\text{-}physiological}$'. Where the former is E_{max} as currently described and calculated in the literature, and the latter is the maximum individual evaporative capacity or 'peak' sweat rate for a given condition, as limited by physiological factors, namely the thermal profile of the individual (thermoafferent stimulus [driving function]), and non-thermal factors (Mekjavić & Eiken, 2006) such as dehydration (Sawka *et al*., 2001) and ethnicity (Taylor, 2006b) and their influence on thermoefferent drive. Under conditions where the $E_{max\text{-}physical}$ is high, such as in conditions of low humidity, it may be $E_{max\text{-}physiological}$ that becomes the limiting factor, whereas under conditions of high humidity it may be the $E_{max\text{-}physical}$ that is limiting, rather than the $E_{max\text{-}physiological}$.

The recruitment of thermoeffector responses is driven by the hierarchy of thermoregulatory centres (Romanovsky, 2007b) and multiple independent thermoeffectors, each of which have a different threshold activation temperature (Kanosue & Crawshaw, 2010). When there is heat gain, afferent signals from the thermoreceptors are integrated centrally and result in efferent output to effector organs that increase cutaneous vasodilation / withdraw vasoconstrictor tone and increase sudomotor activity (Benzinger, 1969; Bligh, 1998; Hardy, 1961). It follows, therefore, that there may be instances where sub-maximal rates of skin blood flow (SkBF) and sweat rate (SR) will be present at the upper limit of the TZ (deep body temperature inflection point), if there has not been a sufficient thermoafferant drive evoked for a maximal thermoeffector response (Figure 7.1). This then is the ‘peak’ thermoeffector response for that thermal profile. Accordingly, in addition to favourably increasing the gradient for heat loss to the skin, the rise in T_c at the upper limit of the TZ would also increase the thermoafferant stimulus for heat loss thermoeffector responses. This hypothesis runs counter to some didactic models (see Figure 2.2), which show SkBF and SR at ‘maximum’ at the upper limit of the TZ (Taylor, Kondo, & Kenney, 2008), which are equated with a ‘loss of thermoregulatory control’, rather than a beneficial thermoregulatory response.

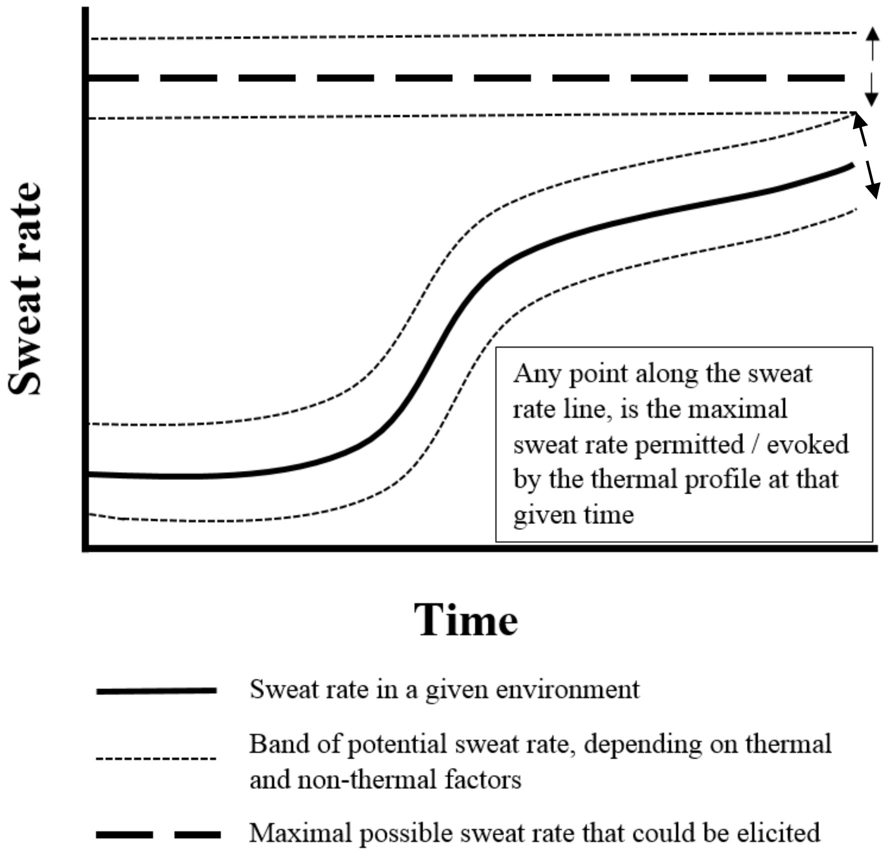


Figure 7.1. Schematic drawing depicting a hypothetical sweating response in comparison to a theoretical 'maximal' individual sweat rate, which is dependent on physiological and physical factors.

Based on the approach originally employed by Belding and Kamon (1973), a number of studies have been conducted to characterise the environmental conditions defining the upper limit of the prescriptive zone (PZ) at a given metabolic rate (Berglund & Gonzalez, 1977; Dougherty, Chow, & Kenney, 2009; Dougherty *et al*., 2010; Kamon *et al*., 1978; Kenney *et al*., 1988, 2004, 1993; Lind, 1963a). However, (as discussed in section 1.2) most have employed inaccurate terminology, and are in fact referring to the upper limit of the TZ. Nonetheless, whilst the biophysical limits defining the upper limits of the PZ and / or TZ under conditions of high and low humidity have received some experimental attention (Berglund & Gonzalez, 1977; Dougherty *et al*., 2009, 2010; Kamon *et al*., 1978; Kenney *et al*., 1988, 2004, 1993; Lind, 1963a), the way in which the environmental conditions interact with an individual's thermal profile to influence the associated thermoeffector responses at this same point, has received considerably less attention. In the original PZ study (Lind, 1963a) the end of test SkBF was estimated using a Whitney strain-gauge (Whitney, 1953) and the figure presented (Fig. 4 p 54 within Lind, 1963a) indicates an increasing rate of change beyond the PZ, but as the limit of the PZ is still within the TZ, the relevance of this strain-gauge data to describe the SkBF response at the boundary of the

TZ is unclear. More recent studies defining the critical limits of thermoregulation (Dougherty *et al*., 2010; Kamon *et al*., 1978; Kenney & Zeman, 2002; Kenney *et al*., 1988, 1993) have not measured SkBF or local SR, despite these being the key autonomic thermoregulatory mechanisms for heat loss; it is therefore not possible to comment on the magnitude of thermoeffector response at the point of uncompensable heat stress. Previous studies, which have estimated sweat loss from mass change, have shown greater (Moyen, Ellis, *et al*., 2014; Moyen, Mündel, *et al*., 2014), or similar (Hayes, Castle, Ross, & Maxwell, 2014), SR levels in high humidity conditions, relative to lower humidity conditions. Additionally, sweat gland activation has been shown to be greater in 85 % RH compared to 55 % RH when individuals exercised in 35 °C, using iodine impregnated paper on the forearm (Moyen *et al*., 2014), though an important consideration is that the water vapour pressure gradient between the skin and the environment would be different between conditions.

Lind (1963a) suggested that the upper limit of the PZ is associated with the minimum bodily thermal gradient compatible with the transfer of adequate amounts of heat from the core to the periphery. Given that T_c is dependent on work rate and independent of ambient temperature (T_a) within the PZ (Lind, 1963a; Nielsen, 1938), Lind's (1963a) assertion suggests that the thermal profile should be similar at the upper limit of the PZ when exercising at a given work rate, even under conditions differing in RH, but the empirical evidence supporting this assertion is limited. For example, Kamon *et al.* (1978) report only that mean T_{re}, and $\overline{T}_{sk}$ were 'similar' at the inflection point in T_{re} as determined by systematically increasing the ambient vapour pressure at seven air temperatures between 36 and 52 °C, whereas in a related study it was noted that $\overline{T}_{sk}$ tended towards 'slightly higher levels' at the inflection point in the higher T_a conditions (Kamon & Avellini, 1976). The effect of humidity on $\overline{T}_{sk}$ is equivocal in studies comparing different environments, with some authors finding it higher in humid conditions when ambient temperature is kept the same / similar (Maughan *et al*., 2012; Moyen, Ellis, *et al*., 2014) and others finding it higher in the lower humidity conditions when ambient temperature is reduced correspondingly as humidity rises (Hayes *et al*., 2014).

In summary, it is well established that environments which combine high T_a and RH challenge the thermoregulatory system, as these conditions markedly reduce dry and evaporative heat losses. What is presently unclear is the way in which differences in ambient humidity affect the thermal profile (T_{re}, $\overline{T}_{sk}$, $\overline{T}_b$ [mean body temperature]) and the associated thermoeffector responses at the upper limit of the TZ, and the way in which this influences the transition from compensable to uncompensable heat stress.

7.1.1. Hypotheses

Accordingly, the present study tested the following hypotheses:

H_1 The ambient T_{db} will differ at the deep body temperature inflection point under low and high humidity conditions.

H_2 The thermal profile of an individual (as indicated by T_{re}, $\overline{T}_b$ and $\overline{T}_{sk}$) will differ at the deep body temperature inflection point under low and high humidity conditions.

H_3 The physiological (thermoeffector) responses will be higher at the deep body temperature inflection point in the low humidity condition compared to the high humidity condition.

H_4 A thermoregulatory 'reserve' capacity, as indicated by sub-maximal rates of skin blood flow and sweat rate, will exist at the deep body temperature inflection point under both low and high humidity conditions.

7.2. Methods

7.2.1. Participants

Sixteen males volunteered to participate in this study and gave written informed consent, in order to match a previous study which compared and detected differences between four different environmental conditions with males of similar fitness (55.6 [7.3] $mL \cdot min^{-1} \cdot kg^{-1}$) at a similar relative work rate (35 % of $\dot{V}O_{2max}$) to those in the present study (Moyen, Ellis, *et al*., 2014) a minimum of thirteen participants were required. All participants were healthy, as determined by an Exercise and Health History questionnaire (Appendix B), non-smokers, and exercised on between two and seven occasions per week at varying intensities. A favourable ethical opinion was given by SFEC (Appendix A) prior to recruiting volunteers for this study. Mean (SD) participant characteristics are shown in Table 7.1.

Table 7.1. Participant characteristics (n=16).

Parameter	Mean (SD)
Age (years)	24.81 (5.32)
Height (m)	1.78 (0.05)
Body mass (kg)	75.74 (9.65)
Body surface area (m^{-2} - DuBois formula)	1.93 (0.13)
Body fat (%) – Seven sites (American College of Sports Medicine, 2000)	9.77 (3.59)
Body fat (%) – Four sites (Durnin & Rahaman, 1967)	14.81 (3.66)
$\dot{V}O_{2max}$ ($L \cdot min^{-1}$)	4.03 (0.82)
$\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	52.63 (8.90)
% of $\dot{V}O_{2max}$ ($L \cdot min^{-1}$) when exercising at 60 W	34.84 (6.93)

7.2.2. Experimental design

Participants were required to visit the Extreme Environments Laboratory on three occasions. On the first visit each participant performed a maximal aerobic capacity test ($\dot{V}O_{2max}$) and had their body fat estimated by measurement of skinfolds, details of which can be found in General methods (section 3.7 and 3.2.5 respectively). The remaining two visits were two experimental conditions designed to enable determination of the T_{re} inflection point under low (~20 % RH) humidity (LH) and high (~80 % RH) humidity (HH) conditions with different starting ambient temperatures (34 °C or 28 °C respectively). This was done to account for the reduced evaporative heat loss capacity provided by the HH environment and to try and ensure that a plateau in T_{re} was achievable in the first hour and there was a similar experimental duration between the humidity conditions. Conditions were undertaken in a balanced order. Participants had at least 48 hours between tests and were always tested in either a morning or afternoon session (fixed within participant) to minimise diurnal variation (Aoki, Stephens, & Johnson, 2001; Little & Rummel, 1971).

7.2.3. Experimental procedures

Details of the experimental procedures can be found in 5.2.3. Based upon the analysis conducted in Chapter Five an inflection in T_{re} was determined by the D_{max} method, due to low CV, limited subjectivity and good face-validity of this approach.

7.2.4. Calculations

A freely available spreadsheet is available to calculate the following variables, this can be found at http://www.sportsci.org/jour/0003/ka.html (Atkins & Thompson, 2000). Heat storage, E_{req} and E_{max} were calculated as described in equations 7.2, 7.3 and 7.4, respectively according to Atkins & Thompson (2000).

Heat storage ($W \cdot m^{-2}$) = ((3474 x body mass x ($\bar{T}_b$ final - $\bar{T}_b$ initial))/t)/A_D *Equation 7.2*

Where: 3474 is average specific heat of body tissue ($J \cdot kg^{-1} \cdot {}^oC^{-1}$), body mass is in kg, $\bar{T}_b$ is mean body temperature (^oC), t is exercise time (s) and A_D is body surface area (m^{-2} [DuBois & DuBois, 1916]).

E_{req} ($W \cdot m^{-2}$) = H - K - R - C -S *Equation 7.3*

Where: H is internal heat production ($W \cdot m^{-2}$), K is heat exchange via conduction ($W \cdot m^{-2}$), R is heat exchange via radiation ($W \cdot m^{-2}$), C is heat exchange via convection ($W \cdot m^{-2}$), and S is body heat storage ($W \cdot m^{-2}$).

E_{max} ($W \cdot m^{-2}$) = f_{pcl} x h_e x (P_s - P_a) *Equation 7.4*

Where: f_{pcl} is permeation efficiency factor of clothing, h_e is evaporative heat transfer coefficient ($W \cdot m^{-2} \cdot kPa^{-1}$), P_s is partial water vapour pressure at the skin surface (kPa), and P_a is partial water vapour pressure of ambient air (kPa).

R ($W \cdot m^{-2}$) = E x s x f_{cl} x f_{eff} x (T_s^4 - T_r^4) *Equation 7.5*

Where E = emittance from the outer surface of a clothed body (0.97), s = Stefan-Boltzmann constant (5.67 x 10^{-8} $W \cdot m^{-2} \cdot K^{-4}$), f_{cl} =clothing area factor (ND) taken to be body surface area as semi-nude, f_{eff} = effective radiation area of a clothed body (0.71), and T_s = surface temperature of the body (^oC) taken to be $\bar{T}_{sk}$ and T_r = mean radiant temperature (^oC) taken to be T_g = globe bulb temperature (^oC).

C ($W.m^{-2}$) = (A_D x f_{cl} x h_c x (T_s - T_{db}))/ A_D *Equation 7.6*

Where A_D = body surface area (m^2), f_{cl} = clothing area factor (ND) taken to be body surface area as semi-nude, h_c = convective heat transfer coefficient ($W.m^{-2}.K^{-1}$), T_s = surface temperature of the body (^oC) taken to be $\bar{T}_{sk}$ and T_{db} = dry bulb temperature (^oC).

7.2.5. Data analyses and statistical methods

All data are presented as mean (SD) unless otherwise stated, with statistical significance accepted at $p<0.05$. Between condition differences values were analysed with paired t-test, or if the data were not normally distributed (as determined by the Shaprio-Wilk test), then differences were analysed by the Wilcoxon test. Data were analysed at set time points: 1) an average taken from 55 to 60 minutes (to compare conditions at the end of the plateau phase); 2) at the inflection point of T_{re}; and 3) during the final minute of each condition. It was assumed that participants would be in a thermal steady state or near-steady state in T_{re} by the 55 to 60 minutes exercise period, therefore the associated thermoeffector responses should also have become stable. Perceptual values and $\dot{V}O_2$ were compared at the discreet time points at which they were collected. Figures 7.2 and 7.3 present data from the rest period up to the 100^{th} minute as that is the last time point where all 16 participants were exercising in both conditions. Thermoregulatory 'reserve' was assessed by a repeated measures ANOVA (time x humidity) comparing values at the point of T_{re} inflection, 10 minutes post inflection, 20 minutes post inflection and at the end of the exercise, these time points were chosen because they included all 16 participants.

7.3. Results

7.3.1. Ambient conditions and experimental control

All participants verbally confirmed that they had followed the pre-test instructions and presented with a urine specific gravity equal to or below 1.020. Participants also presented for each test with similar pre-exercise body mass ($t_{(15)}=0.936$ $p=0.364$) and resting T_{re} ($t_{(15)}=0.936$, $p=0.364$), and lost no more than 1.42 % of their starting nude body weight during a given condition, suggesting that each participant began each condition in a similar physiological state and did not become excessively dehydrated during the conditions. The T_{db} and RH in the LH condition were 34.06 (0.49) °C and 24.05 (2.56) % RH, respectively and the T_{db} and RH over the rest period and first hour of exercise in the HH condition were 28.11 (0.31) °C and 83.18 (2.23) %RH, respectively. Both conditions compared favourably to the target conditions of 28 °C, 80 % RH and 34 °C, 20 % RH respectively. In the last five minutes (average) of the first hour of exercise there were differences in T_{db}, RH and WBGT between conditions ($z=-3.516$, $p=<0.001$; $t_{(15)}=60.103$, $p=<0.001$; $t_{(15)}=15.317$, $p<0.001$) (see Table 7.2).

7.3.2. Thermal profile and thermophysiological responses under steady-state low and high humidity conditions

During 55 to 60 minutes (final 5 minutes of 1st hour of exercise) there was no effect of ambient humidity condition on T_{re} (Figure 7.2), but $\overline{T}_{sk}$ and $\overline{T}_{b}$ were lower ($t_{(15)}$=-10.999, p=<0.001; $t_{(15)}$=-2.910, p=0.011) and HF higher (z=-3.516, p=<0.001) in the HH condition compared to the LH condition (see Figure 7.2 and 7.3). SW was also higher in the HH condition (z=-3.155, p=0.002), although this was likely a function of the higher WBGT ($t_{(15)}$=15.317, p<0.001). However $SR_{Forearm}$ was higher in LH condition ($t_{(15)}$=-2.163, p=0.047), but all other variables were not statistically different between the humidity conditions (see Table 7.2).

Table 7.2. Comparison of the mean (SD) ambient conditions, thermal profiles and physiological responses between the 55-60th minute when cycling at 60 W in a low (20 % RH) and high (80 % RH) humidity condition (n=16). *Significant difference between humidity conditions p<0.05

	Low Humidity	High Humidity	p value
Environmental conditions			
T_{db} (°C)	34.21 (0.48)	28.09 (0.25)	* <0.001
RH (%)	24.06 (2.98)	83.13 (2.07)	* <0.001
WBGT (°C)	23.90 (0.65)	26.69 (0.24)	* <0.001
Thermal profile			
T_{re} (°C)	37.29 (0.32)	37.29 (0.36)	0.976
Δ T_{re} from rest (°C)	0.46 (0.32)	0.41 (0.39)	0.406
$\bar{T}_b$ (°C)	37.12 (0.30)	36.99 (0.27)	*0.011
$\bar{T}_{sk}$ (°C)	35.87 (0.28)	34.93 (0.43)	* <0.001
Heat production ($W \cdot m^{-2}$)	272.94 (52.30)	278.53 (49.37)	0.469
HF ($W \cdot m^{-2}$)	34 (13)	79 (7)	* <0.001
Thermoeffector response			
SR_{Back} ($L \cdot m^{-2} \cdot hr^{-1}$)	0.36 (0.11)	0.36 (0.17)	0.875
$SR_{Forearm}$ ($L \cdot m^{-2} \cdot hr^{-1}$)	0.47 (0.19)	0.42 (0.19)	* 0.047
$SkBF_{Forearm}$ (% of highest 5 mins)	76 (16)	71 (22)	0.320
$SkBF_{Finger}$ (% of highest 5 mins)	74 (19)	71 (20)	0.514
HR ($b \cdot min^{-1}$)	108 (13)	107 (20)	0.872
$\dot{V}O_2$ ($L \cdot min^{-1}$)	1.35 (0.16)	1.34 (0.15)	0.937
Perceptual measures			
TS (VAS- 20cm)	15.25 (8.70)	15.00 (6.80)	0.856
TC (VAS- 20 cm)	11.40 (10.40)	10.50 (11.40)	0.485
SW (VAS- 20 cm)	8.95 (11.20)	10.70 (13.20)	0.002
RPE (Category ratio scale)	12.00 (7.00)	11.00 (8.00)	0.808

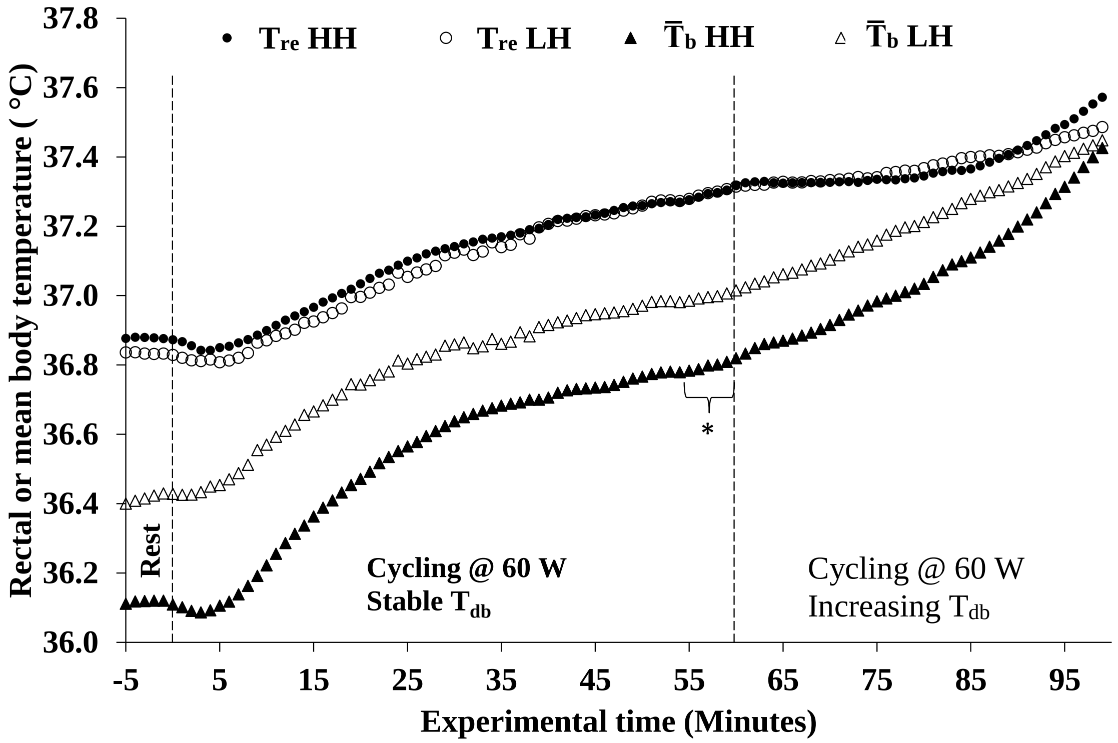


Figure 7.2. Mean rectal (circles) and body temperatures (triangles) for n=16 males whilst resting and cycling at 60 W in LH and HH conditions with stable and increasing ambient temperature. Statistical comparison was made over the 55-60 minute period. *Significant difference in $\bar{T}_b$ in the LH and HH condition ($p<0.05$)

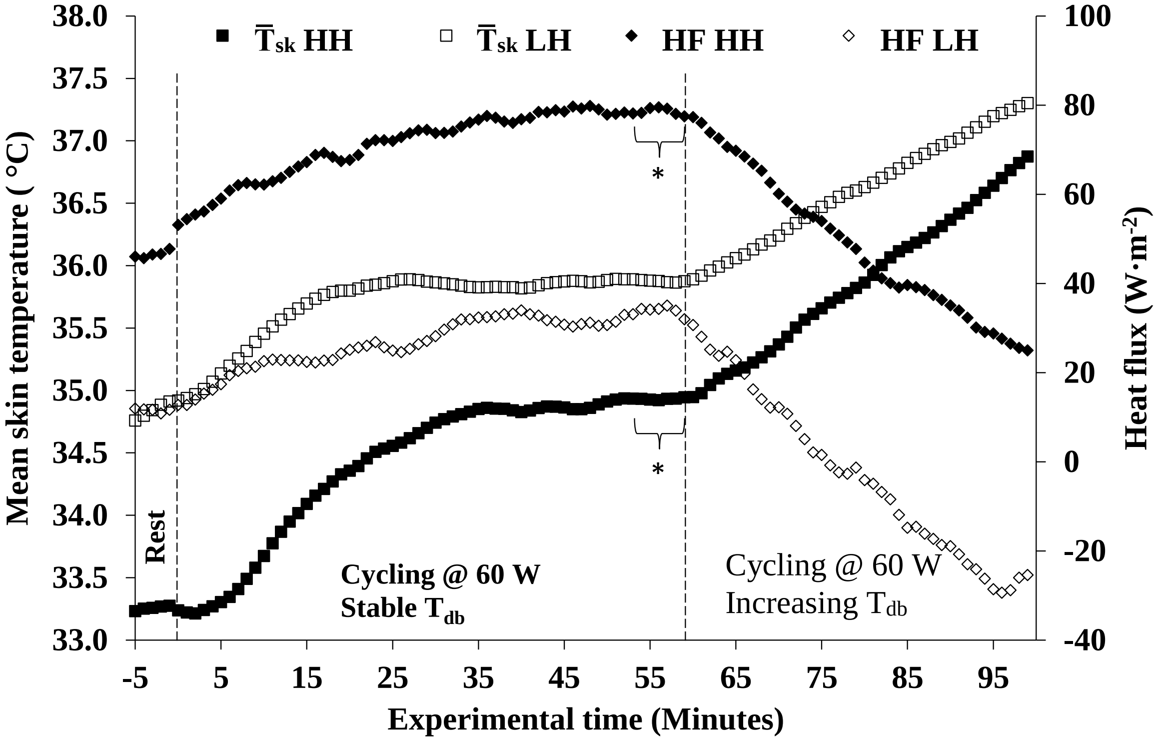


Figure 7.3. Mean skin temperatures (squares) and heat flux (diamonds) for n=16 males whilst resting and cycling at 60 W in LH and HH conditions with stable and increasing ambient temperature. Statistical comparison was made over the 55-60 minute period. * Significant differences between $\bar{T}_{sk}$ and HF in the LH and HH condition ($p<0.05$).

7.3.3. Thermophysiological responses at T_{re} inflection in low and high humidity conditions

In the HH condition, the $\overline{T}_b$ was lower, and WBGT and RH were higher at the T_{re} inflection point in comparison to the LH condition ($t_{(15)}$= -12.435, p=<0.001; $t_{(15)}$=39.467, p=<0.001; $t_{(15)}$=4.225, p=0.001). Although this did not impact on T_{re}, which was similar at the inflection point, the $\overline{T}_{sk}$ and $\overline{T}_b$ were significantly higher in the LH condition ($t_{(15)}$=-6.205, p=<0.001, $t_{(15)}$ =-2.566, p=0.021) with a lower HF (z=-3.516, p=<0.001). Skin wettedness was higher in the HH condition (z=-2.974, p=0.003), but all other variables were not statistically different (see Table 7.3). As the experimental time point at the T_{re} inflection point was not different, the physiological and perceptual responses are not likely to be due to differences in test duration. The E_{req} was significantly higher in the LH condition ($t_{(15)}$-2.707, p=0.016), as was the E_{max} ($t_{(15)}$-22.990, p<0.001), but there was significantly higher heat storage in the HH condition ($t_{(15)}$3.800, p=0.002). The water vapour partial pressure at the skin surface was higher in the LH than the HH condition ($t_{(15)}$-6.190, p<0.001), and as the water vapor partial pressure of the ambient air was lower in the LH than the HH condition ($t_{(15)}$19.693, p<0.001), this indicates a reduced potential for evaporative heat exchange in the HH condition due to the narrower water vapour partial pressure gradient (see Table 7.3). R and C were both significantly different between conditions (z=-3.527, p<0.001; $t_{(15)}$=13.341, p<0.001), with the rates of R and C in the LH condition indicating heat gain.

Table 7.3. Comparison of the mean (SD) thermal, thermoeffector and perceptual responses at the inflection point in T_{re} when cycling at 60 W in a low (20 % RH) and high (80 % RH) humidity condition (n=16). *Significant difference between humidity conditions (p<0.05).

Variable	Low humidity	High humidity	p value
Time at inflection (minutes)	93.00 (13.48)	88.88 (5.81)	0.140
Environmental conditions			
T_{db} (°C)	41.66 (2.77)	34.47 (1.13)	* <0.001
RH %	26.40 (2.94)	80.56 (3.53)	* <0.001
WBGT (°C)	29.69 (2.21)	31.89 (0.92)	* 0.001
Thermal profile			
T_{re} (°C)	37.42 (0.37)	37.40 (0.43)	0.670
ΔT_{re} (°C)	0.57 (0.39)	0.50 (0.45)	0.305
$\bar{T}_b$ (°C)	37.35 (0.38)	37.17 (0.41)	* 0.021
$\bar{T}_{sk}$ (°C)	37.09 (0.53)	36.32 (0.45)	* <0.001
Heat flux ($W \cdot m^{-2}$)	-25 (20)	35 (13)	* <0.001
Partitional calorimetry and calculated variables			
E_{req} ($W \cdot m^{-2}$)	215.92 (35.49)	186.83 (38.39)	* 0.016
E_{max} ($W \cdot m^{-2}$)	133.91 (11.22)	47.95 (8.11)	* <0.001
Heat production ($W \cdot m^{-2}$)	263.55 (35.25)	254.82 (39.23)	0.428
Heat storage ($W \cdot m^{-2}$)	26.95 (8.94)	33.52 (7.91)	*0.002
P_s (kPa)	6.12 (0.14)	5.92 (0.12)	* <0.001
P_a (kPa)	2.15 (0.38)	4.50 (0.28)	* <0.001
R ($W \cdot m^{-2}$)	-0.04 (0.09)	0.02 (0.02)	* <0.001
C ($W \cdot m^{-2}$)	-10.08 (5.30)	4.09 (2.29)	* <0.001
Thermoeffector response			
SR_{Back} ($L \cdot m^{-2} \cdot hr^{-1}$)	0.45 (0.16)	0.51 (0.19)	0.164
$SR_{Forearm}$ ($L \cdot m^{-2} \cdot hr^{-1}$)	0.66 (0.21)	0.68 (0.19)	0.727
$SkBF_{Forearm}$ (% of highest 5 mins)	99 (22)	92 (8)	0.278
$SkBF_{Finger}$ (% of highest 5 mins)	85 (12)	80 (14)	0.325
HR ($b \cdot min^{-1}$)	123 (17)	126 (24)	0.482
$\dot{V}O_2$ ($L \cdot min^{-1}$)	1.51 (0.22)	1.43 (0.20)	0.379

Perceptual measures			
TS (VAS- 20 cm scale)	16.40 (6.40)	16.40 (5.10)	0.776
TC (VAS- 20 cm scale)	8.05 (10.80)	8.15 (11.80)	0.629
SW (VAS- 20 cm scale)	12.85 (11.70)	16.70 (9.70)	* 0.003
RPE (Category ratio scale)	13.00 (8.00)	13.00 (10.00)	0.808

7.3.4. Thermophysiological responses beyond the T_{re} inflection point and at the end of exercise in low and high humidity conditions

Table 7.4 shows the presence of a thermoregulatory reserve beyond the T_{re} inflection point in the thermoregulatory variables for the LH and HH conditions, with the exception of $SkBF_{Forearm}$, where peak vasodilation was already present at the point of inflection. This indicates that there was no plateau in SR beyond the inflection point in T_{re} for either condition, instead a further increase occurred over time as the thermal profile of the exercising individuals increased (Table 7.4). Focusing specifically on the thermoeffector responses, from the inflection in T_{re} to the end of both conditions there was a significant increase in SR_{Back}, $SR_{Forearm}$, and $SkBF_{Finger}$ ($F_{(1,15)}$=45.860, p=<0.001; $F_{(1,15)}$=51.517, p=<0.001 and $F_{(1,15)}$=39.454, p=<0.001) respectively in both HH ($t_{(15)}$=-5.622, p=<0.001; $t_{(15)}$ =-6.945, p=<0.001 and $t_{(15)}$ =-4.315, p=0.001) and LH ($t_{(15)}$=-6.040, p=<0.001; $t_{(15)}$=-5.379, p=<0.001 and $t_{(15)}$=-4.569, p=<0.001) conditions.

At the end of exercise, T_{re} was significantly higher in the HH (38.50 [0.40] °C) than the LH (38.02 [0.41] °C) condition ($t_{(15)}$=4.867, p=<0.001) as was $\overline{T}_{sk}$ and $\overline{T}_b$ (38.79 [0.52] °C *vs.* 38.41 [0.44] °C); (38.56 [0.37] °C *vs.* (38.10 [0.40] °C) significantly different ($t_{(15)}$ =2.865, p= 0.012; $t_{(15)}$=4.749, p= <0.001). The change in T_{re} was also significantly different ($t_{(15)}$=3.791, p= 0.002) with the HH condition demonstrating the greater increase (1.62 [0.55] °C *vs.*1.18 [0.48] °C). This led to a significantly higher SR_{Back} in the HH (0.69 [0.23] $L \cdot m^{-2} \cdot hr^{-1}$) compared to the LH (0.58 [0.21] °C) condition ($t_{(15)}$=2.166, p= 0.047) at the end of the test (see Table 7.4).

Table 7.4. Comparison of the mean (SD) thermoregulatory response at the T_{re} inflection point, the at set time points beyond the T_{re} inflection point and at the end of exercise for participants cycling at 60 W in a low (LH) and high humidity (HH) condition (n=16). * Significantly different from 10 min post inflection, 20 min post inflection and end of test, ** Significantly different from 20 min post inflection and end of test,*** Significantly different from end of test, [a] Significant difference between HH and LH conditions at test termination.

Variable	Condition	Inflection	10 min post inflection	20 min post inflection	End of test
T_{re} (°C)	*LH*	37.42 (0.37) *	37.53 (0.39) **	37.68 (0.42) ***	38.02 (0.41)
	HH	37.40 (0.43) *	37.56 (0.45) **	37.84 (0.48) ***	38.50 (0.40) [a]
Δ T_{re} (°C)	*LH*	0.57 (0.39) *	0.69 (0.44) **	0.83 (0.48) ***	1.18 (0.48)
	HH	0.50 (0.45) *	0.66 (0.48) **	0.93 (0.51) ***	1.62 (0.55) [a]
$\overline{T}_b$ (°C)	*LH*	37.35 (0.38) *	37.52 (0.40) **	37.70 (0.44) ***	38.10 (0.40)
	HH	37.17 (0.41) *	37.42 (0.43) **	37.77 (0.45) ***	38.56 (0.37) [a]
$\overline{T}_{sk}$ (°C)	*LH*	37.09 (0.53) *	37.45 (0.51) **	37.83 (0.57) ***	38.41 (0.44)
	HH	36.32 (0.45) *	36.86 (0.44) **	37.54 (0.46) ***	38.79 (0.52) [a]
Heat production ($W \cdot m^{-2}$)	*LH*	264 (35) ***	-	-	313 (55)
	HH	255 (39) ***	-	-	294 (44)
SR_{Back} ($L \cdot m^{-2} \cdot hr^{-1}$)	*LH*	0.45 (0.16) *	0.49 (0.18) **	0.54 (0.19) ***	0.58 (0.21)
	HH	0.51 (0.19) *	0.58 (0.19) **	0.64 (0.20) ***	0.69 (0.23) [a]
$SR_{Forearm}$ ($L \cdot m^{-2} \cdot hr^{-1}$)	*LH*	0.66 (0.21) *	0.71 (0.20) **	0.75 (0.20) ***	0.80 (0.19)
	HH	0.68 (0.19) *	0.78 (0.19) **	0.82 (0.18) ***	0.85 (0.19)
$SkBF_{Forearm}$ (% of highest 5 mins)	*LH*	98.92 (21.92)	92.98 (8.64)	92.94 (7.27)	91.19 (9.65)
	HH	91.79 (7.82)	92.83 (7.36)	95.52 (6.88)	91.36 (9.61)

$SkBF_{Finger}$ (% of highest 5 mins)	*LH*	84.81 (12.35) ***	85.22 (16.45) ***	85.97 (21.81) ***	103.82 (12.17)
	HH	80.34 (13.64) *	85.20 (14.27) ***	89.18 (13.12) ***	98.89 (7.69)

Finally, over the total experimental duration, total sweat production was significantly higher in the HH (1.09 [0.36] $L \cdot m^{-2} \cdot hr^{-1}$) than the LH (0.88 [0.20] $L \cdot m^{-2} \cdot hr^{-1}$) condition ($t_{(15)}$ =3.725, p=0.002). However, the percentage of sweat produced (L) that evaporated was significantly lower in the HH (66.59 [6.07] %) compared to the LH (83.60 [4.90] %) condition ($t_{(15)}$=-16.079, p=<0.001) as was the absolute amount (0.84 [0.22] *vs.* 1.33 [0.29] kg, $t_{(15)}$=-7.480, p=<0.001).

7.4. Discussion

This was the first study to increase T_{db} incrementally to evoke an inflection in T_{re} whilst simultaneously measuring thermoeffector responses and the thermal profile of the exercising participant under conditions of low and high humidity. This study tested the hypotheses that at the upper limit of the TZ (as identified by the point of inflection in T_{re}), the ambient temperature, as well as the individual thermal profile and the resultant thermoeffector response, would differ between the two humidity conditions. Additionally, it was also hypothesised that there would be evidence of a thermoregulatory ‘reserve’ beyond the T_{re} inflection point under low and high humidity conditions, the presence of further increases in SR and / or SkBF would be evident beyond the T_{re} inflection point, thereby indicating that the inflection point in T_{re} represents a “permissive” thermoregulatory response, which increases the thermoafferent drive as well as the thermal gradient for heat loss, rather than a loss of thermoregulatory control.

The results from the present study demonstrated that the ambient T_{db} was significantly higher in the LH condition than in the HH condition at the T_{re} inflection point, therefore, the first hypothesis - *the T_{db} will differ at the deep body temperature inflection points under low and high humidity conditions* - can be accepted. However, this is, by intention, influenced by the experimental design as the starting T_{db} in the LH condition was approximately 6 °C higher than in the HH condition, to account for the reduced evaporative heat loss capacity provided by the HH environment and to ensure a similar experimental duration between the humidity conditions. However, despite the lower initial T_{db}, the HH condition had a WBGT approximately 3 °C higher than in the LH condition during the first, stable, 60 minute exercise period. WBGT is widely used as an index of thermal stress, but it has limitations when predicting the physiological response to a particular environmental condition and should be used with caution (Parsons, 2003), particularly in humid environments with low air movement and / or high levels of clothing insulation, where it underestimates the level of thermal strain experienced (Budd, 2008; Taylor, 2006a). Indeed, the present study has shown that in the initial stable period of the testing, a higher $\overline{T}_{sk}$ and $\overline{T}_{b}$, and higher rates of $SR_{Forearm}$, were present with the higher T_{db} (LH condition) and not with the higher WBGT (HH condition), indicating these responses may be more driven by T_{db} and its effects on $\overline{T}_{sk}$ than environmental heat stress, as determined by the WBGT.

Lind (1963a) suggested that the upper limit of the PZ is associated with the minimum bodily thermal gradient compatible with the transfer of adequate amounts of heat from the core to the periphery, suggesting that the thermal profile should be similar at the upper limit of the PZ when exercising at a given work rate, even under conditions differing in RH. However, the data from the present study has shown that, the $\overline{T}_{b}$ and $\overline{T}_{sk}$ were significantly higher in the LH condition, compared to the HH condition at the T_{re} inflection point, but there was no difference in the T_{re} at the T_{re} inflection point. Therefore, the second hypothesis - *that the thermal profile of the individual (as indicated by T_{re}, $\overline{T}_{b}$ and $\overline{T}_{sk}$,) will differ at the deep body temperature inflection point under low and high humidity conditions* - can only be accepted in part. Although, time was not the independent variable, T_{db} was increased by 1 °C every 5 minutes after 60 minutes of cycling so time should, therefore, be analogous to T_{db} after the initial stable 60 minute exercise period *i.e.* regardless of the initial differences in starting temperatures between the humidity conditions, after the same amount of experimental time, the T_{db} should have increased by a corresponding amount. Importantly, there was no difference in the experimental time at the T_{re} inflection, despite different T_{db} and WBGT, thus the difference in exercise time is not a confounding factor. Nonetheless, our data are not consistent with Lind's (1963) assertion given that there was a greater thermal gradient between the core (as indicated by T_{re}) and the shell (as indicated by $\overline{T}_{sk}$) in the LH condition than in the HH condition at the T_{re} inflection point, and that there were significant differences in heat flux.

There are two types of study design that have previously been employed in this area of research: 1) those that have conditions differing in % RH and have adjusted the T_{db}

accordingly to ensure that all conditions are matched or similar in terms of WBGT (Backx, McNaughton, Crickmore, Palmer, & Carlisle, 2000; Hayes *et al*., 2014; Stapleton, Wright, Hardcastle, & Kenny, 2012) and, 2) those that have set a fixed T_{db} for all conditions but have differed in the % RH, and WBGT (Maughan *et al*., 2012; Moyen, Ellis, *et al*., 2014). For the studies in the second category, $\overline{T}_{sk}$ was significantly higher in the humid environment (Maughan *et al*., 2012; Moyen, Ellis, *et al*., 2014), presumably due to the reduced evaporative capability of the environment. However, Hayes *et al*. (2014), as well as the present study, demonstrated that the $\overline{T}_{sk}$ was higher in the low humidity condition, presumably as a consequence of the higher T_{db}. Hayes *et al.* (2014) investigated repeated sprint performance in humid (33.7 °C [0.5] °C, 78.2 % [2.3] % RH) and dry (40.2 °C [0.2] °C, 33.1 % [4.9] % RH) ambient conditions with a fixed WBGT (approximately 30 °C, which is similar to the present studies conditions). In the study of both Hayes *et al.* (2014) and the present study (at the T_{re} inflection point), there was still capacity to lose dry heat to the environment in the high humidity environment, but not in the low humidity condition. This is further evidenced in the present study by the significantly lower HF throughout the LH condition, indicating lower levels of heat loss from the skin and from, on average, 76 minutes onwards, the HF data indicates that there was only heat gain (Figure 7.2).While this provides a clear explanation as to why there was significantly higher $\overline{T}_{b}$ and $\overline{T}_{sk}$ in the LH condition, the fact remains that there was no effect on the thermoeffector response (Table 7.3). As T_{re} and Δ T_{re} were not significantly different between humidity conditions at the end of the first hour under the stable T_{db} or at the T_{re} inflection point, this suggests that a change in absolute and rate of increase in T_{re} is required to drive thermoeffector response in the present study. These T_{re} results are similar to previous studies that have investigated the influence of humidity on thermoregulation during exercise in the heat with a matching (or similar) WBGT (Backx *et al*., 2000; Hayes *et al*., 2014; Stapleton *et al*., 2012), where it was reasoned that the similar T_{re} between conditions arose because although the capacity for evaporative heat loss was reduced in the high humidity environments, the rates of dry heat exchange were adequate to compensate for the attenuated evaporative heat loss, and *vice versa* for the low humidity condition.

As expected from the conventional definition, at the inflection point in T_{re} for both conditions, the E_{req} was higher than the E_{max}, meaning ‘uncompensable’ heat stress was present. However, these data show that further heat loss is possible beyond this point (Table 7.4). The E_{req} and E_{max} were lower in the HH condition at the inflection point, with the lower E_{req} due to R and C still being heat loss pathways as opposed to heat gain in the LH condition (Table 7.3). The lower E_{max} is due to the physical ambient properties of the condition which reduces the evaporative capacity of the environment, as described elsewhere (Moyen, Ellis, *et al*., 2014). For the first time then in low and high humidity conditions, it has been shown that there is already heat storage at the point of uncompensable heat stress (T_{re} inflection point) and that this can significantly differ between humidity levels without affecting the absolute or change in T_{re}. Clearly, this must impact on total body heat, with a possible scenario that heat is progressively stored from the shell towards the core and thus doesn't immediately affect the T_{re}, *i.e.* the shell effectively insulates the core. This indicates although $E_{max\text{-}physical}$ was significantly different between conditions, if $E_{max\text{-}physiological}$ does not differ (inferred by a similar thermoeffector response and T_{re}), there will be no difference in T_{re} at the point of T_{re} inflection.

Given that there were no significant differences in the thermoeffector responses between the conditions at the T_{re} inflection point, the third hypothesis - *that the physiological (thermoeffector) responses will be higher at the deep body temperature inflection point in the high humidity condition compared to the low* - is rejected. Interestingly, this lack of difference in thermoeffector responses was evident despite significant differences in the thermal profile of the individual, as indicated by a higher $\overline{T}_b$ and $\overline{T}_{sk}$ in the LH condition. This suggests that, either these thermoeffector responses had reached the maximum possible evoked responses at the T_{re} inflection point, or that the differences in $\overline{T}_b$ and $\overline{T}_{sk}$ were not of sufficient magnitude to invoke a measurable difference in thermoeffector response and that the relative inputs from the core and shell at the T_{re} inflection point are weighted more in favour of the core. Indeed, it is known that cutaneous thermosensitivity is lower than that of the core in driving autonomic responses (Stolwijk & Hardy, 1966; Wyss, Brengelmann, Johnson, Rowell, & Silverstein, 1975). Moreover, it has been shown that once T_c reaches 38 °C during prolonged exercise in the heat, SkBF stops increasing and reaches an upper limit that is below expected maximal SkBF (Brengelmann *et al*., 1977; Johnson, 2010) and that expected to be present during hyperthermia at rest (Taylor, Johnson, O'Leary, & Park, 1984). This is due to competition for blood flow from exercising muscles (González-Alonso *et al*., 2008) and the brain (Nybo & Nielsen, 2001) as a large part of the available blood volume is directed to these areas and so leads to reduction in the removal of heat. In the present study T_{re} did not reach 38 °C until beyond the T_{re} inflection point, $SkBF_{Forearm}$ had already reached a peak rate at the T_{re} inflection point; clearly it is important to consider the rate of rise of T_c and $\overline{T}_{sk}$ rather than simply the thermal profile when comparing studies. However, the SR_{Back}, SRForearm, and $SkBF_{Finger}$ were at submaximal levels at the T_{re} inflection point in both HH and LH conditions.

Unfortunately, studies defining critical limits of thermoregulation (Dougherty *et al*., 2010; Kamon *et al*., 1978; Kenney & Zeman, 2002; Kenney *et al*., 1988, 1993) or studies that have compared thermoregulatory responses at a similar WBGT between conditions with differing levels of RH (Backx *et al*., 2000; Hayes *et al*., 2014; Stapleton *et al*., 2012) have not measured SkBF or local SR, so there is a paucity of literature to compare with. Maughan *et al.* (2012) have reported that $SkBF_{Forearm}$ was not influenced by the relative humidity of the environment, and it appears that a peak rate was reached after approximately 20 minutes of exercise; this is consistent with other work (Périard *et al*., 2012). This was not the case in the present study in which $SkBF_{Forearm}$ and $SkBF_{Finger}$ had a marked increase from the end of the first hour until the T_{re} inflection point, beyond this point $SkBF_{Finger}$ continued to increase, but $SkBF_{Forearm}$ did not significantly change. It is known that an individual's evaporative heat loss is reduced with increasing RH, as the water vapour pressure gradient between the saturated skin and the environment diminishes (Berglund & Gonzalez, 1977; Givoni & Goldman, 1973). This was seen in the present study, with the gradient between P_s and P_a being smaller in the HH condition (Table 7.3), at the T_{re} inflection point. However, this did not affect SR at the point of inflection, but as differences between conditions did become apparent by the end of the exercise period, this is a factor which may have influenced thermoregulation later in the experiment with increasing levels of heat storage.

From the inflection point in T_{re} to the end of each condition there was a significant increase in SR_{Back}, SRForearm, and $SkBF_{Finger}$ in both LH and HH conditions (Table 7.4). Therefore, the final hypothesis - *a thermoregulatory 'reserve' capacity, as indicated by sub-maximal rates of skin blood flow and sweat rate, will exist at the deep body temperature inflection point under low and high humidity conditions* - is accepted. This observation of an apparent 'thermoregulatory reserve' is in contrast to the didactic models which show SkBF and SR at 'maximum' when thermoregulation is no longer possible (Taylor, Kondo, & Kenney, 2008) (Figure 2.2). This is an important observation as it essentially removes the notion of uncompensable heat stress being caused by the inability to sweat further, which leads to increased heat storage due to insufficient evaporative heat loss. As $\overline{T}_{sk}$ rises when exercising in a hot environment, the gradient for dry heat transfer is reduced (González-Alonso, 2012), so it is reasoned that when $\overline{T}_{sk}$ is high, a rising T_c serves to maintain, and / or re-establish, a favourable thermal gradient, thereby reducing the SkBF requirements for heat loss (Cheuvront *et al.*, 2010). As an inflection in T_{re} occurs when there is a clear thermoregulatory reserve and with heat storage already present, it could be argued that it is not a failure of thermoeffector responses to offset heat storage that causes an increase in T_c, but rather that an elevation in T_c causes increased thermoafferent activity. Thus, we contend that the elevation in T_{re} at the inflection point should not be equated with a loss of thermoregulatory control but rather should be considered as a 'permissive' increase in T_{re}.

Comparison between the humidity conditions at the end of the experiment showed that T_{re} was significantly higher in the HH (38.50 [0.40] °C) than the LH (38.02 [0.41] °C) condition. Over that time period, the metabolic heat production had increased significantly from the point of inflection (to ~ 300 $W \cdot m^{-2}$), despite external work rate remaining at 60 W, but it did not differ between conditions. This indicates that efficiency changed over time, which has been observed elsewhere when cycling in the heat (Hettinga *et al.*, 2007) or that CV strain has occurred. Therefore, as the reduction in efficiency was not different between the conditions, the higher end T_{re}, $\overline{T}_b$ and $\overline{T}_{sk}$ in the HH condition must likely be due to the higher thermal strain as a consequence of the higher WBGT °C in the HH condition and reduction in heat loss capacity as reflected by a significantly lower estimated sweat evaporation rate in the HH (66.59 [6.07] %) compared to the LH (83.60 [4.90] %) condition. Higher relative humidity would lead to a reduced capacity of the air for evaporation (Kerslake, 1972) and a reduction in sweating efficiency (Candas *et al.*, 1979). At the end of exercise in the present study, in the HH condition, there was greater SR_{Back} in the final minute of exercise and the comparison of pre and post nude weights suggest greater total overall sweat production compared to the LH condition. It is acknowledged that this could be caused by the higher WBGT in the HH condition and so may not necessarily indicate greater thermoregulatory drive for sweating in the HH condition. However, the fact that, in both conditions, SR increased beyond the inflection point is of interest because it suggests the presence of a thermoregulatory 'reserve'. It is important to note that the test termination point was actually determined in most cases by the physical characteristics of the chamber (reaching its upper limit of 50 °C), which was unable to maintain the required heating rate, rather than exhaustion or an inability to continue, and so comparison in endpoint values between conditions are probably not valid as a measure of fatigue. However, they do serve the purpose to show change / no change over time from the point of inflection in T_{re}.

In summary, the present study has shown: i) the ambient T_{db} at the T_{re} inflection point differs between LH and HH conditions, being lower in the HH condition despite a higher WBGT (as WBGT is 70 % T_{db}); ii) although the T_{re} at the T_{re} inflection point was not different between humidity conditions, differences in the thermal profile were evident as indicated by the $\overline{T}_b$ and $\overline{T}_{sk}$ which were lower in HH condition, presumably due to the lower T_{db}; iii) despite the differences in thermal profile there were no significant differences in thermoeffector responses at the $T_{re,}$ which suggests that either the magnitude of difference in thermal profile was insufficient to induce meaningful differences in thermoeffector response, or that the cutaneous thermoafferent input is of less importance than that of the core in driving autonomic responses; iv) a thermoregulatory ‘reserve’ exists beyond the inflection point in T_{re} (in both a LH and HH environment), which implies that a ‘permissive’ increase in T_{re} serves to re-gain a more favourable thermal gradient between deep body, skin and environment and to evoke a greater thermoeffector responses.

8. Chapter Eight – Influence of Aerobic Fitness on Thermophysiological Responses and Biophysical Characteristics at the Inflection Point in Deep Body Temperature Under Conditions of High and Low Humidity.

8.1. Introduction

In Chapter Seven we examined the effect of the environment, and specifically the influence of humidity, on the thermal profile and thermoeffector responses at the upper limit of the thermoregulatory zone (TZ), but did not take into consideration the aerobic fitness of the participants. Aerobic fitness has long been known to influence thermoregulation (Havenith & van Middendorp, 1990; Jay *et al*., 2011; McLellan, 2001; Piwonka *et al*., 1965). Typically, endurance training results in increased aerobic fitness (Jones & Carter, 2000), leading to greater cardiovascular reserve (Aoyagi *et al*., 1997), as well as improved thermoregulatory function, even when the training takes place in thermoneutral environments (Baum *et al*., 1976; Buono & Sjoholm, 1988; Gisolfi & Robinson, 1969; Piwonka *et al*., 1965; Robinson, Turrell, Belding, & Horvath, 1943; Selkirk & McLellan, 2001). These adaptations may be driven by the improvements in aerobic fitness *per se* (Jones & Carter, 2000), as well as the regular exposure to higher core temperatures during exercise and training (Avellini, 1982).

The adaptations providing aerobically trained individuals with a thermoregulatory advantage over untrained individuals include: plasma volume expansion (Convertino, Keil, & Greenleaf, 1983); a reduction in the deep body temperature (T_c) threshold for skin vasodilation and sweating activation (Henane *et al*., 1977; Lee, Kim, Min, & Yang, 2014); greater skin blood flow at the same relative exercise intensity (Fritzsche & Coyle, 2000); a lower resting (Buono, Heaney, & Canine, 1998) and exercising (Aoyagi *et al*., 1997) T_c; a raised threshold for endotoxin leakage and inflammatory activation during exertional heat stress (Selkirk *et al*., 2008); greater levels of fat oxidation at moderate exercise intensities (Del Coso, Hamouti, Ortega, & Mora-Rodríguez, 2010); a reduction in physiological strain (Piwonka *et al*., 1965); reduced markers of central fatigue during uncompensable heat stress (Wright *et al*., 2012); and quicker adaption to heat acclimation programs (Robinson *et al*., 1943).

The attenuated cardiovascular response to exercise (Jones & Carter, 2000) and increased sudomotor sensitivity (Lee *et al*., 2014; Shin & Lee, 2014) seen with aerobic fitness are similar to adaptations occurring with partial heat acclimatization (Aoyagi *et al.*, 1997; Mora-Rodríguez, 2012; Piwonka *et al.*, 1965). Thus, increased aerobic fitness can enable enhanced heat dissipation (Aoyagi *et al.*, 1997; Piwonka & Robinson, 1967; Shvartz *et al.*, 1977; Shvartz, Magazanik, & Glick, 1974) and improved tolerance of exercise in the heat (Aoyagi *et al.*, 1997; Cheung & McLellan, 1998a; Selkirk & McLellan, 2001). However, whilst aerobically trained individuals typically display many physiological adaptations similar to those seen with heat acclimation (Aoyagi *et al.*, 1997), heat and exercise each induce specific transcriptional programmes (Kodesh & Horowitz, 2010) and, therefore, represent independent stressors.

A key consideration when comparing the thermophysiological responses of individuals with high and low aerobic fitness is the method used to standardise exercise intensity, *i.e.* the use of matched *absolute* or matched *relative* (typically expressed as percentage of an individual's maximal aerobic capacity [% $\dot{V}O_{2max}$]) work rate, as this will influence the thermoregulatory response (Cheung & McLellan, 1998a; Jay *et al.*, 2011; Kacin *et al.*, 2008; McLellan, 2001; Mora-Rodríguez *et al.*, 2010; Mora-Rodríguez, 2012; Périard *et al.*, 2012; Shvartz *et al.*, 1977; Wright *et al.*, 2012). Because work efficiency is largely unaffected by aerobic fitness during cycling exercise (Marsh *et al.*, 2000; Moseley *et al.*, 2004) metabolic heat production should, therefore, be unaffected by the fitness level of the participant when cycling at the same *absolute* external work rate. Accordingly, given the superior thermoregulatory capabilities evident in individuals with high aerobic fitness (Aoyagi *et al.*, 1997; Cheung & McLellan, 1998a; Piwonka & Robinson, 1967; Selkirk & McLellan, 2001; Shvartz *et al.*, 1977, 1974), aerobically fit individuals typically exhibit a reduced T_c and mean skin temperature ($\overline{T}_{sk}$) during exercise at a given external work rate, compared to less aerobically fit individuals, (Havenith *et al.*, 1998; Piwonka *et al.*, 1965; Selkirk & McLellan, 2001; Shvartz *et al.*, 1977; Wright *et al.*, 2012).

In contrast, during exercising at the same *relative* work rate *i.e.* the same percentage of $\dot{V}O_{2\,max}$, individuals with high aerobic fitness will be exercising at a higher *absolute* work rate, thereby generating more metabolic heat than their low aerobic fitness counterparts (Gagnon *et al.*, 2008). This higher *absolute* workload has been shown to result in increased cutaneous blood flow (Fritzsche & Coyle, 2000) and sweat rate (Nadel *et al.*, 1974; Roberts *et al.*, 1977) to counter the increased metabolic heat production. Consequently, when individuals with high aerobic fitness exercise at a given *relative* work rate in an environment which allows their inherent increased heat dissipation capabilities to 'balance' the discrepancy in metabolic heat production (Havenith *et al.*, 1998), such as those permitting high rates of evaporative heat loss, there may be no additional thermal burden compared to those individuals with low aerobic fitness exercising at the same *relative*, but lower *absolute* work rate (Gant *et al*., 2003; Jay *et al*., 2011; Périard *et al*., 2012). Indeed, historically it has been suggested that the equilibrium T_c during exercise under compensable conditions is determined by the *relative* work rate (Saltin & Hermansen, 1966), with the consequence that matched *relative* work rates are often used when comparing independent groups, such as participants of different aerobic fitness levels (Del Coso *et al*., 2010; Fritzsche & Coyle, 2000; Gant *et al*., 2003; Mora-Rodríguez *et al*., 2010), gender (Gerrett *et al.*, 2014; Havenith, Fogarty, Bartlett, Smith, & Ventenat, 2008; Keatisuwan, Ohnaka, & Tochihara, 1996; Kenney & Zeman, 2002), adiposity (Dougherty *et al*., 2009, 2010), or those assigned to different experimental conditions (Avellini, 1982). However, studies which acutely lower the $\dot{V}O_{2max}$ of participants by normobaric (Rowell, Freund, & Brengelmann, 1982) or hypobaric (Greenleaf, Card, & Saltin, 1969) hypoxia have shown that T_c is unaffected by the acute reduction in $\dot{V}O_{2max}$, indicating that $\dot{V}O_{2max}$ and T_c are not causally related.

A further criticism of many studies comparing individuals with different levels of aerobic fitness is that anthropometric differences, such as differences in body mass and body fat percentage (bf %), are often evident between the independent groups (Gass *et al*., 1991; Jay *et al*., 2011; Selkirk & McLellan, 2001). This is an important consideration because due to the lower heat capacity of adipose tissue compared to lean tissue (Havenith, 2001a) individuals with a greater body mass typically have smaller increases in T_c during heat stress (Havenith *et al*., 1998, 1995). Indeed, when groups of high ($\dot{V}O_{2max}$ = 60.1 [4.5] $mL \cdot kg^{-1} \cdot min^{-1}$) and low ($\dot{V}O_{2max}$ = 40.3 [2.9] $mL \cdot kg^{-1} \cdot min^{-1}$) aerobic fitness are matched for body mass and body surface area (BSA) a similar T_c and sweating rate is apparent during cycling exercise at a fixed heat production (~276 $W \cdot m^{-2}$), whereas differences are evident when they are matched for *relative* work rate (Jay *et al*., 2011). In contrast, Gant *et al.* (2003) demonstrated that rectal temperature (T_{re}) did not differ between ‘highly’ ($\dot{V}O_{2max}$ = 72.8 [0.8] $mL \cdot kg^{-1} \cdot min^{-1}$) and ‘moderately’ ($\dot{V}O_{2max}$ = 59.4 [0.7] $mL \cdot kg^{-1} \cdot min^{-1}$) trained runners with similar body mass during treadmill exercise at the same *relative* work rate (65 % $\dot{V}O_{2max}$), whereas a higher T_{re} was evident in the ‘moderately’ trained group when exercising at the same *absolute* work rate (fixed speed of 10.5 $km \cdot hr^{-1}$). However, an important consideration is that individual variability in mechanical efficiency is higher in treadmill running than cycle ergometery (Rowland, Staab, Unnithan, Rambusch, & Siconolfi, 1990; Smoljani, Morris, Dervis, & Jay, 2014) and it may not be appropriate to compare thermophysiological responses between studies using different exercise modes (weight bearing and non-weight bearing). Additionally, the relative homogeneity in $\dot{V}O_{2max}$ values of both participant groups in Gant *et al.* (2003) means that both groups may have optimised the advantageous thermoregulatory adaptions acquired through their aerobic training.

Most of the aforementioned studies investigating the influence of aerobic fitness on thermoregulation were undertaken in a neutral environment and / or under compensable environmental conditions (Gant *et al.*, 2003; Gass *et al.*, 1991; Greenleaf *et al.*, 1969; Jay *et al.*, 2011; Rowell *et al.*, 1982) and the influence of aerobic fitness on the transition to an uncompensable thermal state *i.e.* the upper limit of the TZ, as well as thermoregulation in a hot and uncompensable environment, is less clear. Studies that have determined the upper boundary of the TZ by systematically increasing either T_{db} or ambient water vapour pressure have not directly examined the influence of aerobic fitness. Comparisons have been made between males and females (Kamon *et al*., 1978; Kenney & Zeman, 2002), and lean and obese children (Dougherty *et al.*, 2010), but the inherent differences in aerobic fitness between the comparison groups in these studies are confounded by the gender and anthropometric differences, respectively. Nonetheless, Kamon *et al.*, (1978) suggest that, there is no difference in T_{re} at the point of inflection between men and women when matched for metabolic heat production, but women had significantly higher critical environmental limits and lower sweat rate whilst working at a *relative* work rate of 30 % $\dot{V}O_{2max}$, presumably due to their lower *absolute* metabolic heat production (Kenney & Zeman, 2002). Conversely, when lean and obese children exercised at a relative work rate of 30 % $\dot{V}O_{2max}$, it was shown that the obese children had significantly lower environmental limits than their lean counterparts, which were accompanied by a lower relative ($mL \cdot m^{-2} \cdot h^{-1}$), but not absolute ($mL \cdot h^{-1}$), mean sweating rate (Dougherty *et al.*, 2010).

Finally, the evidence regarding the benefits of aerobic fitness once exercising under uncompensable conditions is equivocal. Mora-Rodriguez *et al.* (2010) compared the thermophysiological responses of trained (60 [6] $mL \cdot kg^{-1} \cdot min^{-1}$) and untrained (44 [3] $mL \cdot kg^{-1} \cdot min^{-1}$) individuals during cycle ergometer exercise under ambient conditions of 36 [1] °C and 25 [2] % RH (airflow 2.5 $m \cdot s^{-1}$) during exercise at matched *relative* work rates. The trained participants had a larger rise in T_{re} at 40, 60 and 80 % $\dot{V}O_{2peak}$ and higher end

T_{re} at 80 % $\dot{V}O_{2peak}$, indicating that the heat production and heat dissipation did not 'equal out', although these findings are potentially confounded by anthropometric differences between the groups as well as differences in hydration, due to higher sweat rates in the trained participants and inadequate fluid replacement (Kuennen, Gillum, Dokladny, Schneider, & Moseley, 2013; McLellan, Cheung, Selkirk, & Wright, 2012). Anthropometric differences were better controlled by Selkirk and McLellan (2001), who compared participants of low and high aerobic fitness ($\dot{V}O_{2peak}$ expressed as mL·kg lean body mass [LBM]$^{-1}$·min^{-1}) and low % bf. The ambient conditions were 40 °C and 30 % RH (airflow <0.1 m·s^{-1}) and all participants wore a military biological and chemical protective ensemble (creating an uncompensable microclimate) and walked on a level treadmill at 3.5 km·h^{-1}. Exercise times were significantly longer for the participants with the high aerobic fitness and low body fat compared to the untrained participants with low body fat and trained participants with high body fat, demonstrating that high aerobic fitness and low body fatness confer a thermoregulatory advantage during exercise in an uncompensable environment at a fixed *absolute* work rate. It is important to note that all of the participants with the high aerobic fitness and low body fat ended exercise due to having reached the studies ethical ceiling for T_{re} of 39.5 °C, whereas other reasons for termination were evident with the other groups (exhaustion, discomfort, HR reaching or exceeding 95 % of maximum for 3 min and nausea). It is therefore possible that exercise times in this case underestimate the thermal tolerance of the trained individuals with low % bf in the study, as previous authors have reported that trained cyclists reach exhaustion at an oesophageal temperature (T_{oes}) of 40.1–40.2 °C (González-Alonso *et al*., 1999).

Accordingly, the present study sought to examine the influence of aerobic fitness on the biophysical characteristics and thermophysiological responses at the inflection point in T_c during exercise in low and high humidity conditions. These environmental conditions were selected to allow for high and low rates of evaporative heat loss, respectively. To negate confounding issues occurring in previous studies particular care was taken to ensure that groups were well matched for anthropometric measures and that comparisons were made for matched *absolute* and *relative* work rates.

8.1.1 The hypotheses tested were:

H_1 The ambient environmental conditions (dry bulb temperature [T_{db}] and wet bulb, globe temperature [WBGT]) at the T_{re} inflection point will differ between the two aerobic fitness groups at the same matched *absolute* and *relative* work rates, for both humidity conditions.

H_2 The thermal profile of the individual, as indicated by T_{re}, mean body temperature ($\overline{T}_b$) and $\overline{T}_{sk}$, will differ at the T_{re} inflection point between the two aerobic fitness groups at the same matched *absolute* and *relative* work rates, for both humidity conditions.

H_3 The physiological (thermoeffector) responses will differ at the T_{re} inflection point between the two aerobic fitness groups at the same matched *absolute* and *relative* work rates, for both humidity conditions.

H_4 There will be a thermoeffector 'reserve' for both groups, in both humidity conditions, at work rates beyond the T_{re} inflection point.

8.2 Methods

8.2.1 Participant characteristics

Sixteen males volunteered to participate in this study and gave written informed consent. This participant number is consistent with similar previous studies which have compared and detected differences between the thermal responses of groups of different fitness levels ([Jay *et al*., 2011; Périard *et al*., 2012] fourteen and sixteen respectively). All participants were healthy (as determined by an Exercise and Health History questionnaire (Appendix B), non-heat acclimated and non-smokers. Eight of the males were classified as 'low aerobic fitness' as they only took part in low to moderate aerobic exercises up to two times per week (less than 2 hours per session). The remaining eight males were classified as 'high aerobic fitness' as they took part in moderate and high intensity aerobic exercise between five and seven times per week. Care was taken to match the groups for body mass, height and bf %. Ethical approval was granted by Science Faculty Ethics Committee (SFEC) (Appendix A). Participant group characteristics are shown in Table 8.1.

Table 8.1. Participant group characteristics (mean [SD]) for Low (n=8) and High (n=8) aerobic fitness groups. * Significant difference between Low aerobic fitness and High aerobic fitness groups ($p<0.05$).

	Low aerobic fitness (n=8)	High aerobic fitness (n=8)	
Age (years)	21.75 (3.96)	27.88 (4.85)	*
Height (m)	1.78 (0.06)	1.78 (0.05)	
Body mass (kg)	74.17 (11.93)	77.31 (7.18)	
Body surface (DuBois formula m^2)	1.91 (0.16)	1.95 (0.10)	
Body fat (%) – Seven sites (ACSM, 2000)	10.27 (4.61)	9.27 (2.39)	
Body fat (%) – Four sites (Durnin & Rahaman, 1967)	15.05 (4.24)	14.58 (3.58)	
$\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	46.53 (6.73)	58.73 (6.28)	*
$\dot{V}O_{2max}$ ($L \cdot min^{-1}$)	3.44 (0.68)	4.62 (0.41)	*
Peak power at $\dot{V}O_{2max}$ (W)	270 (39)	372 (41)	*

8.2.2 Experimental design

Participants were required to visit the Extreme Environments Laboratory on either three or five occasions (dependent upon aerobic fitness group). Participants had at least 48 hours between tests and were always tested in either a morning or afternoon session (fixed within participant) in order to minimise diurnal variation.

On the first visit each participant performed a maximal aerobic capacity test ($\dot{V}O_{2max}$) and had their body fat estimated by measurement of skinfolds, details of which can be found in General methods (section 3.7 and 3.2.5 respectively). The remaining visits were for the experimental conditions which were undertaken in a balanced order. The Low aerobic fitness group exercised on two further occasions: 1) in a Low humidity (LH) environment (~20 % RH); and 2) a High humidity (HH) environment (~80 % RH) at an external work rate of 60 W. Once these trials were completed by all eight members of the Low aerobic fitness group, the mean percentage of $\dot{V}O_{2max}$ elicited during the first hour of cycling during each condition was calculated. Thereafter, the High aerobic fitness group exercised on four further occasions:

1) in a LH environment at an external work rate of 60 W

2) in a HH environment at an external work rate of 60 W

3) in a LH environment at an external work rate set to match the mean *relative* work rate exhibited by the Low aerobic fitness group when working at 60 W.

4) in a HH environment at an external work rate set to match the mean *relative* work rate exhibited by the Low aerobic fitness group when working at 60 W. The Low aerobic fitness group's two visits provided both their *absolute* and *relative* data for both environmental conditions for comparison against the High aerobic fitness group.

8.2.3 Experimental procedures

The pre-experimental procedures, environmental conditions, experimental set up and measurements were identical to those reported in Chapter Five, Six and Seven. Details of which are described in the General Methods. The method used to determine the T_{re} inflection point was the same to that reported in Six and Seven, as recommended in Chapter Five. Partitional calorimetry was undertaken as per the calculations described in 7.2.4.

8.2.4 Data analyses and statistical methods

Pre-test naked body mass and T_{re} were compared between each test for Low and High aerobic fitness groups (separately) by paired samples t-tests. Comparisons between Low and High aerobic fitness groups in LH and HH conditions at i) matched *absolute* work rates and ii) matched *relative* work rates were tested with separate two way mixed model ANOVAs (within effect = humidity condition [LH or HH], between effect=aerobic fitness [Low aerobic fitness or High aerobic fitness]). For data that were not appropriate for parametric tests, a Friedman ANOVA (within effect=humidity) and Kruskal-Wallis Test (between effect=fitness) were used. If *post hoc* testing was required, paired (within) and independent (between) samples t-tests were used for parametric data, or a Wilcoxon (within) and Mann Whitney (between) tests for non-parametric testing. The presence of a thermoregulatory reserve beyond the T_{re} inflection was assessed by paired t-tests comparing values at the point of T_{re} inflection, 10 minutes post inflection, 20 minutes post inflection and at the end of the exercise in the LH and HH conditions (separately), these time points were chosen because they contained all 16 participants.

8.3 Results

8.3.1 Ambient conditions, experimental control and work rate matching

All participants verbally confirmed that they had followed the pre-test instructions, and presented with a urine specific gravity equal to or below 1.020 SG. For the Low aerobic fitness group, there was no difference in starting body mass ($t_{(7)}$=-0.329, p= 0.752) or starting T_{re} ($t_{(7)}$=-1.902, p= 0.099) between the HH and LH conditions respectively. Similarly, for the High aerobic fitness group there was no difference in starting body mass ($t_{(7)}$=-0.526, p=0.615; $t_{(7)}$=-0.507, p=0.627) or starting T_{re} ($t_{(7)}$=-0.608, p=0.563; $t_{(7)}$=-0.365, p=0.726) in the HH and LH conditions, respectively, or between the matched *absolute* and *relative* conditions. During the experimental conditions the participant's body mass decreased on average by 0.62 (0.64) %, suggesting that each participant began the trials in a similar physiological state and did not become excessively dehydrated during each condition. The T_{db} over the rest period and first hour of exercise in the LH condition was 34.04 (0.45) °C and RH was 24.02 (2.28) %, with a T_{db} of 28.15 (0.29) °C and RH of 82.98 (2.39) % in the HH condition, which compared favourably to the target conditions of 34 °C, 20 % RH and 28 °C, 80 % RH, respectively.

The mean *relative* exercise intensity in both ambient conditions at the matched *absolute* work rate of 60 W was 39.72 (6.11) % $\dot{V}O_{2max}$ and 29.96 (3.33) % $\dot{V}O_{2max}$ for the Low and High aerobic fitness groups, respectively, which was significantly different between the groups ($F_{(1,7)}$=21.228, p=0.002). However, the metabolic heat production elicited at this work rate was not significantly different between fitness groups in either the LH ($t_{(14)}$=-0.887, p= 0.390) or HH (z=-0.840, p=0.442) condition (Table 8.2). In order to match the *relativ*e exercise intensity of the Low aerobic fitness group, the external work rate was increased for the High aerobic fitness group to a mean of 99 (14) W, for additional tests under both LH and HH conditions. This elicited a *relative* exercise intensity of 39.31 (4.54) % $\dot{V}O_{2max}$; matching was successful as there was no significant difference in the *relative* exercise intensity between the Low and High aerobic fitness groups for either the LH ($t_{(14)}$=-0.175, p=0.864) and HH ($t_{(14)}$=0.443, p=0.664) conditions. However, metabolic heat production at this matched *relative* work rate was significantly higher (as intended) in the High aerobic fitness group in both the LH conditions ($t_{(14)}$=-5.499, p=<0.001) and HH ($t_{(14)}$=-3.495, p=0.004) conditions (Table 8.2).

Table 8.2. Mean (SD) metabolic heat production for Low (n=8) and High (n=8) aerobic fitness individuals when exercising at matched *absolute* (60 W) and *relative* work rates under conditions of Low and High humidity in the first hour of testing when air temperature was stable. * Significantly greater ($p<0.05$) than low fitness group and high fitness group at 60 W, within humidity condition.

Humidity	**Low humidity (LH)**			**High humidity (HH)**		
Group and work rate condition	**Low aerobic fitness 60 W**	**High aerobic fitness 60 W**	**High aerobic fitness 40 % VO_{2max}**	**Low aerobic fitness 60 W**	**High aerobic fitness 60 W**	**High aerobic fitness 40 % VO_{2max}**
Metabolic heat production ($W \cdot m^{-2}$)	231 (22)	241 (24)	315 (37) *	246 (40)	229 (18)	311 (35) *

8.3.2 The influence of aerobic fitness on the T_{re} inflection point in LH and HH conditions during cycling exercise at a matched absolute work rate (60 W).

Environmental conditions, thermal profile, thermophysiological and perceptual responses at the T_{re} inflection point for the Low and High aerobic fitness groups during exercise at the same *absolute* work rate under LH and HH conditions are presented in Table 8.3. There was a significant main effect of humidity condition on T_{db}, ($F_{(1,14)}=156.796$, $p<0.001$), RH ($F_{(1,14)}=1466.333$, $p<0.001$), and WBGT ($F_{(1,14)}=18.172$, $p=0.001$) at the T_{re} inflection, but there were no significant effects of aerobic fitness, or interaction effects between aerobic fitness and humidity on the environmental conditions at the T_{re} inflection point. *Post hoc* analysis of the main effect of humidity showed that the T_{db} in LH condition was significantly higher than in HH ($t_{(15)}=-12.435$, $p<0.001$), whereas RH ($t_{(15)}=-39.467$, $p<0.001$) and WBGT ($t_{(15)}=-4.225$, $p=0.001$) were lower in LH than in HH at the T_{re} inflection point.

There was no significant effect of humidity condition or aerobic fitness on T_{re} at the inflection point, however there was a significant interaction effect of humidity and aerobic fitness ($F_{(1,14)}=12.509$, $p=0.003$). *Post hoc* analysis of the interaction effect showed that, in the HH condition only, the Low aerobic fitness group had a higher T_{re} than the High aerobic fitness group at the T_{re} inflection ($t_{(14)}=2.980$, $p=0.010$). There was a significant main effect of humidity on the $\overline{T}_b$ at the T_{re} inflection point ($F_{(1,14)}=12.160$, $p=0.004$). Although the main effect for aerobic fitness was not significant there was an interaction effect between humidity and aerobic fitness on the $\overline{T}_b$ at the T_{re} inflection ($F_{(1,14)}=13.694$, $p=0.002$). *Post-hoc* analysis showed that $\overline{T}_b$ at the T_{re} inflection point was significantly higher in LH than HH ($t_{(15)}$=-2.566, p=0.021), and within the HH condition the Low aerobic fitness group had a higher $\overline{\overline{T}}_b$ than the High aerobic fitness group at the T_{re} inflection point ($t_{(14)}$=3.113, p=0.008). Similarly, there was a main effect of humidity condition on $\overline{T}_{sk}$ at the T_{re} inflection point ($F_{(1,14)}$=53.59, p<0.001), and an interaction effect between humidity and fitness ($F_{(1,14)}$=6.88, p=0.020), whereas the main effect for aerobic fitness was not significant. The *post hoc* analysis showed that $\overline{T}_{sk}$ was significantly higher in LH than HH ($t_{(14)}$=-6.205, p<0.001) and within the HH condition the Low aerobic fitness group had a higher $\overline{T}_{sk}$ than the High aerobic fitness group ($t_{(14)}$=2.304, p=0.037), at the T_{re} inflection point. There was a significant effect of humidity condition on the HF at the T_{re} inflection point ($F_{(1,14)}$=156.285, p<0.001), but no effect of fitness or interaction effect between humidity condition and fitness. The *post hoc* analysis showed that HF was significantly higher in HH than LH ($t_{(15)}$=-12.711, p<0.001).

Analysis of the thermophysiological responses at the T_{re} inflection point showed a main effect of aerobic fitness on SR_{Back} ($F_{(1,14)}$=300.752, p=0.003), but no effect of humidity condition, or interaction effect. *Post hoc* analysis showed that the Low aerobic fitness group had a lower SR_{Back} than the High aerobic fitness group at the T_{re} inflection point under LH ($t_{(14)}$=-3.357, p=0.005) and HH ($t_{(14)}$=-2.407, p=0.030) conditions. There were no significant main effects or interaction effects for $SR_{Forearm}$, $SkBF_{Forearm}$, $SkBF_{Finger}$ or $\dot{V}O_2$ at the T_{re} inflection point. However, the HR data indicated that there was a significant effect of aerobic fitness ($F_{(1,14)}$=1456.586, p<0.001) and an interaction effect between humidity condition and aerobic fitness ($F_{(1,14)}$=20.551, p<0.001), but no effect of humidity condition. *Post hoc* analysis showed that HR was significantly lower for the High aerobic fitness group than the Low aerobic fitness group in the LH condition ($t_{(14)}$=2.987, p=0.010) and HH condition ($t_{(14)}$=5.554, p<0.001).

There was a main effect of humidity condition ($F_{(1,14)}$=7.410, p=0.017), but not fitness, or an interaction effect in E_{req}. *Post hoc* analysis showed that there was greater E_{req} in the LH condition ($t_{(15)}$=-2.707, p=0.016). Similarly, there was a main effect of humidity condition ($F_{(1,14)}$=495.556, p<0.001), but not fitness, or an interaction effect in E_{max}. *Post hoc* analysis showed that there was greater E_{max} in the LH condition ($t_{(15)}$=-22.990, p<0.001).

There was no main effect of aerobic fitness, humidity condition, or an interaction effect on heat production. However, there was a main effect of humidity condition ($F_{(1,14)}$=15.250, p=0.002), but not fitness, or an interaction effect in heat storage. *Post hoc* analysis showed that there was greater heat storage in the HH condition ($t_{(15)}$=-3.800, p=0.002), and within that HH condition, the Low fitness group had a higher level of heat storage compared to the High fitness group ($t_{(14)}$=2.282, p=0.039).

Analysis of the perceptual responses at the T_{re} inflection point showed that there were no effect of fitness or humidity condition on TS. However, TC was lower in the Low aerobic fitness group compared to the High aerobic fitness group in the HH condition ($H_{(1)}$=7.456, p=0.006) and SW was higher in the HH condition than the LH condition ($Fr_{(1)}$=6.250, p=0.012), but there was no effect of aerobic fitness. RPE was lower in the High aerobic fitness group compared to the Low aerobic fitness group in the HH condition only ($H_{(1)}$=9.231, p=0.002).

Table 8.3. Mean (SD) environmental conditions, thermal profile, thermophysiological responses and perceptual responses at the point of T_{re} inflection during cycling exercise at the same *absolute* work rate (60 W) under LH and HH conditions, for participants of Low (n=8) and High (n=8) aerobic fitness. [a] Significant main effect for humidity conditions; [b] Significant main effect of aerobic fitness; [c] Significant interaction effect between humidity and aerobic fitness; [d] Significant difference between Low and High aerobic fitness groups in HH environment; [e] Significant difference between Low and High aerobic fitness groups in LH environment.

	Low humidity (LH)		**High humidity (HH)**		
	Low aerobic fitness	**High aerobic fitness**	**Low aerobic fitness**	**High aerobic fitness**	
Environmental conditions					
T_{db} (°C)	40.69 (2.93)	42.62 (2.93)	34.13 (0.85)	34.80 (1.21)	a
RH (%)	26.59 (4.06)	26.21 (1.42)	81.24 (4.07)	79.88 (3.02)	a
WBGT (°C)	28.96 (2.57)	30.42 (1.61)	31.74 (0.73)	32.04 (1.11)	a
Thermal profile					
T_{re} (°C)	37.51 (0.40)	37.33 (0.33)	37.66 (0.43)	37.12 (0.24)	c,d
$\bar{T}_b$ (°C)	37.41 (0.41)	37.29 (0.36)	37.43 (0.36)	36.92 (0.29)	a,c,d
$\bar{T}_{sk}$ (°C)	37.04 (0.57)	37.14 (0.53)	36.55 (0.25)	36.09 (0.49)	a,c,d
Heat flux ($W·m^{-2}$)	-18 (24)	-32 (14)	39 (8)	31 (16)	a
Thermophysiological responses					
SR_{Back} ($L·m^2·hr^{-1}$)	0.35 (0.09)	0.55 (0.14)	0.42 (0.12)	0.62 (0.20)	b,d,e
$SR_{Forearm}$ ($L·m^2·hr^{-1}$)	0.64 (0.16)	0.69 (0.25)	0.68 (0.17)	0.67 (0.22)	
$SkBF_{Forearm}$ (FU)	166 (60)	221 (77)	190 (121)	251 (89)	
$SkBF_{Finger}$ (FU)	231 (127)	261 (92)	207 (52)	280 (72)	

$SkBF_{Forearm}$ (% highest 5 min)	105 (29)	93 (12)	91 (9)	92 (7)	
$SkBF_{Finger}$ (%highest 5 min)	85 (12)	84 (13)	82 (12)	79 (16)	
HR ($b \cdot min^{-1}$)	133 (16)	113 (0)	145 (18)	106 (9)	b,c,d,e
$\dot{V}O_2$ ($L \cdot min^{-1}$)	1.52 (0.26)	1.50 (0.18)	1.52 (0.20)	1.35 (0.16)	
Partitional calorimetry and calculated variables					
E_{req} ($W \cdot m^{-2}$)	218 (43)	213 (28)	201 (41)	173 (32)	a
E_{max} ($W \cdot m^{-2}$)	136 (14)	132 (7)	51 (9)	45 (7)	a
Heat production ($W \cdot m^{-2}$)	269 (42)	258 (30)	273 (43)	236 (26)	
Heat storage ($W \cdot m^{-2}$)	29 (11)	25 (7)	38 (5)	30 (8)	a,d
Perceptual measures					
TS (VAS-20cm)	16.30 (1.40)	16.55 (6.40)	16.90 (2.50)	15.95 (5.10)	
TC (VAS-20 cm)	7.60 (9.80)	9.85 (5.80)	6.20 (5.60)	10.55 (8.90)	d
SW (VAS-20 cm)	13.10 (10.10)	12.50 (10.90)	16.75 (7.00)	16.60 (8.20)	a
RPE (Category ratio scale)	14 (3)	12 (8)	14 (4)	12 (7)	d

8.3.3 *The influence of aerobic fitness on the T_{re} inflection point in LH and HH conditions during cycling exercise at a matched relative work rate (~40 % $\dot{V}O_{2max}$)*

Environmental conditions, thermal profile, and thermophysiological and perceptual responses at the point of T_{re} inflection during exercise at the same *relative* work rate under LH and HH conditions are presented for the Low and High aerobic fitness groups in Table 8.4. There was a significant main effect of humidity condition on the T_{db} ($F_{(1,14)}$=327.657, p<0.001), RH ($F_{(1,14)}$=1572.306, p<0.001) and WBGT ($F_{(1,14)}$=14.759, p=0.002) at the T_{re} inflection point, with significant interaction effects of humidity condition and aerobic fitness for T_{db} ($F_{(1,14)}$=8.792, p=0.010) and WBGT ($F_{(1,14)}$=7.475, p=0.016). *Post hoc* analysis showed that the T_{db} in the LH condition was significantly higher than in the HH condition ($t_{(15)}$=-14.685, p<0.001) at the T_{re} inflection point, with the Low aerobic fitness group inflecting at a higher T_{db} and WBGT than the High aerobic fitness group in the HH condition ($t_{(14)}$=-2.851, p=0.013; $t_{(15)}$=3.211, p=0.006). *Post hoc* analysis of the RH showed that, as intended, LH was significantly less humid than HH at the T_{re} inflection point ($t_{(15)}$=41.028, p<0.001), whereas *post hoc* analysis of the WBGT data showed that WBGT was lower in the LH condition than in HH, whilst within HH the T_{re} inflection occurred at a higher WBGT for the Low aerobic fitness group than the High aerobic fitness group ($t_{(15)}$=3.211, p=0.006).

There was no significant effect of humidity condition or aerobic fitness on T_{re} at the inflection point, although there was a significant interaction effect between humidity condition and aerobic fitness ($F_{(1,14)}$=6.137, p=0.027), however *post hoc* analysis did not reveal any significant differences between or within groups. There was a significant main effect of humidity condition and an interaction effect between humidity and aerobic fitness respectively on $\overline{T}_b$ at the T_{re} inflection point ($F_{(1,14)}$=7.031, p=0.019; $F_{(1,14)}$=8.167, p=0.013), although the main effect of aerobic fitness was not significant. *Post hoc* analysis showed that the $\overline{T}_b$ at the T_{re} inflection point was significantly higher in the LH condition than the HH condition ($t_{(15)}$=-2.181, p=0.046), but there was no difference between aerobic fitness groups within each humidity condition. Analysis of the $\overline{T}_{sk}$ at the T_{re} inflection point demonstrated a main effect of humidity condition ($F_{(1,14)}$=51.550, p<0.001) and an interaction effect between humidity ($F_{(1,14)}$=6.671, p=0.022), but no main effect of aerobic fitness. The *post hoc* analysis showed that the $\overline{T}_{sk}$ in LH was significantly higher than in HH ($t_{(15)}$=-6.116, p<0.001) at the T_{re} inflection point, and that the Low aerobic fitness group had a higher $\overline{T}_{sk}$ than the High aerobic fitness group in HH at the T_{re} inflection point ($t_{(14)}$=-2.834, p=0.013). There was a significant effect of humidity condition and aerobic fitness, but no interaction effect between humidity condition and fitness on HF at the point of T_{re} inflection ($F_{(1,14)}$=143.495, p<0.001; $F_{(1,14)}$=19.598, p=0.30). The *post hoc* analysis showed that the HF in HH was significantly higher than in LH ($t_{(15)}$=11.692, p<0.001) at the T_{re} inflection point, and that the Low aerobic fitness group had a lower HF than the High aerobic fitness group in HH at the T_{re} inflection point ($t_{(14)}$=-3.873, p=0.002).

Analysis of the thermophysiolgical responses at the T_{re} inflection indicated that there was a significant main effect of aerobic fitness on SR_{Back} ($F_{(1,14)}$=313.664, p<0.001), but no main effect of humidity condition or interaction effect. *Post hoc* analysis showed that Low aerobic fitness group had a lower SR_{Back} than the High aerobic fitness group in both the LH ($t_{(14)}$=-5.494, p<0.001) and HH ($t_{(14)}$=-4.192, p=0.001) conditions. There was a significant main effect of humidity condition on $SR_{Forearm}$ ($F_{(1,14)}$=5.470, p=0.035), but no main effect of aerobic fitness or an interaction effect, *post hoc* analysis showed that $SR_{Forearm}$ at the T_{re} inflection point was significantly lower in the LH condition compared to the HH condition ($t_{(15)}$=2.419, p=0.029). There was no significant difference in $SkBF_{Forearm}$ or $SkBF_{Finger}$

between humidity conditions and fitness groups when compared in the arbitrary Flux Units (FU), or when normalised as a percentage of the highest 5 minute value. There was an interaction effect between humidity condition and fitness of the heart rate at the point of T_{re} inflection ($F_{(1,14)}$=8.133, p=0.013) but no significant main effect of humidity condition or fitness. The *post hoc* analysis showed that the Low aerobic fitness group had a higher heart rate than the High aerobic fitness group in HH at the T_{re} inflection point ($t_{(14)}$=2.330, p=0.035). There was significant effect of fitness on $\dot{V}O_2$ at the point of T_{re} inflection ($F_{(1,14)}$=23.115, p<0.001), but no effect of humidity condition or an interaction effect between humidity condition and fitness. The *post hoc* analysis showed that the Low aerobic fitness group had a lower $\dot{V}O_2$ than the High aerobic fitness group in LH and HH conditions at the T_{re} inflection point ($t_{(14)}$=-3.694, p=0.002; $t_{(14)}$=-4.461, p=0.001).

There was a significant effect of fitness ($F_{(1,14)}$=51.956, p<0.001), humidity condition ($F_{(1,14)}$=69.700, p<0.001) and an interaction effect ($F_{(1,14)}$=40.998, p<0.001) on E_{req}. The *post hoc* analysis showed that the E_{req} was significantly higher in the LH condition ($t_{(15)}$=-4.360, p=0.001) and that the E_{req} was significantly higher for the High fitness group in both the HH ($t_{(14)}$=-3.128, p=0.007) and LH ($t_{(14)}$=-9.816, p<0.001) conditions. There was a significant effect of environment ($F_{(1,14)}$=538.564, p<0.001), but no significant main effect of fitness or an interaction effect on E_{max}. The *post hoc* analysis showed that the LH condition had a significantly higher E_{max} than the HH condition ($t_{(15)}$=-22.373, p<0.001). There was a significant effect of fitness ($F_{(1,14)}$=1372.057, p<0.001), but no significant main effect of humidity condition or an interaction effect on heat production. The *post hoc* analysis showed that the Low aerobic fitness group had a lower heat production than the High aerobic fitness group in LH condition ($t_{(14)}$=-2.979, p=0.010) and the HH condition ($t_{(14)}$=-4.538, p<0.001) at the T_{re} inflection point. There was a significant effect of humidity condition $F_{(1,14)}$=20.247, p<0.001), but no significant main effect of fitness or an interaction effect on heat storage. The *post hoc* analysis showed that there was greater heat storage in the HH condition ($t_{(15)}$=-4.444, p=0.001).

Analysis of the perceptual response indicated that there were no differences in TS or RPE between humidity conditions or aerobic fitness groups.TC was higher in the High aerobic fitness group compared to the Low aerobic fitness group in the HH condition ($H_{(1)}$=3.982, p=0.046) and overall SW ($Fr_{(1)}$=6.250, p=0.012) was higher in HH condition compared to the LH condition at the T_{re} inflection point.

Table 8.4. Mean (SD) environmental conditions, thermal profile, thermophysiological responses and perceptual response at the point of T_{re} inflection during cycling exercise at the same *relative* work rate (~40 % $\dot{V}O_{2max}$) under LH and HH conditions, for participants of Low (n=8) and High (n=8) aerobic fitness. [a] Significant main effect for humidity conditions; [b] Significant main effect of aerobic fitness; [c] Significant interaction effect between humidity and aerobic fitness; [d] Significant difference between low and high fitness groups in HH environment; [e] Significant difference between low and high fitness groups in LH environment.

	Low humidity (LH)		**High humidity (HH)**		
	Low aerobic fitness	**High aerobic fitness**	**Low aerobic fitness**	**High aerobic fitness**	
Environmental conditions					
T_{db} (°C)	40.69 (2.93)	41.64 (1.61)	34.13 (0.85)	32.52 (1.36)	a,d
RH (%)	26.59 (4.06)	27.85 (4.06)	81.24 (4.07)	82.22 (3.11)	a,c
WBGT (°C)	28.96 (2.57)	30.01 (1.34)	31.74 (0.73)	30.48 (0.93)	a,c,d
Thermal profile					
T_{re} (°C)	37.51 (0.40)	37.74 (0.40)	37.66 (0.43)	37.63 (0.28)	c
$\bar{T}_b$ (°C)	37.41 (0.41)	37.59 (0.37)	37.43 (0.36)	37.29 (0.30)	a,c
$\bar{T}_{sk}$ (°C)	37.04 (0.57)	37.06 (0.59)	36.55 (0.25)	36.00 (0.48)	a,c,d
Heat flux ($W \cdot m^{-2}$)	-17 (24)	1 (33)	39 (8)	72 (22)	a,b,d
Thermophysiological responses					
SR_{Back} ($L \cdot m^2 \cdot hr^{-1}$)	0.35 (0.09)	0.72 (0.17)	0.42 (0.12)	0.74 (0.18)	b,d,e
$SR_{Forearm}$ ($L \cdot m^2 \cdot hr^{-1}$)	0.64 (0.16)	0.78 (0.18)	0.68 (0.17)	0.82 (0.19)	a
$SkBF_{Forearm}$ (FU)	166 (60)	248 (99)	190 (121)	231 (76)	
$SkBF_{Finger}$ (FU)	231 (127)	283 (115)	207 (52)	271 (99)	
$SkBF_{Forearm}$ (%highest 5 min)	105 (29)	88 (9)	91 (9)	87 (11)	
$SkBF_{Finger}$ (%highest 5 min)	85 (12)	83 (23)	82 (12)	86 (16)	
HR ($b \cdot min^{-1}$)	133 (16)	130 (10)	145 (18)	128 (11)	c,d
$\dot{V}O_2$ ($L \cdot min^{-1}$)	1.52 (0.26)	1.99 (0.14)	1.52 (0.20)	1.86 (0.16)	b,d,e
Partitional calorimetry and calculated variables					
E_{req} ($W \cdot m^{-2}$)	218 (43)	277 (22)	201 (41)	232 (22)	a,b,c,d,e
E_{max} ($W \cdot m^{-2}$)	136 (14)	130 (8)	51 (9)	55 (5)	a
Heat production ($W \cdot m^{-2}$)	269 (43)	351 (30)	273 (43)	329 (31)	b,d.e
Heat storage ($W \cdot m^{-2}$)	29 (11)	33 (9)	38 (5)	38 (11)	a

Perceptual measures					
TS (VAS- 20cm)	16.30 (1.40)	16.20 (2.50)	16.90 (2.50)	17.00 (3.30)	
TC (VAS- 20 cm)	7.60 (9.80)	8.25 (7.40)	6.20 (5.60)	9.80 (6.90)	b,d
SW (VAS- 20 cm)	13.10 (10.10)	15.75 (7.50)	16.75 (7.00)	17.95 (5.20)	a
RPE (Category ratio scale)	14 (3.00)	14 (10.00)	14.00 (4.00)	12.00 (7.00)	

8.3.4 Thermophysiological responses beyond the T_{re} inflection point.

Table 8.5 and 8.6 show the thermal profile, thermophysiological and perceptual responses at discrete time points beyond the individual T_{re} inflection point for individuals of Low or High aerobic fitness working at matched *absolute* (table 8.5) and *relative* (table 8.6) work rates in LH and HH conditions. In both humidity conditions, all thermal responses (T_{re}, $\bar{T}_b$, $\bar{T}_{sk}$) increased significantly beyond the T_{re} inflection point, regardless of work rate or aerobic fitness level. Concomitantly, there was evidence of a thermoregulatory 'reserve' beyond the T_{re} inflection point. Specifically, there was evidence for a significant increase in SR_{Back} and $SR_{Forearm}$ beyond the T_{re} inflection point for both aerobic fitness groups in both humidity conditions when exercising at matched *absolute* and *relative* work rates. There were also increases over time in $SkBF_{Finger}$ in both humidity conditions. Specifically, in the LH condition, both fitness groups at both work rates increased over time when expressed as a % of highest 5 minutes and in the FU. Whereas, in the HH condition, just the high and low fitness group at the matched *absolute* work rate increased over time when expressed as a % of highest 5 minutes, however this increase was only seen in the high aerobic fitness group at the relative work rate when expressed in the FU. However, there were no differences in $SkBF_{Forearm}$ as a % of highest 5 minutes or in the FU for both fitness groups in either humidity conditions under any of the work rates.

Table 8.5. Mean (SD) thermal profile and thermophysiological responses at the T_{re} inflection point, and for discrete points beyond the T_{re} inflection point for Low (n=8) and High (n=8) aerobic fitness individuals exercising at a matched *absolute* (60 W) and a matched *relative* exercise intensity in a High Humidity environment (80 % RH). * Significantly different from 10 min post inflection, 20 min post inflection, and end of test. ** Significantly different from 20 min post inflection and end of test. *** Significantly different from end of test. ## Significantly different from 10 min post inflection and end of test.

Variable	Aerobic fitness (work rate)	T_{re} inflection point	10 min post inflection	20 min post inflection	End of test
Thermal profile					
T_{re} (°C)	*Low (60 W)*	37.66 (0.43) *	37.84 (0.45) **	38.10 (0.50) ***	38.52 (0.38)
	High (60 W)	37.13 (0.24) *	37.29 (0.25) **	37.57 (0.28) ***	38.48 (0.45)
	High (Av. 99 W)	37.63 (0.28) *	37.83 (0.30) **	38.11 (0.34) ***	38.73 (0.40)
Δ T_{re} (°C)	*Low (60 W)*	0.69 (0.51) *	0.84 (0.55) **	1.11 (0.61) ***	1.55 (0.56)
	High (60 W)	0.35 (0.36) *	0.48 (0.34) **	0.75 (0.33) ***	1.69 (0.56)
	High (Av. 99 W)	0.82 (0.34) *	0.99 (0.39) **	1.26 (0.45) ***	1.92 (0.53)
$\bar{T}_b$ (°C)	*Low (60 W)*	37.43 (0.36) *	37.68 (0.40) **	38.03 (0.43) ***	38.53 (0.29)
	High (60 W)	36.92 (0.29) *	37.16 (0.28) **	37.52 (0.31) ***	38.59 (0.45)
	High (Av. 99 W)	37.29 (0.30) *	37.57 (0.30) **	37.95 (0.34) ***	38.69 (0.35)
$\bar{T}_{sk}$ (°C)	*Low (60 W)*	36.55 (0.25) *	37.07 (0.34) **	37.73 (0.39) ***	38.58 (0.41)
	High (60 W)	36.10 (0.49) *	36.65 (0.44) **	37.34 (0.47) ***	39.00 (0.55)
	High (Av. 99 W)	36.00 (0.48) *	36.60 (0.43) **	37.34 (0.48) ***	38.53 (0.27)
Heat production ($W \cdot m^{-2}$)	*Low (60 W)*	273 (43) ***	-	-	311 (49)
	High (60 W)	236 (26) ***	-	-	278 (34)
	High (Av. 99 W)	329 (31) ***	-	-	382 (36)

Thermoeffector response					
SR_{Back} ($L \cdot m^2 \cdot hr^{-1}$)	*Low (60 W)*	0.42 (0.12) *	0.48 (0.14) **	0.51 (0.15)	0.54 (0.18)
	High (60 W)	0.62 (0.20) *	0.69 (0.18) **	0.77 (0.17) ***	0.85 (0.16)
	High (Av. 99 W)	0.74 (0.18) *	0.80 (0.17) **	0.83 (0.18)	0.85 (0.18)
$SR_{Forearm}$ ($L \cdot m^2 \cdot hr^{-1}$)	*Low (60 W)*	0.68 (0.17) *	0.79 (0.15)	0.83 (0.14)	0.85 (0.13)
	High (60 W)	0.67 (0.22) *	0.77 (0.23) **	0.82 (0.23) ***	0.86 (0.24)
	High (Av. 99 W)	0.82 (0.19) **	0.84 (0.19)	0.86 (0.19)	0.89 (0.17)
$SkBF_{Forearm}$	*Low (60 W)*	91 (89)	91 (7)	95 (8)	90 (8)
(% of highest 5 mins)	*High (60 W)*	92 (7)	95 (8)	96 (6)	93 (11)
	High (Av. 99 W)	87 (11)	96 (14)	93 (5)	94 (14)
$SkBF_{Finger}$ (% of highest 5 mins)	*Low (60 W)*	82 (12) ***	88 (12)	91 (12)	97 (10)
	High (60 W)	79 (16) ##	83 (17) ***	87 (15) ***	101 (5)
	High (Av. 99 W)	86 (16)	93 (9)	96 (9)	96 (6)
$SkBF_{Forearm}$ (FU)	*Low (60 W)*	190 (121)	189 (121)	198 (124)	186 (120)
	High (60 W)	251 (89)	254 (84)	261 (94)	255 (105)
	High (Av. 99 W)	231 (76)	254 (97)	247 (82)	251 (104)
$SkBF_{Finger}$ (FU)	*Low (60 W)*	207 (52)	223 (61)	232 (58)	247 (59)
	High (60 W)	280 (72) ##	293 (68) ***	310 (64) ***	362 (73)
	High (Av. 99 W)	271 (99)	294 (92)	301 (95)	305 (100)

Table 8.6. Mean (SD) thermal profile and thermophysiological responses at the T_{re} inflection point, and for discrete points beyond the T_{re} inflection point for Low (n=8) and High (n=8) aerobic fitness individuals exercising at a matched *absolute (60 W)* and a matched *relative* exercise intensity in a Low Humidity environment (80 % RH). * Significantly different from 10 min post inflection, 20 min post inflection, and end of test. ** Significantly different from 20 min post inflection and end of test. *** Significantly different from end of test. # Significantly different than 10min post inflection

Variable	Aerobic fitness (work rate)	T_{re} Inflection point	10 min post inflection	20 min post inflection	End of test
Thermal profile					
T_{re} (°C)	*Low*	37.51 (0.40)	37.63 (0.43)	37.78 (0.48)	38.05 (0.32)
	(60 W)	*	**	***	
	High	37.33 (0.33) *	37.44 (0.35)	37.58 (0.37)	37.99 (0.51)
	(60 W)		**	***	
	High	37.74 (0.34)	37.88 (0.34)	38.08 (0.35)	38.68 (0.49)
	(Av. 99 W)	*	**	***	
ΔT_{re} (°C)	*Low*	0.66 (0.47)	0.77 (0.53)	0.90 (0.58)	1.20 (0.50)
	(60 W)	*	**	***	
	High	0.52 (0.33)	0.61 (0.34)	0.75 (0.38)	1.17 (0.49)
	(60 W)	*	**	***	
	High	0.90 (0.36)	1.02 (0.36)	1.21 (0.36)	1.85 (0.49)
	(Av. 99 W)	*	**	***	
$\bar{T}_b$ (°C)	*Low*	37.41(0.41)	37.58 (0.43)	37.79 (0.50)	38.13 (0.29)
	(60 W)	*	**	***	
	High	38.56 (0.36) *	37.45 (0.37)	37.63 (0.39)	38.08 (0.51)
	(60 W)		**	***	
	High	37.59 (0.37)	37.80 (0.36)	38.05 (0.37)	38.74 (0.48)
	(Av. 99 W)	*	**	***	
$\bar{T}_{sk}$ (°C)	*Low*	37.04 (0.57)	37.41 (0.54)	37.83 (0.66)	38.41 (0.25)
	(60 W)	*	**	***	
	High	37.14 (0.53) *	37.49 (0.51)	37.83 (0.50)	38.42 (0.59)
	(60 W)		**	***	
	High	37.06 (0.59)	37.48 (0.61)	37.93 (0.93)	38.92 (0.61)
	(Av. 99 W)	*	**	***	
Heat production	*Low*	269 (42)	-	-	295 (41)
	(60 W)	***			
($W \cdot m^{-2}$)	*High*	259 (30)	-	-	330 (64)
	(60 W)	***			
	High	351 (30)	-	-	392 (57)
	(Av. 99 W)	***			

Thermoeffector response					
SR_{Back} ($L·m^2·hr^{-1}$)	*Low (60 W)*	0.35 (0.09) *	0.37 (0.08) **	0.42 (0.11)	0.47 (0.15)
	High (60 W)	0.55 (0.14) *	0.60 (0.17) **	0.66 (0.19) ***	0.69 (0.22)
	High (Av. 99 W)	0.72 (0.17) #	0.77 (0.18)	0.80 (0.22)	0.79 (0.24)
$SR_{Forearm}$ ($L·m^2·hr^{-1}$)	*Low (60 W)*	0.64 (0.16) *	0.69 (0.16) **	0.72 (0.17)	0.77 (0.15)
	High (60 W)	0.69 (0.25) *	0.74 (0.24) **	0.79 (0.23) ***	0.83 (0.23)
	High (Av. 99 W)	0.78 (0.18) *	0.83 (0.16)	0.87 (0.14)	0.91 (0.10)
$SkBF_{Forearm}$ (% of highest 5 mins)	*Low (60 W)*	105 (29)	92 (7)	91 (8)	87 (10)
	High (60 W)	93 (12)	94 (10)	95 (6)	96 (7)
	High (Av. 99 W)	88 (9)	88 (8)	86 (12)	88.74 (8.07)
$SkBF_{Finger}$ (% of highest 5 mins)	*Low (60 W)*	85 (12) ***	83 (22)	86 (28)	102 (11)
	High (60 W)	84 (13) ***	88 (8) ***	86 (15)	106 (14)
	High (Av. 99 W)	83 (23) ***	82 (19) ***	94 (9)	104 (8)
$SkBF_{Forearm}$ (FU)	*Low (60 W)*	166 (60)	152 (69)	151 (69)	144 (69)
	High (60 W)	221 (77)	224 (74)	229.35 (80.63)	233.84 (94.40)
	High (Av. 99 W)	248 (99)	253 (112)	245 (106)	252 (102)
$SkBF_{Finger}$ (FU)	*Low (60 W)*	231 (127) ***	204 (90)	209 (106)	264 (124)
	High (60 W)	261 (92) ***	278 (112) ***	276 (116) ***	321 (97)
	High (Av. 99 W)	283 (115) ***	282 (113)	321 (93)	358 (106)

Below is a brief summary of key findings at the inflection point to assist the reader during the discussion section.

Table 8.7. Summary of key environmental conditions, thermal profile, thermophysiological responses and perceptual response at the point of T_{re} inflection during cycling exercise at the same *absolute* work rate 60 Watts) under LH and HH conditions, for participants of Low (n=8) and High (n=8) aerobic fitness. Where = indicates no difference between Low and High fitness groups and > or < indicated greater or smaller value respectively.

Variable	**Low Humidity (LH)**	**High Humidity (HH)**
T_{db}	=	=
T_{re}	=	Low > High
$\overline{T}_{sk}$	=	Low > High
HF	=	=
SR_{Back}	Low < High	Low < High
$SR_{Forearm}$	=	=
$SkBF_{Forearm}$	=	=
$SkBF_{Finger}$	=	=
HR	Low > High	Low > High
Heat storage	=	Low > High

Table 8.8. Summary of environmental conditions, thermal profile, thermophysiological responses and perceptual response at the point of T_{re} inflection during cycling exercise at the same *relative* work rate (~40 % $\dot{V}O_{2max}$) under LH and HH conditions, for participants of Low (n=8) and High (n=8) aerobic fitness. Where = indicates no difference between Low and High fitness groups and > or < indicated greater or smaller value respectively.

Variable	**Low Humidity (LH)**	**High Humidity (HH)**
T_{db}	=	Low > High
T_{re}	=	=
$\overline{T}_{sk}$	=	Low > High
HF	=	Low < High
SR_{Back}	Low < High	Low < High
$SR_{Forearm}$	=	=
$SkBF_{Forearm}$	=	=
$SkBF_{Finger}$	=	=
HR	=	Low > High
Heat storage	=	=

8.4. Discussion

This was the first study to systematically compare the thermoregulatory and thermoeffector responses of Low and High aerobic fitness individuals (46.53 [6.73] *vs.* 58.73 [6.28] $mL \cdot kg^{-1} \cdot min^{-1}$ respectively) when exercising at matched *absolute* and *relative* work rates at and beyond the inflection point in T_c during exercise under high (HH) and low (LH) humidity conditions. Additionally, care was taken to ensure that groups were well matched for anthropometric measures, in order to negate this as a confounding factor (Jay *et al*., 2011). The hypotheses tested were that: 1) the ambient temperature; 2) the thermal profile, and; 3) the thermoeffector response, would differ at the T_{re} inflection point between the two aerobic fitness groups at the same matched *absolute* and *relative* work rates, for both humidity conditions. It was anticipated that the advantageous thermoregulatory adaptations associated with increased aerobic fitness, in particular, the increased sudomotor response, would be delay the transition from compensable to uncompensable conditions when exercising at a given absolute work rate under conditions permitting high rates of evaporative heat loss, and might negate the additional thermal burden when exercising at a matched relative work rate. Additionally, it was also hypothesised that, 4) in line with the observation in Chapter Seven, there would be a thermoeffector 'reserve' for both aerobic fitness groups, in both humidity conditions, beyond the T_{re} inflection point.

At the matched *absolute* work rate, there was no effect of fitness on T_{db} at the point of T_{re} inflection in either humidity condition. However, at the matched *relative* work rate, the Low aerobic fitness group inflected at a higher T_{db} and WBGT than the High aerobic fitness group in the HH condition. Thus, in contrast to our anticipated finding, there was no apparent benefit of aerobic fitness on the T_{db} precipitating the transition to uncompensability under either humidity condition during exercise at a given *absolute* work rate. However, at a given *relative* work rate aerobic fitness was able to offset the additional thermal burden (of a higher absolute work rate) under LH conditions, to the extent that the T_{db} at the T_{re} inflection was not different, but this was not the case under the HH conditions. Therefore the first hypothesis - *the ambient environmental conditions (T_{db} and WBGT) at the T_{re} inflection point will differ between the two aerobic fitness groups at the same matched absolute and relative work rates, for both humidity conditions* - can only be accepted in part.

As was the case in the data presented in Chapter Seven, there was an effect of environment on T_{db} at the point of T_{re} inflection. This was, by intention, so that the inflection occurred at similar time points within the protocol. The T_{db} in the LH condition being approximately 6 °C higher and the WBGT 3 °C lower than the HH condition (as discussed in 7.4). Interestingly, in both LH and HH when heat production is similar (*absolute* condition) and dissimilar (*relative* condition) the inflection in T_{re} occurred when the aerobic fitness groups were at similar levels of heat storage. This is consistent with the observation that aerobic fitness increases heat loss (Sawka *et al*., 1992), though this can come at a higher 'physiological cost' (Mora-Rodríguez *et al*., 2010). While an inflection in T_{re} at a lower T_{db} in the matched *relative* work rate condition may eventually predispose individuals of High aerobic fitness to a higher absolute T_{re} and greater rates of rise in T_{re} when exercising at a given level of physiological stress, previous studies have proposed that aerobically trained participants show a greater ability to tolerate high core temperatures (Cheung & McLellan, 1998b; Selkirk & McLellan, 2001) and the practical significance of the lower T_{re} at the inflection point is unclear.

The T_{re} at the T_{re} inflection point was not affected by environment *per se* during exercise at a given *absolute* work rate, likewise, there was no main effect of aerobic fitness, although an interaction effect demonstrated that the Low fitness group had a significantly higher T_{re} at the T_{re} inflection point than the High aerobic fitness group, in the HH condition. There was no significant difference between groups in T_{db} at the T_{re} inflection point, however the Low fitness group had significantly higher heat storage at this point than the High fitness, which is likely to be due to the profile at which T_{re} increased (a steeper curve than the High fitness participants). Neither environment nor aerobic fitness influenced the T_{re} at the T_{re} inflection point during exercise at a given *relative* work rate; although there was a significant interaction effect, the location of this could not be detected in *post hoc* analysis. In the matched *absolute* work rate condition, $\overline{T}_b$ and $\overline{T}_{sk}$ were lower in the HH condition than LH condition with the Low aerobic fitness group having higher $\overline{T}_b$ and $\overline{T}_{sk}$ than the High aerobic fitness group in the HH condition due to the differences in the environmental profile at that point (higher T_{db}). Similarly, in the matched *relative* work rate condition, $\overline{T}_b$ and $\overline{T}_{sk}$, were lower in the HH condition, than in the LH condition, but there was no difference between aerobic fitness groups in the either condition. Thus, the second hypothesis - *the thermal profile of the individual, as indicated by T_{re}, $\overline{T}_b$ and $\overline{T}_{sk}$, will differ at the T_{re} inflection point between the two aerobic fitness groups at the same matched* *absolute and relative work rates, for both humidity conditions* - can only be partly accepted.

Previous work has shown that when cycling, there is equal work efficiency between trained and untrained cyclists (Marsh *et al*., 2000; Moseley *et al*., 2004). This was the case in the present study, with similar levels of metabolic heat production evident in the High and Low aerobic fitness groups at the matched *absolute* work rates. Therefore, it was expected that the thermoregulatory advantage afforded by aerobic training (Sawka *et al*., 1992) should be evident as reduced deep body and skin temperature (Havenith *et al*., 1998). This was only the case in the HH condition, with the Low fitness individuals having a significantly greater T_{re} than the High fitness individuals at the point of inflection in T_{re}. This coincided with significantly greater levels of heat storage ($W \cdot m^{-2}$) in the Low fitness group in the HH condition, as seen in Table 8.3, which may have been caused by a significantly lower SR compared to the High fitness group. When comparing independent groups who are working at a matched *relative* work rate, it is key to match for anthropometric characteristics (Jay *et al*., 2011). This was done in the present study, but has historically been over-looked by others (Fritzsche & Coyle, 2000; Gant *et al*., 2003; Mora-Rodríguez *et al*., 2010).

The findings in the present study – that there was no difference in T_{re} between High and Low aerobic fitness individuals at the point of inflection when exercising at a matched *relative* work rate - are in contrast to some previous studies where participants of High and Low aerobic fitness who are matched for anthropometric characteristics are compared during exercise at a matched *relative* work rate (Greenleaf *et al*., 1969; Jay *et al*., 2011; Rowell *et al*., 1982), or studies where individuals are compared against themselves following an acute reduction in $\dot{V}O_{2max}$ (Greenleaf *et al*., 1969; Rowell *et al*., 1982). A crucial difference between these previous studies and the present study is that the environmental condition in this previous work was ‘neutral’, with low humidity, and designed to be compensable heat stress; Jay *et al*. (2011) for example, had environmental conditions of 24.8 (± 0.6) %, 26 (10) % RH. The extant research that has considered the impact of humidity upon the transition from compensable to uncompensable conditions is limited. Cheung and McLellan (1998a) have shown that when evaporative heat loss is restricted by wearing protective clothing (McLellan, Pope, Cain, & Cheung, 1996), high aerobic fitness results in a significant improvement in exercise heat tolerance, regardless of hydration or acclimation status. In the present study however, there were no differences in

T_{re} (or heat storage) at the point of inflection in the *relative* condition between the fitness groups, despite an inflection occurring at a lower T_{db} for the High aerobic fitness group at HH. This suggests that an inflection in T_{re} may have occurred in this group under these less stressful ambient conditions to preserve the gradient for heat exchange from core to shell, under conditions where an increased SR was not sufficient to maintain heat loss and where the T_{re} was not at critically high levels.

During exercise at both the matched *absolute* and *relative* work rate, the $\overline{T}_{sk}$ at the T_{re} inflection point was significantly higher in the LH condition than the HH condition, presumably due to the difference in T_{db} and greater mismatch between E_{max} and E_{req}. Additionally, the Low aerobic fitness group had a higher $\overline{T}_{sk}$ than the High aerobic fitness group in the HH condition at the T_{re} inflection point, possibly caused by lower rates of evaporative cooling due to the lower level of SR compared to the High fitness group (see Table 8.3 and 8.4) coupled with the reduced E_{max} of the environment and therefore reduced % of sweat produced evaporating. The effect of humidity on $\overline{T}_{sk}$ in the literature is unclear, with reports of higher (Maughan *et al*., 2012; Moyen, Ellis, *et al*., 2014) and lower (Hayes *et al*., 2014) $\overline{T}_{sk}$ when exercising in a high humidity environment, this discrepancy appears, however, to be dependent on whether the T_a is matched. Interestingly, in the HH condition, the core to shell gradient was similar between High and Low aerobic fitness groups at the matched *absolute* work rate (1.03 and 1.11 °C respectively), but when exercising at the same *relative* work rate the High aerobic fitness group had a notably larger core to shell gradient than the Low aerobic fitness group (1.63 and 1.11 °C respectively) at the T_{re} inflection point. This elevated core to shell gradient in combination with higher sweat rates for the High fitness group at the inflection point, points towards a greater 'physiological cost' for the High fitness group yet indicates that the increased SR was not sufficient to maintain the required heat loss in the HH environment under the matched *relative* work rate conditions, presumably due to the reduced capacity for evaporative heat losses.

In both humidity conditions, the High aerobic fitness group had significantly higher SR_{Back} than the Low aerobic fitness group at the T_{re} inflection point during exercise at matched *absolute*, and matched *relative*, work rates. There was also an effect of fitness on $SR_{Forearm}$, which was higher in the HH than the LH condition when participants exercised at the matched *relative* work rate only. However, there was no significant difference in SkBF at the T_{re} inflection point between aerobic fitness groups in either humidity condition, at either work rate. Therefore, the third hypothesis - *the physiological (thermoeffector)* *responses will differ at the T_{re} inflection point between the two aerobic fitness groups at the same matched absolute and relative work rates, for both humidity conditions* - can be partly accepted. The significantly higher SR exhibited by the High aerobic fitness group is in agreement with previous studies that have shown increased sweat production with increased aerobic fitness (Aoyagi *et al*., 1997; Cheung & McLellan, 1998a; Piwonka & Robinson, 1967; Selkirk & McLellan, 2001; Shvartz *et al*., 1977, 1974). The increased SR observed in High aerobic fitness individuals is thought to be due to increased cholinergic sensitivity (Buono & Sjoholm, 1988). The site specific differences that we observed are consistent with previous work which has shown that sweating on the forehead, but not the forearm, is altered by aerobic fitness (Cramer *et al*., 2012) in a neutral environment (Mean 25 °C, 1 kPa), at matched *relative* (at 60 % of $\dot{V}O_{2max}$) and *absolute* (a set metabolic heat production per unit surface area) work rates. While this method for matching the *absolute* work rate is different to the present study's approach, they are functionally similar, as participants in the present study were also matched for anthropometric characteristics and so there was no difference in the metabolic heat production between groups at the matched *absolute* work rate. The increased SR appears to have been effective in offsetting the increased thermoregulatory stress of the higher metabolic heat production, resulting in a similar T_{re} at the T_{re} inflection point between groups in the matched *relative* work rate condition, and indeed a lower T_{re} for the High aerobic fitness group in the HH environment at the matched *absolute* work rate. However, despite the improved sweating response, aerobic fitness did not offer any benefit in terms delaying the transition to uncompensable heat stress when exercising a given *absolute* work rate and did not prevent an inflection in T_{re} occurring at a lower T_{db} at the matched *relative* work rate.

Of course, an increased sweat rate (compared to Low fitness individuals) may ultimately lead to dehydration if sufficient fluid replacement is not available. Dehydration levels exceeding 2 % of body mass can exacerbate the cardiovascular response and impair performance (Cheuvront *et al*., 2010), however, the maximal body mass reduction was 1.93 % for one individual in one condition, with the overall mean (SD) for all participants as 0.60 (0.59) % in the current study and urine specific gravity was equal to or below 1.020 SG for all participants at the start of exercise with fluid (with electrolytes) consumed throughout the protocol. Thus, dehydration is unlikely to have been a contributing factor to thermal / thermoeffector responses, as changes in body mass have been shown to accurately reflect sweat losses and hydration status (Baker *et al*., 2009; Sawka *et al*., 2007).

The present study showed no effect of aerobic fitness or ambient humidity on $SkBF_{Forearm}$ and $SkBF_{Finger}$ at either work rate at the point of T_{re} inflection. Périard *et al.* (2012) also found no differences between aerobically trained and untrained groups when comparing $SkBF_{Forearm}$ (FU) when exercising at 60 and 75 % $\dot{V}O_{2max}$. However, in contrast, (Mora-Rodríguez *et al*., 2010) have found differences in $SkBF_{Forearm}$ (as a percentage of resting values) between aerobically trained and untrained groups exercising at 40, 60 and 80 % $\dot{V}O_{2peak}$. The reasons for these contrasting findings are not entirely clear, although as discussed previously (section 5.4), there is a relatively high CV % for this measurement with the method conducted in this series of studies, whereas the technique employed by Mora-Rodríguez *et al*., (2010), in which the left forearm to which the laser Doppler probe was attached was supported by a sling at heart level may have reduced the 'noise' of their measurement, thereby improving the ability to detect meaningful differences. However, it was felt that this approach was impractical, for the present study due to the prolonged exercise duration and associated discomfort, although this should be considered for future work.

Beyond the T_{re} inflection point, significant increases was observed in T_{re}, Δ $T_{re,}$ $\overline{T}_{b,}$ $\overline{T}_{sk}$, heat production, SR_{Back}, $SR_{Forearm}$ and $SkBF_{Finger}$ (but not $SkBF_{Forearm}$) in both fitness groups and work rates in the HH and LH condition. Therefore, the final hypothesis, - *there will be a thermoeffector 'reserve' for both groups, in both humidity conditions, at work rates beyond the T_{re} inflection point* can be accepted. It is suggested that uncompensability in a hot dry environment is limited by maximal sweating (Gagnon, 2012), therefore aerobically trained individuals have a thermoregulatory advantage over aerobically trained individuals in this environment (Aoyagi *et al*., 1997). However, as discussed in 2.4.1, the notion that sudomotor activity achieved during exercise is determined by the required evaporation for heat balance (E_{req}) or maximal evaporation possible within the given environment (E_{max}) (Cramer *et al*., 2012; Jay *et al*., 2011) is flawed because the body is unable to 'sense' E_{max} or even humidity (Newton, 2011). In the current study, at the point of T_{re} inflection E_{max} was lower than E_{req}, and positive heat storage had already taken place. Supporting the suggestion put forward in the Literature Review that the transition into uncompensable heat stress should not be associated with maximal thermoeffector response, the data from this Chapter and Chapter Seven demonstrate a clear increase in SR beyond the inflection point in T_{re}, suggesting that this effector response increases with both the absolute and rate of change of body temperature. It is important to have observed this 'reserve' in both High and Low fitness individuals, as it shows that regardless of training status an inflection in T_{re} occurs when there is already heat storage present and further increases thermoeffector responses are possible.

In summary, despite improved thermoregulatory function as indicated by greater sweating rate regardless of humidity or work rate, when body weight and body composition are controlled, aerobic fitness does not offer any benefit in terms of the T_{db} eliciting the transition to uncompensable heat stress when exercising at a given *absolute* work rate under conditions of high or low humidity. The improved thermoregulatory function (increased sweating rate) of aerobically fit individuals means that their T_{re} inflection occurs at the same T_{db} as less aerobically fit individuals during exercise at a given *relative* work rate under LH conditions, despite the higher metabolic heat production. Under HH conditions, which limit evaporative heat loss, the improved sweating rate of aerobically fit individuals does not provide a thermoregulatory advantage, and does not offset the increased metabolic heat production during exercise at a matched relative work rate, whereby the T_{re} inflection may occur at a lower T_{db}. It therefore is essential to ensure participants are matched for key anthropometric characteristics and an appropriate method for standardising work rate is chosen to compare individuals of different fitness levels. Finally, a thermoregulatory 'reserve', as indicated by sub-maximal rates of skin blood flow and sweat rate at the point of T_{re} inflection exist in both fitness groups and so strengthens the argument for rejecting the notion that uncompensable heat stress occurs when thermoeffector responses are maximally evoked.

9. Chapter Nine – General discussion and future work

9.1. General Summary and Discussion

Given that each experimental chapter of this thesis has a discussion, in an attempt to avoid repetition, this section is limited to a general summary and associated brief discussion.

The upper edge of the thermoregulatory zone (TZ) is thought to define the limit of human thermoregulation when working and exercising in the heat; the identification of this boundary is regarded as a key safety and experimental design consideration. The work presented in this thesis comprehensively investigated the upper boundary of the TZ by manipulating key variables in the heat balance equation (W, M, C, K, E and R). In the Literature Review it was noted that, despite the clear importance of being able to identify this boundary in a reliable and valid manner, and to understand the factors influencing it, substantial gaps remain in our understanding of the thermophysiology at, and beyond, the upper edge of the TZ. For example, from a methodological viewpoint it was unclear whether the upper limit of the TZ could be reliably determined or was influenced by the nature of the protocol (continuous or incremental increase in ambient temperature or humidity) used to establish it.

9.2. Summary of key findings and their implications

Having manipulated clothing evaporative resistance and $\overline{T}_{sk}$ through the addition of minimal clothing and use of ambient air temperatures above and below $\overline{T}_{sk}$, we found no observable effect on $\overline{T}_{sk}$ and thermoeffector (SR and SkBF) function between two clothing conditions when participants exercised in 40 °C air. Although the additional clothing coverage provided increased insulation, it may have also acted as a barrier to heat *gain* from the environment. This result was not reproduced in 30 °C air; when clothing acted as a barrier to heat loss. The implication of this result is that clothing should not necessarily be viewed as a thermoregulatory burden when exercising in hot environments. Clothing may, in fact, be beneficial in some circumstances, as it can protect the wearer from high radiant heat loads, advantageously redistribute sweat and prevent some sweat from dripping from the body and therefore not provide any cooling. The results also give an insight into the relative importance of $\overline{T}_{sk}$ (and $\overline{T}_{b}$) for the thermoeffector responses. Finally, this study allowed the development of a useful, less exhausting, experimental protocol for the examination of the TZ in subsequent experiments.

The protocol employed in these later experiments to determine the upper edge of the TZ, and used by previous authors (Dougherty *et al*., 2010; Kamon *et al*., 1978; Kenney & Zeman, 2002; Kenney *et al*., 1988, 1993), manipulates the physical aspects of the environment, and so the potential for heat loss. The work presented in this thesis has, for the first time, established that this protocol is reliable and reproducible; the inflection in T_c could be reliably detected using visual analysis and the D_{max} method. In previous studies it was not clear if the inflection point in T_c at the edge of the TZ was an artefact of the continuously rising ambient temperature or humidity protocol. We have shown that the inflection in T_{re} is synonymous with uncompensable heat stress and that T_{re} rises linearly beyond the inflection point, regardless of whether T_a continues to rise or is held constant when T_{re} inflects.

Knowing the protocol for establishing the upper edge of the TZ is reproducible allowed examination of the reproducibility of the physiological responses to exercise at this point, such data were not prevalent in the literature. Our findings show that, even when environmental conditions and thermal profiles (T_{re} and $\overline{T}_{sk}$) are similar between experimental conditions, there is variability in the thermoeffector response. There is a requirement for further research to establish what proportion of this variability can be accounted for by human error (*e.g.* sensor placement) and/or biological variability.

Importantly, we have shown that at the inflection point some of the thermoeffector responses are submaximal. Thus, at the point of "uncompensability" the thermoeffector responses are limited by the thermal profile (afferent input); further increases in T_c and $\overline{T}_{sk}$ are "permissive" in allowing greater effector responses and thereby dry and evaporative heat loss. This is in contrast to the view held by some (Cheung *et al*., 2000; Cheung & McLellan, 1998a; Givoni & Goldman, 1973; Kraning & Gonzalez, 1991) that uncompensability occurs when heat gain (metabolic or environmental) exceeds the capacity of the body to lose heat to the environment (See Figure 2.2 and 9.1). Thus, the body could delay the point of uncompensability in some circumstances by evoking maximum effector responses earlier, but it is unable so to do because of the afferent thermal input (thermal profile) at the time. Therefore, an important and novel finding from the present work is that this point of uncompensability at the upper edge of the TZ does not equate with a maximum evoked thermoeffector response. Indeed, we have shown that humidity, aerobic fitness and work rate impact on the thermal profile of the individual and the T_{re} inflection point in different ways but, in all instances the inflection in T_{re} occurs when there is a clear thermoregulatory reserve and with heat storage already present in low and high humidity conditions, with participants of low and high aerobic fitness. The elevation in T_{re} at the inflection point therefore, should not be equated with a "loss of thermoregulatory control" but rather should be considered as an increase in $\overline{T}_b$ that is "permissive" in terms of the thermoeffector responses, that is, another distinct phase in the model of thermoregulatory control/response. A suggested amendment to the area highlighted in red in Figure 9.1 is suggested in Figure 9.2, in light of the experimental work in this thesis.

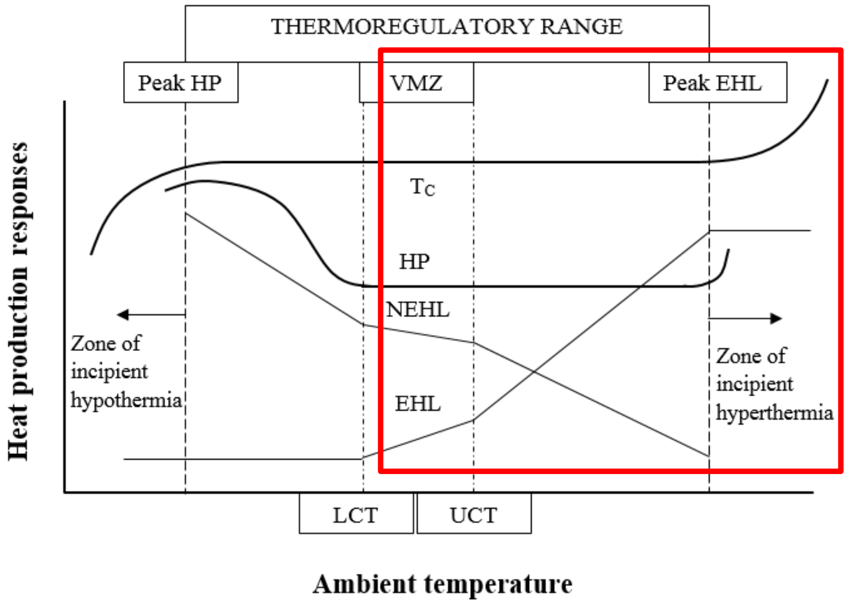


Figure 9.1 Representation of maintenance of core temperature (T_c) in the autonomic thermoregulatory range. Where HP = heat production, EHL = evaporative heat loss, VMZ = vasomotor zone, NEHL = non-evaporative heat loss, LCT = lower critical temperature, UCT = upper critical temperature. With red box indicating the area of interest to this thesis. (Redrawn from Mekjavić, Tipton, & Eiken, 2003)

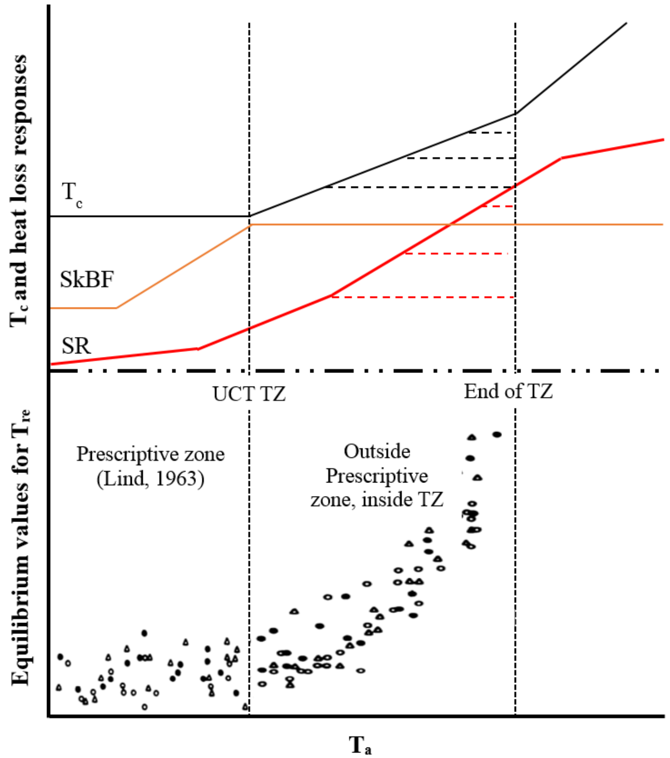


Figure 9.2. Suggested update to a diagram of an overview of thermoeffector and body temperature responses across a range of air temperatures Where T_c = deep body temperature, SkBF = skin blood flow, SR = sweat rate, T_a = ambient temperature, dashed lines = possible re-establishment of thermal balance depending on environment heat production, effector output and thermal gradients established, UCT TZ = upper critical temperature, TZ = Thermoneutral zone, T_a = ambient temperature. Data redrawn from Lind (1963a) showing equilibrium T_{re} for n=3 over a range of environmental temperatures at an energy expenditure of 300 kcal·hr^{-1}.

Humidity does not appear to affect the T_{re} at the T_{re} inflection point, but beyond this point, high humidity (80 % RH) causes a greater rate of rise in T_{re} in males (with a range of aerobic fitness). It is known that aerobic fitness offers thermoregulatory advantages, at rest and during exercise (Aoyagi *et al.*, 1997). However, it was shown in this thesis that individuals with high aerobic fitness did not transition into uncompensable heat stress later compared to their low fitness counterparts when working at the same absolute work rate in, despite higher SR. Additionally, when exercising in a low humidity (20 % RH) there is no difference in T_{re} at the T_{re} inflection point, between individuals of high and low aerobic fitness, regardless of working at matched relative and absolute work rates.

T_a had been held constant above the inflection of T_{re}, the rate of rise in $\overline{T}_{sk}$ diminished, as did the rate of rise in SR and $SkBF_{Forearm}$. The reduction in these thermoeffector responses in the presence of a linearly rising T_c supports the "permissive" nature of the afferent thermal input to the effector response, and is also a measure of the relative importance of $\overline{T}_{sk}$ as a thermoafferant input. It also supports the concept that $\overline{T}_b$ is the controlled variable of the thermoregulatory system. That $\overline{T}_b$ has to become "uncompensable" before effector responses (primarily in local sweating) are maximally evoked suggests that the 'gain' of the thermoregulatory system is not as sensitive as other systems such as, for example, those that control ventilation and cerebral blood flow (arterial CO_2) in which the controlled variable changes little when maximal effector responses are evoked. Two of the abiding questions of thermophysiology include: why does the thermoregulatory system not initiate full vasodilation and sudomotor function at the first instance of heat gain? In addition to the teleological answers of "*this would be wasteful of water and minerals and not necessary as behavioural thermoregulation is so powerful*" we can add the answer, "*because it is not permitted to*". As $\overline{T}_b$ increases, heat loss effector responses are permitted to increase, and heat loss increases, at some point heat gain and loss may reach a new equilibrium. Because most thermoregulatory research occurs with T_c below 40 °C it is possible that, in certain circumstances, data collection ceases before T_c stabilises. In this case "uncompensability" is a consequence of experimental design rather than heat balance. Much as $\dot{V}O_{2peak}$ is defined as an increase in work rate with unchanging (maximal) $\dot{V}O_2$, perhaps uncompensability should be defined as an increase in T_c with unchanging (maximal) effector responses.

By investigating the point at which individuals move from compensable and uncompensable heat stress, we have gained an insight into how the thermoregulatory system responds under stress. This transition, defined by an inflection point in T_c, simply represents the point at which heat production exceeds the rate of heat loss permitted by the temperature profile of the individual. We now have a better insight into the complex interaction of the physical and physiological factors influencing the regulation of $\overline{T}_b$.

9.3. Possible future work

9.3.1. The effect of heat acclimation at the T_c inflection point

Chapter Eight investigated the effect of aerobic fitness on the point of uncompensable heat stress as aerobically trained individuals have beneficial thermoregulatory adaptions compared to low fitness individuals, driven by the improvements in aerobic fitness *per se* (Jones & Carter, 2000), as well as the regular exposure to higher core temperatures during exercise and training (Avellini, 1982). Whilst the physiological adaptations typically displayed by aerobically trained individuals are similar to those seen with heat acclimation (Aoyagi *et al*., 1997), heat and exercise each induce specific transcriptional programmes (Kodesh & Horowitz, 2010), thus, represent independent stressors. This future work is proposed because Chapter Eight demonstrated that aerobic fitness was not sufficient to give a thermoregulatory advantage in conditions of high humidity, and it is hypothesised that heat acclimation, with its potentially greater impact on thermoregulatory function compared to aerobic fitness (Aoyagi *et al*., 1997), may be sufficient to delay the transition into uncompensable heat stress. These data could be collected as part of a larger heat acclimation study. These data could be added to the psychrometric chart of critical loci as presented by Kenney *et al*. (2004)

9.3.2. The effect of protective clothing

In Chapter Four, males exercised in two ambient conditions in two different levels of clothing insulation and increased metabolic heat production at set points to create a compensable then uncompensable environment within the same experimental condition. This methodology was not continued in the thesis, however as many participants became fatigued before they were uncompensable. It is proposed that a repeated measures design experiment is conducted with multiple levels of clothing as with Corbett *et al*. (2014) but with the incrementally increasing ambient temperature as in Chapter Five to Eight. While studies have been conducted using similar methods (Kamon *et al*., 1978; Kenney *et al*., 1987, 1988, 1993) no data are available to assess the thermoeffector response pre-, at and post- the point of T_c inflection. With the original work of Lind (1963a) in mind, it is logical to predict that levels SkBF and SR may differ between clothing conditions, despite T_c being similar. Additionally, these data could be added to the psychrometric chart of critical loci as presented by Kenney *et al*. (2004).

10. Chapter Ten – Delimitations, Assumptions and Limitations

10.1. Delimitations

Delimitations are those limits that the researcher knowingly imposes upon an experiment to keep it within the scope of work and for the medical well-being and safety of the participants.

For all studies in this thesis, healthy, active male participants (18-34 years old) were recruited, the majority of which were University of Portsmouth students between 2012 and 2014 (*i.e.* when and where this thesis was undertaken) and as such represented a homogenous sample of convenience.

To effectively test the hypotheses presented in this thesis, participants were initially exposed to a thermal load that was sufficient to cause a rise in T_c but stay within the thermoregulatory zone (TZ). In order to take the participants out of the TZ into uncompensable heat stress, they either increased the endogenous thermal strain by increasing the external work rate (Chapter Four) or were exposed to an increasing exogenous thermal strain through manipulating clothing (Chapter Four) or ambient T_{db} (Chapters Five to Eight). Furthermore, cycling was primarily chosen as the mode of exercise due to the low level of skill and familiarisation required, but also due to the ease with which work intensity could be monitored and controlled.

Deep body temperature (T_c) was monitored at the rectum (T_{re}) in all experiments, and was the primary measure in Chapters Five to Eight. It is acknowledged that this site is associated with slower responses to thermal change because the rectum is surrounded by a large mass of abdominal tissue with low thermal conductivity. However, T_{re} was monitored because: 1) it is widely used and considered a robust site for deep body temperature measurement when probes are inserted 15 cm beyond the rectum (Taylor *et al*., 2014); 2) it is an established measurement site for T_c which allows for comparison of our T_{re} results with other studies; 3) the majority previous work in this area (with adults) has used this measure (Belding & Kamon, 1973; Kamon *et al*., 1978; Kenney *et al*., 1987, 1988, 1993); and, 4) because pilot work indicated that few of the participants were able to tolerate the oesophageal probe. An initial feasibility study compared measurements of temperature at the rectum, the auditory canal (T_{au}) and the oesophagus (T_{oes}). Due to the more rapid response time and because the ambient T_{db} was fixed within a trial T_{au} was used as the primary measure presented in Chapter Four. However, despite surrounding the ear with insulation, the auditory temperature probe was influenced by the environmental conditions and/or local heat exchange around the ear, as evidenced by the differences in T_{au} between trial conducted at 30°C (50 % RH) and those conducted at 40°C (29 % RH), prior to reaching an uncompensable thermal state. T_{oes} was an optional measure for participants in all other studies, several tried but were unable to tolerate it or declined its use.

Skin temperature and HF was measured at eight sites and attached to the skin using Tegaderm patches and Transpore tape. It is acknowledged that this may artificially increase the local skin temperature measured (Tyler, 2011), but reducing the area covered was not satisfactory due to the movement and high sweat rates of the participant causing thermistors to become detached from the skin if not securely fastened.

Prior to each study all participants undertook a maximal aerobic capacity test in room temperature conditions (~20-21 °C and ~45-50% RH) with a fan available for cooling. From these data, relative work rates during the experiments, which took place in warm and hot environments, were calculated. While warm and hot environments have been shown to decrease $\dot{V}O_{2max}$, these are in instances of preheating or after prolonged duration in that environment (Arngrímsson *et al*., 2004, 2003; Nybo *et al*., 2001). Due to the lack of preheating, the short test duration and experimental evidence (Pirnay et al., 1970) it seems unlikely that the $\dot{V}O_{2max}$ values from a maximal aerobic capacity undertaken in room temperature would be significantly different from a maximal aerobic capacity undertaken in a hot environment. It is acknowledged however that the percentage of $\dot{V}O_{2max}$ would alter during the experiment and so the work rate may have been in fact be at a higher percentage $\dot{V}O_{2max}$ than currently stated. But to collect hyperthermic $\dot{V}O_{2max}$ data throughout or at the end of the test in order to have an indication of this change would have been impracticable and distract from the main aim of the experiments.

Finally, experimental end-points based on physiological responses were imposed under advice from the Department's Independent Medical Officer. These were: 1) if any measure of deep body temperature was >39.5 °C or if on reaching 39.0 °C, the rate of rise was 0.15 °C for a further two five minute readings 2) the $\dot{V}O_{2\,max}$ test or age predicted maximum HR (220 minus age), whichever was greater and 3) if any skin temperature reached 42 °C.

10.2 Assumptions

Assumptions refer to those facts or statements which are taken for granted, or without proof *per se*.

It was assumed that each participant was truthful in their completion of the Exercise and Health History Questionnaire (Appendix B), particularly their frequency of exercise (Question 3) as in Chapter Eight as this was an inclusion criteria, and when verbally confirming prior to each testing session that they had followed the pre-test instructions, *e.g.* refraining from alcohol, caffeine and exercise.

It was assumed that each participant would reach a thermal steady state in at least the first exercise stage in Chapter Four and within the first hour of the protocol in Chapters Five – Eight. If this had not happened, the experiment would have ended after 60 minutes (or at request of the participant or any of the other stopping criteria).

It was also assumed that each there was no change in the mechanical efficiency of the cycle ergometers used. Regular maintenance was performed and the cycle ergometers were removed from the high humidity environment swiftly after each experiment and wiped down.

10.3 Limitations

Limitations are those factors which are externally imposed upon each of the studies in this thesis; they also relate to matters of experimental design, or measurement techniques available. As an example, each experiment in this thesis was undertaken in a laboratory that was not equipped for measuring receptor function or brain activity, so it was not possible to measure thermoeffector activity directly, only a surrogate measure of afferent output as indicated by local sweat rate and changes in skin blood flow.

Despite effort to avoid this issue, there is the possibility that there were confounding issues with the insulation of the auditory canal temperature measurement and variation in pre-exercise T_{re} (within participant, between conditions) which may have influenced the results of Chapter Four. In following experimental work, care was taken to ensure participants started with a T_{re} no more than ±0.3 °C different to previous tests, if this was violated, the test would not take place until this rule had been satisfied.

It has been acknowledged in this thesis that there are valid criticisms of using thermometry to calculate heat storage (Jay, DuCharme, Webb, Reardon, & Kenny, 2010; Jay, Gariépy, *et al*., 2007) and to view a weighted combination of shell (peripheral) and deep body (brain and viscera) temperatures as mean body temperature. The majority of thermophysiological studies use this approach as the alternative requires a whole body calorimeter. In Chapters Seven and Eight it was observed that heat storage was already present at the point of T_{re} inflection. This may have been an artefact of the slower responses of rectal thermometry (the chosen measurement site for deep body temperature) to thermal change, because the rectum is surrounded by a large mass of abdominal tissue with low thermal conductivity (Taylor *et al*., 2014).

On a few occasions, some participants had to stop or slow the cycling cadence for short periods of time, these were for the following reasons: 1) participants needing to urinate; 2) participants needing to adjust their rectal thermistor; 3) quick maintenance of the cycle ergometer (adjusting flywheel rope, cleaning cadence sensor). For all instances, the period of time was recorded in the experimenter's notes. Due to urination requiring the participants to stop cycling completely for a time period of 1-4 minutes, metabolic heat production would not be consistent between individuals or trials. In an attempt to reduce the impact on: 1) measurements of oxygen uptake - participants were asked to urinate just after or at least 5 minutes before Douglas bag collections, 2) the point of inflection - to urinate in the first hour of the protocol and 3) comparison between conditions, if they needed to urinate in subsequent conditions, participants were asked that they did so at the same time point, but were not asked to stop if they did not need to pass urine.

Due to the functional limitations of the environmental chamber available for the experiments in this thesis, ambient temperature was the manipulated variable, rather than water vapour pressure as used in many previous studies (Belding & Kamon, 1973; Dougherty *et al*., 2010; Kamon *et al*., 1978; Kamon & Avellini, 1976; Kenney & Zeman, 2002; Kenney *et al*., 1987, 1993). This of course means that when the ambient temperature is increased and RH is controlled (as was done in experiments reported in Chapters Five to Eight), ambient water vapour pressure (Pa) would have also increased. Evaporation is determined by the gradient between Pa and the saturated water vapour pressure at the skin ($P_{sk,s}$). During the temperature ramp, $\overline{T}_{sk}$ increased and so $P_{sk,s}$, therefore the gradient between these parameters would also change. Unfortunately, this was unavoidable (chamber limitations) but was shown to be repeatable across conditions and within participants.

11. Chapter Eleven - References

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12. Chapter Twelve – Appendices

A – Letters of favourable ethical opinion

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26 June 2012

Miss Ella Walker
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ETHICAL APPROVAL – BSREC 12/050b

Protocol Title: A feasibility study: Reliability and procedure of key measures and evaluation of thermometry and partitional calorimetry – Amendment 2 – Addition of laser Doppler skin blood flow and Q-sweat capsule.
Date Resubmitted: 25 June 2012.

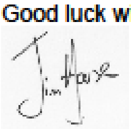
Thank you for submitting your protocol amendment for ethical review. As all elements of the protocol adhere to the Schedule of Approved Procedures the protocol has been reviewed by the undersigned in accordance with current procedures[1] on behalf of the BioSciences Research Ethics Committee.

The Committee is happy to approve your application under Approval Code **BSREC 12/050b**.

In your protocol you should include details of the equipment used to measure these two new variables. You should also be more specific on the placement of the Doppler probe on the forearm *e.g.* is it on the dorsal or ventral surface of the forearm? Also, you should include details of how both systems are calibrated (as you should for the VO2 measurements) – all will be required for your thesis.

Finally, on the participant information sheet, you may want to mention that blood flow is measured by shining a low powered laser light (like a weak laser pointer) into the skin and that no needles are used or blood samples taken. The mention of "blood flow" may put-off some potential participants who think that blood sampling is required.

Good luck with your study.



Dr Jim House
Co-chair of the BioSciences Research Ethics Committee

Information: Dr Corbett

[1] The Schedule of Approved Procedures (Annex C to *Procedures for Ethical Review)*. Biosciences Research Ethics Committee, University of Portsmouth, October 2011).

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11 October 2012

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ETHICAL APPROVAL – BSREC 12/077b

Protocol Title: Influence of clothing and relative work rate on the ability to thermoregulate below 39.5 °C when cycling in the heat.
Date Submitted: 9 October 2012.

Thank you for submitting your protocol amendment No.2 for ethical review. As all elements of the protocol adhere to the Schedule of Approved Procedures the protocol has been reviewed by the undersigned in accordance with current procedures[1] on behalf of the BioSciences Research Ethics Committee.

Thank you for advising of the change of conditions for your experiment and the rationale for this change.

Further to my verbal approval, the Committee is happy to formally approve your application under Approval Code **BSREC 12/077b**.

Good luck with your study.

Jim House

Dr Jim House
Co-chair of the BioSciences Research Ethics Committee

Information:

Dr J Corbett
Dr M Barwood
Prof M Tipton

[1] The Schedule of Approved Procedures (Annex C to *Procedures for Ethical Review*). Biosciences Research Ethics Committee, University of Portsmouth, October 2011).

Faculty of Science
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Date 01/10/2014
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FAVOURABLE OPINION

Protocol Title:	The effect of a different ambient air temperature profiles on thermoregulation and themoeffector responses in exercising males SFEC 2014 – 043a
Date Reviewed:	16/09/2014

Dear Ms Walker,

Thank you for your submission for ethical review. Having completed their review, members of the Science Faculty Ethics Committee have reached a Favourable opinion of your proposed research.

Please notify the committee of any substantial amendments to the proposed procedures, send an annual report to the committee regarding study progress and a final study report once the study has concluded. Please send these to sci.fac@port.ac.uk. Thank you and the committee wishes you well with your study.

Dr Chris Markham – Chair of SFEC

CC -
Dr Jim House – Vice Chair of SFEC
Holly Shawyer – Faculty Administrator

Scientific and Ethical Review Committee
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19th November 2013

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SCIENTIFIC AND ETHICAL REVIEW

Protocol Title: The effect of aerobic fitness and exercise intensity on thermoregulation in hot-dry and hot-humid environments
Date Submitted: 18th November 2013

Thank you for submitting your protocol amendment to the Departmental Scientific and Ethical Review Committee (SERC) for review. As all elements of the protocol adhere to the Schedule of Approved Procedures[1] it has been reviewed by the undersigned in accordance with current procedures on behalf of the Science Faculty Ethics Committee (SFEC)[2,3].

The Committee is happy to give favourable ethical opinion for your study under Opinion Code SFEC 2013-041B on behalf of the SFEC.

Good luck with your study.

Dr Clare Eglin
Member of the DSES Scientific and Ethical Review Committee
Vice-Chair / Member of the Science Faculty Ethics Committee

Information:

Any co-workers / supervisors

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[1] The Schedule of Approved Procedures, Scientific and Ethics Review Committee, Department of Sport & Exercise Science, University of Portsmouth, November 2012).
[2] Procedures for Ethical Review, Scientific and Ethics Review Committee, Department of Sport & Exercise Science, University of Portsmouth, October 2012.
[3] Procedures for Ethical Review, Science Faculty Ethics Committee, University of Portsmouth, October 2012.

B – Questionnaire used to screen all participants

Exercise and Health History Questionnaire

University of Portsmouth

July 2014 version Department of Sport & Exercise Science

All pages of this questionnaire should be completed by volunteers of certain physically active or stressful activities conducted by the Department of Sport and Exercise Science, primarily experiments, consultancy testing / demonstrations, and some taught laboratories. This information allows the activity leader (supervisor) to determine if it is safe for the volunteer to participate, or whether medical advice is required before proceeding. The information provided also gives a suitable history should a medical examination be required for particular studies. If required, the additional medical examination report is completed by the Independent Medical Officer and stapled to this document as pages (7 and 8)

.

IT IS VERY IMPORTANT THAT VOLUNTEERS ANSWER ALL OF THE QUESTIONS FULLY, AND TO THE BEST OF THEIR KNOWLEDGE. If you have particularly sensitive responses to any of the questions that you would rather not divulge in this record please mark the questions appropriately, and we may be able to arrange for you to discuss this directly, and in confidence, with an appointed medical doctor.

ALL INFORMATION PROVIDED IS TREATED AS MEDICAL-IN-CONFIDENCE (THE SAME AS YOUR PERSONAL MEDICAL RECORDS).

VOLUNTEERS ARE NOT TO PARTICIPATE IN ANY STUDY UNTIL THE DETAILS IN THIS COMPLETED FORM HAVE BEEN CHECKED AND COUNTERSIGNED BY A MEMBER OF THE DEPARTMENT'S TECHNICAL OR ACADEMIC STAFF (PAGE 6)

*Note for questions marked *, please delete as necessary*

Participant's Details

Full Name …………………………………………………………

Telephone Number.…………………..……..

Date of Birth ………………………………………

Age………………Email…………………………………

Date of completing this questionnaire………………………………….

For UoP Students Only:

Year of study:* *1 2 3 M PhD Student ID Number…………….. Personal Tutor……..*

Q1 How would you describe your current physical fitness status?

Very unfit (sedentary) / Unfit / Moderately Fit / Very Fit (*e.g.* competitive sportsperson)*

Q2 Do you undertake regular physical exercise? Yes / No*

If Yes Please tell us what type of exercise?

Light activities: *heart beats slightly faster than usual, you can talk/sing while you are active (walking leisurely, stretching, vacuuming or light gardening)*

Moderate activities: *your heart beats faster than normal, you can talk while you are active (fast walking, aerobics, strength training, swimming gently)*

Vigorous activities: *your heart rate increases a lot, you cannot talk or your talking is broken up by large breaths while you are active (stair machine, jogging or running, tennis, squash, badminton, basketball, cycling)*

Q3 How frequently do you exercise?

Q4 How often do you undertake exercise of a maximal nature?

Never / Sometimes / Often*

Q5 How would you consider your present body weight?

Underweight / Ideal weight / Slightly overweight / Very overweight*

Q6 Are you a regular smoker? Yes / No* – if yes number per day………

Q7 Are you an occasional smoker? Yes / No* – if yes average per week…….

Q8 Are you a previous smoker? Yes / No* – if yes how long since stopping ..… years

---

Q9 Do you drink alcoholic drinks*?* Yes / No*

If yes do you: have the occasional drink? Yes / No*
have a drink every day? Yes / No*

Q10 Have you consulted your doctor within the last 6 months (*except than for contraception*)? Yes / No*

If "yes" please give details *e.g.*
- Why did you consult your doctor?
- What medication was prescribed, for how long, and when did you finish taking it?
- Did your GP place any restriction on you (activities, diet *etc*.)?
- When was the problem resolved?

Q11 Do you have any allergies? Yes / No*

If Yes, please give details. Please include any allergies to dressings *e.g.* elastoplasts.

Q12 Are you currently taking any form of medication including both prescribed and over the counter preparations? Yes / No* – if yes give details (**No need to disclose contraceptive medication**)
e.g.
- What you are taking?
- What are you taking it for?
- Dose?
- How long have you been taking it?

Q13 Have you routinely taken any medication in the past 2 years? Yes / No* – if yes give details
e.g.
- What did you take?
- What did you take it for?
- Dose?
- How long did you take it?
- When did you stop taking it?

Q14 Have you ever been told to give up sports because of health problems? Yes / No* – if yes give details *e.g.*
- What were you advised to stop?

- When?

- Why?

Q15 Do you get tired more quickly than your friends do during exercise? Yes / No* – if yes give details

---

Q16 Have you ever suffered from any of the following?

Asthma	Yes / No*
Diabetes	Yes / No*
Hypertension (high blood pressure)	Yes / No*
Any form of heart disorder	Yes / No*
High blood cholesterol	Yes / No*
Epilepsy	Yes / No*
Have you ever had a seizure	Yes / No*
Fainting	Yes / No*

If yes to any of the above, please give full details of the condition / episode so that we can determine whether it is safe for you to participate in the activity, or if we need to seek medical advice:

<u>If you reported "yes" to asthma</u>, have you:
- been prescribed any medication for this in the past 4 years?
- if so, what have you been prescribed specifically (inhaler name and colour)?
- how often do you / did you use the medication?
- when did you last use the medication?

Q17 Have you ever been told you have a heart murmur? Yes / No* – if yes give details

---

Q18 Have you ever been told you have a heart arrhythmia? Yes / No* – if yes give details

---

Q19 Do you have any other history of heart problems? Yes / No* – if yes give details

---

Q20 Have you had a severe viral infection (*e.g.* myocarditis or mononucleosis) within the last month? Yes / No* – if yes give details

---

Q21 Have you ever been told you had rheumatic fever? Yes / No* – if yes give details

---

Q22 Have you ever suffered from the following?

Heat stroke, heat exhaustion or sunstroke	Yes / No*
Cold Illness or injury (non freezing cold injury or frostbite)	Yes / No*
Poor Circulation (including Raynaud's phenomenon)	Yes / No*
Peripheral neuropathy	Yes / No*

If yes, please give details *e.g.*
- When did this occur?

- What was the outcome?
- What caused this?
- Have you any restrictions placed upon you by your doctor because of this?

Q23 Please give details of any overnight hospital admissions you have had *e.g.*
- When?
- Duration of stay?
- What for?
- When were you fully recovered?

Q24 Have you any other past medical history we have not already asked you about? Yes / No* – if yes give details

---

Q25 Do you have any muscle, joint or back injury at present? Yes / No* – if yes give details

---

Q26 Have you had to suspend any normal activity due to ill health or injury in the last month? Yes / No* – if yes give details

---

Q27 Is there a history of heart disease or sudden cardiac death in your family? Yes / No* – if yes give details *e.g.*
- Which blood relative?
- What happened / was diagnosed?
- Age of relative at incident / diagnosis?

Q28 Are both parents still alive? Yes / No* If No, please give cause of death and age at death.

Q29 Does any of your parents or brother/sister (blood relatives only) suffer from a serious medical condition? If so please can you provide details *e.g.*
- Who?
- What?
- Age when diagnosed?

Q30 Has anyone in your immediate family (*parent, child or brother/sister*) less than 50 years of age:

Died suddenly and unexpectedly? Yes / No*
Been treated for recurrent fainting? Yes / No*
Had unexplained seizure problems? Yes / No*
Had unexplained drowning while swimming? Yes / No*
Had unexplained car accident? Yes / No*
Had heart transplant? Yes / No*
Had pacemaker or defibrillator implanted? Yes / No*
Been treated for irregular heart beat? Yes / No*
Had heart surgery? Yes / No*

If you have answered yes to any of the above questions, please give details *e.g.*
- Who?
- What?
- Age at incident / diagnosis?

Q31 Do you suffer from, or have you ever suffered from the following

Chest pain Yes / No*
Chest pain on exercising Yes / No*
Unexpected breathlessness on exertion Yes / No*
Undue dizziness on exertion Yes / No*
Collapse whilst exercising Yes / No*
Palpitations (irregular heart beat) Yes / No*

If you have answered yes to any of the above questions, please give details.

Q32 Are you a blood donor? Yes / No*

If yes, have you donated blood in the last week? Yes / No*

Q33 <u>For females (only required for studies involving thermal stress, hypoxia and arduous exercise)</u>

Please state here whether you <u>may</u> be pregnant: Not pregnant / Unsure / Pregnant*

Q34 **To the best of your knowledge are there any other reason(s) that may prevent you from successfully completing the tasks that have been explained to you by the lead academic / principal investigator and as described in the Participant Information Sheet?**

Yes / No* **If yes, please give details**

<u>Emergency Contact Details</u>

Please supply the name, address and telephone number of an emergency contact: (please print)

Name ……..………………………………… Relationship …………………….……

Address
……
…

……….………………………………………………………………………………………………
…………..

Telephone number(s) ………………………………………………………….

DECLARATION

I understand that it is my responsibility to fully disclose information about my health in this questionnaire and that knowingly failing to do so may place me at risk during trial experiments.

I also understand that if anything changes in my health circumstances between this screening questionnaire and / or medical examination and the date of my participation in the experiment, test series or taught laboratory, it is my responsibility to fully inform the leader of the activity and that failure to do so, whether knowingly or unknowingly may place me at risk during the activity.

Signature of Participant ……………………………………………….. Date …………………………

VOLUNTEERS ARE NOT TO PARTICIPATE UNTIL THE DETAILS COMPLETED ABOVE HAVE BEEN CHECKED AND COUNTERSIGNED BY A MEMBER OF THE DEPARTMENT'S TECHNICAL OR ACADEMIC STAFF BELOW

Note to Technical/Academic checker: Please check that the details above do not show any contra-indications that might jeopardise the volunteer's health or safety. If in any doubt whatsoever, please seek the advice of colleagues, and ultimately a member of the DSES Scientific and Ethical Review Committee. If there are any contra-indications, or remaining doubt please thank the volunteer for their time **BUT ADVISE THEM THAT THEY CANNOT PARTICIPATE IN THE STUDY AT THE MOMENT UNTIL FURTHER MEDICAL ADVICE IS SOUGHT**. In that case, please seek medical advice in accordance with current procedures[1].

Name of Academic / Technical Staff ………………………………

Signature of Academic / Technical Staff …………………………. Date ………………………..

ONLY PAPER RECORDS OF THIS CAN BE RETAINED SECURELY. RETENTION OF MEDICAL RECORDS ELECTRONICALLY REQUIRES SPECIALIST IT PROCEDURES WHICH ARE NOT IN PLACE

[1] DS&ES Guidelines for medical assessment and medical cover for human tests.

C – Clothing provided to participants

a. Full length cycle jersey

b. Cycling leggings

c. Sport sock

d. Cycling shorts

- Garments a, b and c were worn for the Clothed condition in the experiment reported in Chapter Four.
- Garments c and d were worn in the Semi-nude condition for experiment reported in Chapter Four and was the only clothing condition for experiments reported in Chapter Five – Eight

D – Reliability

The most appropriate approach for assessing reliability is contentious (Atkinson and Nevill, 2000; Hopkins 2000b). Broadly, approaches to assessing reliability can be split into measures of either relative reliability - the degree to which an individual maintains their position within a sample over repeated measurement, or measures of absolute reliability - the variation in an individual’s measurements across multiple trials (Baumgartner, Jackson, Mahar, & Rowe, 2003; Hopkins, 2000a). Relative reliability is commonly described by correlation coefficients, which can range from +1 (perfect positive correlation) to -1 (perfect negative correlation); if each participant has identical values in test-retest situation the plot of the values of the two trials would form a straight line and the correlation coefficient would have a value of 1. This method is widely used for test-retest reliability because it allows for comparison between different methods, but it has been criticised for its sensitivity to the between-participant heterogeneity (Altman & Bland, 1983; Ball & Scurr, 2010; Hopkins, 2000a) and because it does not provide any information on change in the mean between trials or systematic bias (Batterham & George, 2000; Weir, 2005), which might be expected with learning effects, training effects, or with acclimation to heat (Batterham & George, 2000; Currell & Jeukendrup, 2008; Hopkins, 2000a; Weir, 2005). Moreover, it is important to note that the correlation coefficient is not a measure of agreement; it is a measure of association (Altman & Bland, 1983) and so is not appropriate for use in analysing test-retest data. The same criticism holds true for interclass correlation (ICC), although this has been refuted because the ICC is not designed to provide an absolute index of measurement error, but rather, it provides information regarding inferential statistics (Weir, 2005).

Measures of absolute reliability can be assessed using the limits of agreement (LoA) (Bland & Altman, 1986), although this approach was not designed to examine test-retest reliability *per se*, but to examine agreement between two different techniques (Altman & Bland, 1983; Bland & Altman, 1986). Moreover, the values of the LoA depend on the sample size from which they are estimated and so is considered an inappropriate statistic for low participant numbers (Hopkins, 2000b). A sample of over 40 participants is recommended for this statistic (Atkinson & Nevill, 1998), meaning it is often impractical for human physiology experiments. Thus, the preferred measure for assessing absolute reliability is typical error of the measurement (TEM), also known as standard error of measurement (SEM), or typical error (Hopkins, 2000a). The TEM is calculated by dividing the standard deviation of the difference score (differences between trial 1 and 2 for example) by $\sqrt{2}$ (Hopkins, 2000a, 2000b) and so remains in units of measurement, but can also be expressed as a coefficient of variation (CV %), which presents the values expressed as a percentage of their respective means (Hopkins, 2000a) and, as a dimensionless value, allows comparison to other research (Hopkins, 2000a). The TEM and CV % are unaffected by the sample size and range, are easier to extrapolate to new participants (Hopkins, 2000a), and can be used on heteroscedastic data if the absolute values are log transformed (Atkinson & Nevill, 1998; Hopkins, 2000a)

E – UPR16

FORM UPR16

Research Ethics Review Checklist

Please include this completed form as an appendix to your thesis (see the Postgraduate Research Student Handbook for more information

University of Portsmouth

Postgraduate Research Student (PGRS) Information			**Student ID:**	471361
PGRS Name:	Ella Walker			
Department:	DSES	**First Supervisor:**	Dr Jo Corbett	
Start Date: (or progression date for Prof Doc students)		**February 2012**		

Study Mode and Route:	Part-time	☒	MPhil	☐	MD	☐
	Full-time	☐	PhD	☒	Professional Doctorate	☐

Title of Thesis:	On the Edge of Thermoregulation: a Matter of Physiology and Physics
Thesis Word Count: (excluding ancillary data)	62,699

If you are unsure about any of the following, please contact the local representative on your Faculty Ethics Committee for advice. Please note that it is your responsibility to follow the University's Ethics Policy and any relevant University, academic or professional guidelines in the conduct of your study

Although the Ethics Committee may have given your study a favourable opinion, the final responsibility for the ethical conduct of this work lies with the researcher(s).

UKRIO Finished Research Checklist:
(If you would like to know more about the checklist, please see your Faculty or Departmental Ethics Committee rep or see the online version of the full checklist at: http://www.ukrio.org/what-we-do/code-of-practice-for-research/)

a) Have all of your research and findings been reported accurately, honestly and within a reasonable time frame?	YES NO	☒ ☐
b) Have all contributions to knowledge been acknowledged?	YES NO	☒ ☐
c) Have you complied with all agreements relating to intellectual property, publication and authorship?	YES NO	☒ ☐
d) Has your research data been retained in a secure and accessible form and will it remain so for the required duration?	YES NO	☒ ☐
e) Does your research comply with all legal, ethical, and contractual requirements?	YES NO	☒ ☐

Candidate Statement:

I have considered the ethical dimensions of the above named research project, and have successfully obtained the necessary ethical approval(s)

Ethical review number(s) from Faculty Ethics Committee (or from NRES/SCREC):	BSREC 12/050b, BSREC 12/077b, SFEC 2014-041b and SFEC 2014-043a

If you have *not* submitted your work for ethical review, and/or you have answered 'No' to one or more of questions a) to e), please explain below why this is so:

Signed (PGRS):	Ella	**Date:** 25.9.15