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Optimising strategies to enhance endurance running performance under hot and humid conditions

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Abstract

The purpose of this thesis was to optimise preparation for endurance runners competing in a hot environment. A multidisciplinary approach was adopted, investigating acute and chronic interventions to identify the most effective preparation. The determinants of endurance performance model (Bassett & Howley 2000) facilitated simultaneous investigation of the efficacy of interventions as well as the physiological mechanisms through which interventions influence performance. Finally, a retrospective analysis was conducted to identify the relationship between physiological markers and endurance running in the heat, in order to inform training prioritisation.

Study 1 investigated the validity and reliability of skin temperature (T_{SKIN}) measurement tools at rest and during treadmill running, to identify an appropriate measurement tool for use in subsequent studies, without restrictive trailing wires. A thermal camera provided insufficient levels of validity and reliability for safe monitoring of T_{SKIN}, while telemetry thermistors provided a viable and more convenient alternative to the criterion measure of hard-wired thermistors.

Study 2 investigated the effect of evidenced and practical internal (eg. ice slurry ingestion) and external precooling (eg. ice packs, forearm immersion and cold garments) strategies during incremental running in the heat. No differences were observed across the determinants of endurance performance between cooling techniques, with both eliciting modest reductions in blood lactate accumulation, compared with no cooling, which may prevent a decrement in the lactate turnpoint speed under heat stress. However, external cooling elicited a greater reduction in T_{SKIN} and thermal sensation, alleviating perceived thermal strain further than internal cooling.

Study 3 investigated ischaemic preconditioning (IP), which may enhance endurance performance through haemodynamic and/or metabolic mechanisms, prior to incremental running in the heat. IP had no effect on the determinants of endurance performance. The thermoregulatory responses to exercise-induced hyperthermia may supersede previously reported effects of IP, which is not recommended as an ergogen prior to exercise in the heat.

Study 4 investigated the effect of a chronic strategy, involving five days of controlled hyperthermia heat acclimation (HA), on the determinants of endurance performance and a 5 km time trial in the heat. Heat acclimation improved $\dot{V}O_{2max}$, the lactate thresholds and 5 km performance. Alleviated physiological, thermal and metabolic strain were observed, likely attributable to enhanced cardiovascular stability and heat dissipation. Five days of controlled hyperthermia HA appears to be a potent strategy to support endurance running in the heat.

Study 5 directly compared acute (external precooling), chronic (HA) and combined (precooling & HA) strategies during 5 km running. Precooling did not improve performance following HA, despite modest alleviation of physiological strain, which may indicate an inappropriate pacing strategy. Heat acclimation improved performance further than precooling (HA +6.6%, precooling +3.7%), indicating HA should be prioritised, although precooling appears beneficial when acclimation is not possible.

Multiple, linear regression indicated the physiological determinants of endurance performance do not accurately predict endurance performance in the heat (R^2=0.72, standard error of the estimate =105.6 s). The unexplained variation during endurance performance in the heat may reflect a greater prominence of perceptual measures to influence running in the

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heat. Preparation for competition in the heat should therefore prioritise improving perceived thermal and exertional strain under heat stress, alongside improving traditional physiological markers.

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List of abbreviations

ANOVA	Analysis of variance
$b.min^{-1}$	Beats per minute
BHC	Body heat content
BHS	Body heat storage
BM	Body mass
CI	Confidence interval
CNS	Central nervous system
CO	Cardiac output
CO_2	Carbon dioxide
CV	Coefficient of variation
CON	Control
CV	Cardiovascular
d	Cohen's *d* effect size
η2	Eta squared effect size
EXT	Mixed methods external cooling
GXT	Graded exercise test
HW	Hard wired thermistor system
HA	Heat acclimation
HA+PC	Heat acclimation and precooling
HR	Heart rate
HRmax	Maximum heart rate
Hb	Haemoglobin
Hct	Haematocrit
ICC	Intraclass correlation coefficient
ICE	Ice slurry drink
IP	Ischemic preconditioning
IR	Infrared
Kg	Kilogram
$Km.h^{-1}$	Kilometres per hour
LOA	Limits of agreement
$L.min^{-1}$	Litres per minute
LT	Lactate threshold
LTP	Lactate turnpoint

LTHA	Long term heat acclimation
MHP	Metabolic heat production
Min	Minutes
mL.kg.$^{-1}$min.$^{-1}$	Millilitres of oxygen per kilogram of body mass per minute
mmHg	Millimetre of mercury
O_2	Oxygen
OBLA	Onset of blood lactate accumulation (4 mMol.L^{-1})
PC	Precooling
PSI	Physiological strain index
PTV	Peak treadmill velocity
RE	Running economy
RER	Respiratory exchange ratio
RH	Relative humidity
RPE	Rating of perceived exertion
S	Seconds
SD	Standard deviation
STHA	Short term heat acclimation
SV	Stroke volume
TC	Thermal camera
T_{CORE}	Core temperature
TEE	Typical error of the estimate
TEL	Telemetry thermistor system
TEM	Typical error of the measure
TS	Thermal sensation
T_{SKIN}	Skin temperature
TT	Time trial exercise protocol
TTE	Time to exhaustion exercise protocol
$\dot{V}O_2$	Oxygen consumption
$\dot{V}O_{2max}$	Maximal oxygen consumption
$\dot{V}O_{2peak}$	Peak oxygen uptake
$v\dot{V}O_{2max}$	Estimated velocity at maximal oxygen consumption
WBGT	Wet bulb globe index

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education and were a driving force behind me continuing in education and I dedicate this thesis to you.

Declaration

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

Signed:

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1   Introduction

For millennia humans have been required to complete strenuous physical work under extreme environmental conditions, not least due to a necessity to hunt prey and survive (Lieberman 2015). In more recent years, this challenge remains in occupational environments, and is experienced by individuals across a range of industries that includes, but is not limited to; construction, firefighting, military, mining and sport (Health and Safety Executive, 2015). Within these fields, in particular, a deleterious effect of hot and humid conditions on prolonged exercise duration and sporting performance is well-established (Galloway & Maughan 1997), which is pertinent given that premier sporting competitions such as the Olympic Games and IAAF World Championships routinely occur in the summer months and often in hot environments. Moreover, competing under high ambient temperatures and humid conditions presents potential impairments to an athlete's health, as well as their exercise performance (Bergeron 2014). Therefore, across many endurance sports, prolonged metabolic heat production that arises from energy metabolism for locomotion, may exceed the cooling capacity of the human body in a given environment (Mountjoy et al. 2012). This results in considerable body heat storage and heightens the risk of hyperthermia, heat exhaustion or heat stroke (Bergeron et al. 2012). To mitigate against these risks, many sporting governing bodies, notably those of athletics, triathlon, soccer and tennis (Mountjoy et al. 2012), as well as volleyball (Bahr & Reeser 2012), implement sport-specific heat stress policies which aim to protect athletes when environmental conditions exceed predefined thresholds. However, whilst these policies may reduce instances of extreme heat illness and medical emergencies (Casa et al. 2015), they do not account for individual differences in body shape and size (Racinais et al. 2015a). Moreover, the detrimental effects of heat stress on athletic performance are widely observed in competitions that occur below these *extreme* threshold temperatures (Guy et al. 2015; Ely et al. 2007; El Helou et al. 2012; Nassis et al. 2015; Mohr et al. 2012). Consequently, across a range of sports, the potential remains for those who are better prepared and adapted, to experience a smaller decrement in exercise performance, and yield a competitive advantage when competing in the heat.

To prepare to perform in hot environments, humans have traditionally undergone repeated heat exposures across a number of days, to promote physiological adaptations that will enhance heat dissipation and work capacity (Wyndham et al. 1954; Fox et al. 1963; Greenleaf & Greenleaf 1970). The origins of such strategies may in part, be traced back to the work of Aldo Dreosti, a Physician working for Rand Mines Limited in Witwartersrand, South Africa. Dreosti encouraged miners to undertake novel heat training sessions, progressively increasing their

exposure to hot conditions across 2 weeks, as well as developing a heat tolerance protocol to screen prospective miners, as he sought to reduce the number of fatalities in the 1930s (Schneider & Moseley 2014). Subsequently, throughout the mid-19th century, similar strategies have been researched and refined, notably for military personnel (Fox et al. 1963; Greenleaf & Greenleaf 1970; Taylor et al. 1995) and astronauts (Sciaraffa et al. 1980).

More recently, athletes who compete in a hot environment will undertake long term, heat acclimatization on location, or *a priori* artificial heat acclimation programmes as part of their preparation (Armstrong 1998). Undertaking specialised preparation such as these is necessary given the fine margins that determine success at elite level (Hopkins 2005), as well as the regularity with which major sporting events and competitions are scheduled for summer months and/or hot locations. For example, at the 2008 Beijing Olympics ambient temperature ranged between 21-31°C with relative humidity exceeding 60% (Ross et al. 2011). Environmental conditions across the sites of the Rio de Janiero Olympics at the time of the London Olympics indicate further thermal challenges during the 2016 Olympics, with peak temperatures of 38°C and humidity exceeding 80%, displaying large variation from mean daily ambient temperature of 26°C (National Institute of Meteorology Brazil 2015). This trend looks set to continue with the 2019 IAAF World Championships and 2022 FIFA World Cup taking place in Doha, Qatar.

Both acclimation and acclimatisation training[1] are effective strategies to induct the heat acclimation phenotype (Garrett et al. 2011), which is characterised by systemic thermoregulatory, cardiovascular and sudomotor adaptations (Sawka et al. 2011). Evidence advocating the use of heat acclimation strategies for improving sporting performance in the heat is ubiquitous, within both endurance performance (Lorenzo et al. 2010; Schmidt et al. 2015) and higher-intensity team sports (Buchheit et al. 2011; Sunderland et al. 2008). Recent evidence suggests the traditional approach of acclimating through repeated, prolonged low intensity training may be optimised through manipulation of the primary stressors; environmental conditions (temperature, radiant heat and humidity) and physical work (intensity, duration and frequency). Accordingly, this manipulation may proffer a more individual-specific adaptation, pertaining to sporting type (Sunderland et al. 2008) or sex (Mee et al. 2015a), as well as reducing the overall training volume (Gibson et al. 2015b). However, despite the emergence of more efficient strategies such as these, repeated acclimation or acclimatisation training in a hot environment, across 5- 14 days, remains time-consuming and costly. Furthermore, the transient nature of the heat acclimation phenotype necessitates this

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[1] Heat acclimation develops following exposure to controlled experimental conditions, whereas heat acclimatization is produced in the naturally occurring environment (Sawka et al. 2011).

training occurs in the weeks prior to competition, which opposes a typical competition taper phase (Spilsbury et al. 2015). Consequently, for endurance athletes competing in the heat, the potential of acute interventions that do not have these restrictions, is being realised.

Acute internal (Siegel et al. 2012) or external (Minett, Duffield, et al. 2012) cooling of the body in the hour prior to exercise, has been shown to mitigate against heat strain[2] through lowering core body temperature, permitting a greater heat storage capacity, and improving thermal comfort during exercise. Accordingly, precooling techniques have been shown to improve both self-paced endurance time trial performance (Cotter et al. 2001, Duffield et al. 2010) and time to exhaustion (Siegel et al. 2010). Despite the widespread support for adopting both chronic (Chalmers et al. 2014; Garrett et al. 2011) and acute strategies (Tyler et al. 2015; Jones et al. 2012) prior to exercise in the heat, there has yet to be a direct comparison between techniques. Furthermore, a uni-disciplinary approach is apparent, with only a modest amount of literature pertaining to the additive effect of combining interventions. This limited research has been focussed upon intermittent sprint cycling (Castle et al. 2011; Brade et al. 2012) and endurance cycling (Schmit et al. 2015), but does not provide conclusive evidence supporting combining interventions. However, endurance running is characterised by a greater absolute metabolic heat production (Millet et al. 2009) and reduced convective cooling, versus cycling, which will result in an expedited elevation of body temperature (Cramer & Jay 2015). Furthermore, given that endurance performance in the heat remains impaired in the acclimated individual (Sawka et al. 1985, Lorenzo et al. 2010), the investigation of a multi-disciplinary approach involving the combination of precooling and acclimation appears warranted for runners.

However, such a multi-disciplinary approach may extend beyond specific thermal interventions. For example, alternative, non-thermal acute interventions such as the supplementation of caffeine (Hulston & Jeukendrup 2008) and sodium (Sims et al. 2007) have both been highlighted as potential ergogens, and could complement an acclimation strategy. However, the effectiveness of both caffeine (Roelands et al. 2011) and sodium supplementation (Earhart et al. 2015) during exercise in the heat have been questioned. An alternative acute intervention, the application of which in an exercise performance context is relatively novel, is ischaemic preconditioning. Ischaemic preconditioning involves repeated bouts of occlusion to major limbs and is established within clinical practice to prepare cardiac muscle for subsequent stresses arising from surgical hypoxia, infarction and reperfusion

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[2] Heat stress refers to environmental (including clothing) and metabolic conditions that tend to increase body temperatures; heat strain refers to physiological (e.g., body temperature) consequences of heat stress (Sawka et al. 2011).

(Hausenloy & Yellon 2008). Notable improvements have been observed in endurance exercise performance under normothermic conditions following preconditioning (Bailey et al. 2012a; de Groot et al. 2010), whilst emerging evidence indicates alleviation of physiological strain in other extreme environments, such as hypoxia (Foster et al. 2011; Foster et al. 2014). Specifically, maximal oxygen uptake ($\dot{V}O_{2max}$) may be enhanced following preconditioning (de Groot et al. 2010), whilst blood lactate concentration has been shown to be reduced during endurance running (-1.07 mMol.L^{-1}), prior to improved 5 km running time (2.5%, Bailey et al. 2012a). Prominent purported mechanisms underpinning the effects of preconditioning are improved peripheral haemodynamics improving blood flow to active skeletal muscles, thereby increasing oxygen delivery and the removal of waste products (Bailey et al. 2012b), improved metabolism through increased mitochondrial efficiency that proffers greater oxygenation of the muscle (Cooper & Brown 2008), as well as the potential for desensitising of afferent feedback that may facilitate the maintenance of a greater self-selected exercise intensity (Tocco et al. 2014). Whilst the precise mechanisms underpinning exercise performance following preconditioning have recently become a topic of fierce debate (Salvador et al. 2015), improved muscle blood flow, greater aerobic capacity arising from improved muscle oxygenation and a reduced perceived strain respectively, all have the potential to ameliorate endurance performance impairments afforded by cardiovascular, metabolic and perceptual alterations that characterise endurance performance in the heat (Nybo et al. 2014). However, this technique has yet to be examined under heat stress. Ultimately, it would appear that the potential exists to optimise existing endurance performance strategies, prior to competing in the heat, and a multi-disciplinary approach should be investigated as a method of achieving this.

Endurance running performance is typically assessed through time trial and time to exhaustion trials (Casa 1999; Maughan & Shirreffs 2004), with these occurring in a laboratory, or artificially hot environment, such as an environmental chamber. Such protocols offer an ecologically valid assessment of endurance performance by allowing free pacing (time trial), or help to discern the physiological mechanisms that determine any changes in performance from an intervention (time to exhaustion), but do not permit both (Stevens & Dascombe 2015). Alternatively, both performance and physiological changes may be encapsulated within a single test, by examining the physiological markers of the lactate turnpoint, running economy and $\dot{V}O_{2max}$, with running economy and $\dot{V}O_{2max}$ combining to provide a valid estimate of endurance performance in velocity at $\dot{V}O_{2max}$ (Bassett & Howley 2000; Denadai et al. 2004; Jones et al. 2006; Joyner & Coyle 2008). Collectively, these markers, known as the determinants of endurance performance, account for a large proportion of the variability in endurance cycling (Coyle 1999) and running performance (McLaughlin et al. 2010). Given that the lactate

turnpoint (Lorenzo et al. 2011) and $\dot{V}O_{2max}$ (Nybo et al. 2014) are distinctly impaired in the heat, and the lactate turnpoint remains a strong predictor of endurance performance in both hot and cold environments (Lorenzo et al. 2011), this would appear a sensitive and valid model for holistically evaluating endurance performance under heat stress.

Therefore, this thesis will investigate how acute and chronic strategies, both individually and collectively, can mediate the decline in endurance performance in the heat, in order to optimise competition preparation for an endurance runner who will compete in the heat. Furthermore, the determinants of endurance performance exercise model will facilitate a better understanding of how these interventions impact on physiology, and in turn performance.

The following literature review will provide an overview of relevant literature pertaining to endurance running under heat stress and the potential strategies that may be used to enhance heat tolerance and ameliorate the deleterious effects to running performance. The review will begin by examining the mechanisms underpinning endurance performance and the physiological markers that combine effectively to determine performance. The review will then consider the range of exercise models that can be adopted to validly and reliably assess endurance running, before introducing the physiological manifestations of environmental heat stress. Methods of quantifying heat stress and subsequent heat strain will be discussed, as well as typical endurance performance decrements observed from running in the heat. Finally, chronic and acute strategies that may benefit endurance running in the heat will be introduced and evaluated.

2 Literature review

2.1 Endurance performance

2.1.1 Determinants of endurance performance

Energy for human locomotion is provided by catabolic reactions that harvest energy from cellular respiration. In turn, this energy facilitates the movement of skeletal muscle filaments that proffers muscular action of the appropriate limbs. Although a myriad of factors including gait, limb anatomy and environmental conditions help to determine the individual's velocity (Saunders et al. 2004a), a collection of physiological markers may be used to quantify the underlying biochemical process. These markers accurately define the characteristics required for successful endurance performance (McLaughlin et al. 2010; Bassett & Howley 2000; Joyner & Coyle 2008; Jones 1998, Coyle 1999), such that they have been termed the determinants of endurance performance and include; the lactate threshold (LT), lactate turnpoint (LTP), running economy (RE) and maximum oxygen uptake ($\dot{V}O_{2max}$).

Traditionally, $\dot{V}O_{2max}$ has been considered to be the most important physiological characteristic for an endurance athlete (Saltin 1967) and a high $\dot{V}O_{2max}$ is still considered a pre-requisite to success at elite level (Joyner and Coyle 2008). Given that $\dot{V}O_{2max}$ cannot be sustained beyond 5-10 min (Joyner 1991) and to better explain differences in performance between those of similar $\dot{V}O_{2max}$, emphasis has shifted towards sub-maximal markers such as blood lactate indices and running economy (Burnley and Jones 2007). A simplification of these relationships is shown in Figure 1, providing an interpretation on how the LT and $\dot{V}O_{2max}$ collectively determine running velocity.

Figure 1: Simplification of interplay between determinants of endurance performance to influence distance running performance. Modified from Jones (2006), which extends the previous model of Coyle (1995) by incorporating $\dot{V}O_2$ kinetics and acknowledging the contribution of anaerobic energy provision in determining running performance.

The determinants of endurance performance model demonstrates how an individual's $\dot{V}O_{2max}$ determines the upper limit of aerobic metabolism, beneath which the LT determines the $\dot{V}O_2$ that can be sustained at a given velocity, termed fractional utilisation of $\dot{V}O_{2max}$ (%$\dot{V}O_{2max}$), or performance $\dot{V}O_2$. In one interpretation of this relationship, Londeree (1986) suggests '*a runner's potential is limited by $\dot{V}O_{2max}$, but other factors will determine how close to his potential a runner is at a particular point in time*'. The running speed that can be maintained, and consequently performance velocity, is then determined by how efficiently the corresponding oxidative adenosine triphosphate (ATP) turnover at the performance $\dot{V}O_2$, is converted to locomotion (running economy). An individual's ability to meet changes in exercise intensity, through aerobic energy provision is determined by $\dot{V}O_2$ kinetics. Therefore, this component is most pertinent to events that experience regular changes in intensity. It is important to note however, this model utilises the LTP as a representative marker of the metabolic stress experienced by the muscle, and does not implicate lactate in the process of fatigue above LTP. Trained runners demonstrate the ability to sustain a higher %$\dot{V}O_{2max}$ across an event, relative to untrained individuals (Bassett and Howley 2000).

It has been suggested that $\dot{V}O_{2max}$ is primarily limited by cardiovascular factors, whilst the performance $\dot{V}O_2$ may be more closely linked to peripheral adaptation in the muscles (Holloszy & Coyle 1984). Consequently, larger changes in %$\dot{V}O_{2max}$ than $\dot{V}O_{2max}$ itself are observed across months of endurance training, as demonstrated by Figure 2 from Astrand and Rodhal (1970).

Figure 2: $\dot{V}O_{2max}$ (termed maximal aerobic power) as denoted by top black line, may improve in the first months of training, but larger changes are observed in %$\dot{V}O_{2max}$, denoted by lower black line.

The determinants of endurance performance combine to predict running speed, and have been used by Joyner (1991) to predict a faster world marathon record, based on best-known values for each component, using the following formula.

Equation 1: Calculation of marathon running speed (Joyner 1991).

Running speed = $\dot{V}O_{2max}$ (mL.kg^{-1}.min^{-1}) x %$\dot{V}O_{2max}$ at LT x RE (km.h^{-1} $\dot{V}O_2^{-1}$ [mL.kg^{-1}.min^{-1}])

This formula demonstrates how the determinants integrate to determine the running speed, when confounding individual factors such as motivation, dehydration, hyperthermia and carbohydrate depletion are excluded. More recently, further determinants of endurance performance have been proposed, including the predicted velocity at $\dot{V}O_{2max}$ (v$\dot{V}O_{2max}$) (Billat & Koralsztein 1996), peak treadmill velocity (PTV) (Noakes et al. 1990) and oxygen kinetics (Burnley & Jones 2007), with v$\dot{V}O_{2max}$ the most widely adopted (Jones & Carter 2000; Bassett & Howley 2000). This may reflect the potential for anaerobic energy sources to contribute to PTV (Jones & Carter 2000), as well as oxygen kinetics appearing most relevant to middle distance events such as 800-1500 m, where there is a significant anaerobic contribution (Jones & Carter 2000, Jones 2009).

The use of $\dot{V}O_{2max}$ LT and RE within a model is well supported for explaining endurance performance (Coyle et al. 1988; Coyle et al. 1991; Stratton et al. 2008; Denadai et al. 2004; Jacobs et al. 2011; Joyner & Coyle 2008). In one notable study, McLaughlin et al. (2010) demonstrated that the determinants were able to collectively account for 95.4% of the variation in 16 km running time trial performance. The composite measure of RE and $\dot{V}O_{2max}$, v$\dot{V}O_{2max}$, was the strongest individual predictor explaining 94.4% of the variance, whilst $\dot{V}O_{2max}$ and LT both accounted for ~90% of the variation. A breakdown of these results is shown below in Table 1. Within these data, %$\dot{V}O_{2max}$ was the weakest predictor of performance, to which the authors attributed the homogeneity of intensity within which all participants completed the run (76 – 88 %$\dot{V}O_{2max}$).

Table 1: Pearson product–moment correlations between the determinants of endurance performance and 16 km time trial performance (McLaughlin et al. 2010).

	R
$v\dot{V}O_{2max}$ (m·min^{-1})	−0.972
Velocity at LT (m·min^{-1})	−0.907
$\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1})	−0.902
PTV (m·min^{-1})	−0.892
Velocity at 3 mM LA (m·min^{-1})	−0.887
Velocity at 2 mM LA (m·min^{-1})	−0.854
RE (mL·kg^{-1}·km^{-1}) at 82% $\dot{V}O_{2max}$	0.812
%$\dot{V}O_{2max}$ at LT	0.136

An appropriate model of endurance performance can facilitate assessment of endurance performance in a laboratory, but also allows practitioners and researchers to quantify how training and/or interventions that promote specific and divergent physiological adaptations, influence performance. Moreover, since LT, LTP and $\dot{V}O_{2max}$ can be used as physiological markers to demarcate exercise intensity domains, they represent relevant variables upon which to determine training zones, to facilitate appropriate overload, with regard to training intensity and volume (Davison et al. 2009; Denadai et al. 2004). Finally, the determinants of endurance performance model allows practitioners to predict endurance performance, which may be accurate to as much as 0.2–0.4% of the actual race finishing time (Jones 2009), when appropriate correction factors are adopted (Londeree 1986). In the following section, the determinants of endurance performance from the traditional model will be individually introduced and the respective contribution to endurance running discussed.

2.1.2 $\dot{V}O_{2max}$

Through a series of experiments in the 1920s, the eminent physiologist AV Hill first reported an increase in oxygen consumption, accompanying in a linear fashion, increases in exercise intensity. This relationship was maintained until a plateau was achieved, whereby oxygen consumption ($\dot{V}O_2$) appeared to reach a 'ceiling', despite an increase in exercise intensity (Hill et al. 1924). This was termed 'maximal oxygen intake' and this concept has become embedded within the exercise physiology literature across the last century, with it considered the best measure of the functional limit of the cardiovascular system (Rowell 1974), and a widely used measure of cardiorespiratory fitness (Howley et al. 1995). The standardised nomenclature for this phenomenon has become maximum oxygen uptake ($\dot{V}O_{2max}$); defined as the highest rate at which oxygen can be taken up and utilised by the body during severe exercise (Bassett & Howley 2000). The concept is succinctly encapsulated by the Fick principle ($\dot{V}O_{2max} = CO \times a - \overline{V}O_2$ difference) which states $\dot{V}O_{2max}$ to be the product of cardiac output (CO),

minus the arterio-venous difference. Therein, $\dot{V}O_{2max}$ represents the integrative ability of the cardiovascular system to oxygenate the blood, expedite the delivery of this blood through a high cardiac output and skeletal muscle blood flow, as well as extract oxygen at the muscle. $\dot{V}O_{2max}$ would appear to be predominantly limited by CO, through an inability to deliver sufficient oxygenated blood to the active muscles for aerobic respiration to continue to match exercise intensity, which may account for as much as 70–85% of the limitation in $\dot{V}O_{2max}$ (Cerretelli & Di Prampero 1987). An insufficient CO prevents sarcoplasmic reticulum calcium release (Allen et al. 2007), impairs sodium/potassium pump activity (McKenna et al. 2008), and slows cross-bridge cycling (Fitts 2007). In turn, such localised stress activates muscle afferents leading to cessation of central motor drive and voluntary effort (Amann & Calbet 2008). Therefore, training and interventions that enhance CO or blood oxygen carrying capacity have a direct impact on $\dot{V}O_{2max}$. A classic study by Ekblom et al. (1968) demonstrated an 8% increase in CO (pre 22.4 L.min^{-1}: post 24.2 L.min^{-1}) following 16 weeks of physical training, contributing to a 16% increase in $\dot{V}O_{2max}$ (pre 3.15 L.min^{-1}: post 3.68 L.min^{-1}). Moreover, improvements in $\dot{V}O_{2max}$ within the first months of endurance training primarily arise through an increased stroke volume mediated by an increase in systemic blood flow, with peripheral changes of increased capillary density leading to enhanced oxygen extraction taking longer to present (Ekblom 1968). Thus, it is unsurprising to observe that the larger $\dot{V}O_{2max}$ values in endurance athletes compared with lesser-trained individuals, predominantly arises from a larger end-diastolic volume, due to a greater cardiac compliance and a distensible pericardium (Levine et al. 1991). Trained athletes benefit from rapid diastolic relaxation with vigorous suction (Ferguson et al. 2001), because of a remodelling of the heart which increases the equilibrium volume of the left ventricle; the volume of blood in the heart when transmural filling pressure is 0 mmHg (Yellin et al. 1990). This affords athletes the potential to utilise the Frank-Starling mechanism to produce a large stroke volume and thus CO consistently.

Enhancing the factors that determine oxygen transport within the body has also been shown to increase $\dot{V}O_{2max}$. Consequently, the more pronounced cardiovascular stress arising from high intensity interval training, appears to produce faster and larger adaptations that enhance $\dot{V}O_{2max}$ compared with continuous endurance training (Milanović et al. 2015). This also includes, increasing red blood cell volume through altitude training (Rusko et al. 2004) or blood doping (Gledhill 1985). Similarly, interventions such as heat acclimation that induce hypervolaemia through an increase increased plasma volume (PV) have been shown to increase $\dot{V}O_{2max}$ by helping maintain CO at maximal intensities, as PV expansion is not of a magnitude to elicit haemodilution (Lorenzo et al. 2010). For example, Coyle et al. (1990) demonstrated that

PV expansion by 200-300 ml of 6% dextran caused increases in stroke volume of 10-15%, accompanied by haemodilution of only a 4%, but there was still a significant increase in $\dot{V}O_{2max}$ (4%). Such an observation is also supported by arterial oxygen saturation not being a major limiting factor to $\dot{V}O_{2max}$, therefore modest haemodilution does not elicit a detrimental effect on oxygen delivery (Saltin and Wagner 2006). Whilst cellular oxygen delivery is also dependent upon initial pulmonary diffusion, this rarely becomes a limiting factor as arterial oxygen saturation is maintained around 95% during maximal exertion (Powers et al. 1989). In elite athletes however, a limitation from pulmonary diffusion has been proposed, because of an extremely high CO reducing blood transit time through the lungs (Dempsey & Wagner 1999).

Although central cardiac factors appear to form the predominant limitation to $\dot{V}O_{2max}$, other functional limitations may also contribute. This assertion is supported by Wagner (1992) who suggests no single limitation to $\dot{V}O_{2max}$, rather that every step in the oxygen pathway contributes in an integrated way, and a reduction in the transport capacity of any of the steps will reduce $\dot{V}O_{2max}$. Supplementary limiting factors may include oxygen uptake at the muscle, with Honig et al. (1992) identifying the importance of maintaining an elevated partial pressure of oxygen in the blood, relative to that in the sarcolemma. Furthermore, small adaptations in mitochondrial enzymes (Holloszy & Coyle 1984) as well as the structure and volume of capillary beds (Saltin 1985), which increase muscular blood transit time rather than blood flow, are associated with improved submaximal exercise performance and may contribute to an enhanced $\dot{V}O_{2max}$. Traditionally, the importance of peripheral limitations to $\dot{V}O_{2max}$ may have been overestimated, considering almost all oxygen is extracted from blood perfusing the active skeletal muscles during maximal exercise (Bassett & Howley 2000). Such findings may reflect methodological artefacts of experimental trials, which involved small muscle mass or individual limb exercise, which do not appropriately replicate severe whole body maximal exercise with respect to CO and cardiovascular strain (Richardson & Saltin 1998; Bassett & Howley 2000). Ferretti (2014) has subsequently suggested that the role of peripheral factors may be magnified when the active muscle mass is predominantly formed of smaller muscles, which may help explain such findings. To summarise, evidence indicates that central factors, and specifically maximal CO, predominantly limit $\dot{V}O_{2max}$, rather than peripheral factors such as the ability of muscle mitochondria to consume oxygen (Wagner 1992; Bassett & Howley 2000; Lorenzo et al. 2010).

Endurance runners are characterised by high $\dot{V}O_{2max}$ values, typically between 70-85 mL.kg^{-1} min^{-1} for male athletes (Saltin & Astrand 1967), which may be double that of recreationally active individuals. $\dot{V}O_{2max}$ appears to set the upper limit for energy production for an individual,

but does not necessarily determine the final performance (Bassett & Howley 1997). A high $\dot{V}O_{2max}$ would appear necessary to sustain the rate of ATP production necessary to complete endurance events in a competitive time. Bassett & Howley (2000) suggest that a 2:15 hour marathon time would require a $\dot{V}O_2$ of approximately 60 $mL.kg^{-1}.min^{-1}$ to be sustained, which given that the marathon is typically completed at 80-85% of $\dot{V}O_{2max}$ would necessitate a $\dot{V}O_{2max}$ in the region of 70-75 $mL.kg^{-1}.min^{-1}$. As previously detailed in the model of Bassett & Howley (2000) in Figure 1, the rate of energy production is dependent on the fractional utilisation of $\dot{V}O_{2max}$ that an individual can sustain. Therefore, sub-maximal factors such as lactate turnpoint and running economy may better predict running performance in a group containing individuals with similar $\dot{V}O_{2max}$ values (Coyle 1999).

Due to the reliance of $\dot{V}O_{2max}$ tests on the participant to voluntarily work to maximal effort, objective criteria to validate $\dot{V}O_{2max}$ measurements would seem appropriate, especially where investigations attempt to establish the effects of an intervention, such as a training regime. This situation is exacerbated by the apparent regularity with which a proportion of a population or relevant participant cohort, do not display a plateau in $\dot{V}O_2$ (Edvardsen et al. 2014). Therefore, a range of secondary criteria have been developed and utilised throughout the exercise science literature, pertaining to; the respiratory exchange ratio, post exercise blood lactate concentration, maximum observed heart rate and perceived exertion of the test (Howley et al. 1995; Edvardsen et al. 2014). The use of $\dot{V}O_{2max}$ criteria is not without contention (Poole et al. 2008; Smirmaul et al. 2013), due to the potential for incorrectly accepting or refuting a test as maximal depending upon the criteria adopted (Midgley, McNaughton, Polman, et al. 2007). A more appropriate method of establishing whether a test can be deemed maximal has been suggested to be through the completion of a subsequent verification (Midgley & Carroll 2009), or constant-load test (Poole et al. 2008), above the highest attained intensity during the incremental trial. However, such procedures may not be appropriate for certain experimental designs, such as those under heat stress, where a manipulated variable such as body temperature could influence the results of a verification. Therefore, recent evidence advocates caution when interpreting results using secondary criteria. Following a large analysis of secondary criteria applied across 861 treadmill tests, to mitigate against erroneous classification of maximal tests, Edvardsen et al. (2014) recommend that when secondary criteria should be specific to the population and fitness level, in accordance with the appropriate normative data.

2.1.3 Blood lactate response

The presence and role of lactic acid in human muscle metabolism has been reported and debated since early in the 20th century (Ryffel 1910; Hill et al. 1924). The formation of lactic acid may occur momentarily as a by-product of metabolism following glycolysis, before it dissociates in a reversible reaction into lactate and hydrogen ions (Robergs et al. 2004). This follows an increased energetic contribution from glycolysis, arising from an inability of the rate of fat oxidation to meet the ATP demands of contracting muscles, as exercise intensity increases. Concomitantly carbohydrate, rather than fat, becomes the primary fuel source, typically at relatively modest exercise intensities, around 40% of $\dot{V}O_{2max}$ (Holloszy & Kohrt 1996). Glycolysis occurs in the cell cytoplasm, and produces the intermediate metabolite, pyruvic acid, with the rate of energy requirement determining whether this is converted into acetyl coenzyme A by the enzyme pyruvate dehydrogenase, for complete oxidative breakdown of the glucose molecule in the mitochondria. Alternatively, when the energy demand cannot be met by the energy yield from the Krebs cycle and electron transport chain, pyruvic acid converts into lactic acid, facilitated by lactate dehydrogenase. Being a relatively weak acid, lactic acid promptly dissociates due to the aqueous solution, releasing hydrogen ions, and subsequently combines with sodium or potassium ions to produce the base lactate. In turn lactate can be used as a fuel source within the liver, heart, brain and slow twitch muscle fibres via shuttling of oxidisable substrate from areas of glycogenolysis to areas of high cellular respiration, as well as sustain blood glucose levels through the Cori cycle (Brooks 1986; Brooks 2009).

Glycolysis is an ongoing process, with the relative contribution increasing with the metabolic rate in accordance with exercise intensity, but not necessarily to oxygen availability (Gladden 2000; Robergs et al. 2004). Therefore, when exercise intensity is relatively low, energy provision may be met predominantly through aerobic metabolism, resulting in a smaller production of lactate, matched by removal. As intensity increases, energy production derived from pyruvate delivered to the mitochondria does not meet the energy requirement, resulting in an increased contribution of glycolysis and associated lactate and hydrogen ion accumulation (Hermansen & Stensvold 1972; Holloszy & Coyle 1984; Robergs et al. 2004). The release of hydrogen ions contributes to a lowered pH in the muscle, which coincides with metabolic acidosis and the onset of fatigue (Robergs et al. 2004). Acidosis is thought to impair muscle filament movement by interfering with contraction coupling between actin and myosin filaments (Robergs et al. 2004). An increase in muscle lactate concentration is reflected in the circulating blood lactate concentration, with which it is highly correlated, although a consistent difference remains (Ivy et al. 1980). This reflects that a negative gradient is necessary for lactate to transfer to the blood, aided by lactate being directly removed from the blood at the

liver, and that the blood lactate level is dependent on body water status (Jacobs 1986). Consequently, blood lactate concentration represents the balance between lactate production in the muscle and utilisation, or conversion, to pyruvate in the liver.

Blood lactate, as sampled from whole blood in the capillary, appears to be a valid surrogate measure of the ability of an individual to yield energy from carbohydrate, once the metabolic rate has exceeded what can be supplied through lipolysis (Bentley et al. 2007). The blood lactate response to incremental exercise permits identification of the corresponding intensities that elicit changes in an individual's metabolic function (Bentley et al. 2007). The point at which blood lactate level rises above steady-state and demonstrates a curvilinear response, demarcates an intensity where an increased contribution from glycolysis is required (Wasserman et al. 1985) and therefore the onset of metabolic acidosis, as the energy demand exceeds that provided by aerobic metabolism (Beaver et al. 1985). Although a multitude of names have been ascribed to this event, the common nomenclature is to term this the lactate threshold (Jones 1998, Saunders & Green 2013). Exercise above this threshold is associated with an increased respiratory, perceptual and metabolic strain (Jones & Carter 2000). As intensity continues to increase, similarly the contribution of glycolysis increases, resulting in an exponential increase in the muscle and circulating lactate concentration (Beaver et al. 1985). The exercise intensity at which an exponential increase begins, as identified on a blood lactate versus exercise intensity plot, has been termed the lactate turnpoint (Jones 1998, Saunders & Green 2013). This event has also been termed the onset of blood lactate accumulation (OBLA) due to the association of this with a blood lactate concentration of 4 $mMol^{-1}$, although this index is now more widely used as a training marker (Heck et al. 1985). Figures demonstrating these methods are shown below (Sections 2.1.3.1 - 2.1.3.3). Exercise above these thresholds is associated with expedited fatigue, through metabolic acidosis (Sahlin 1992), or an accelerated depletion of muscle glycogen (Boyd et al. 1974), which is why the lactate turnpoint represents a fractional utilisation of the maximum oxidative capacity that can be maintained during exercise (Bassett & Howley, 2000).

Considerable debate surrounds the validity of lactate threshold concepts, with Faude et al. (2009) suggesting these thresholds represent a continuous transition, and therefore the term 'threshold' may be misleading. Consequently, Faude et al. (2009) describes an aerobic-anaerobic transition, using the terms lactate threshold aerobic and lactate threshold anaerobic to represent the lactate threshold and lactate-turn point respectively, within this transition framework. Despite discrepancies concerning the nomenclature, it is apparent that such markers are useful for tracking exercise performance as the oxidative capacity of skeletal

muscle appears to determine the absolute level of the lactate thresholds and therefore, the sustainable exercise intensity (Kindermann et al. 1979, Holloszy & Coyle, 1984). These thresholds therefore, have been shown to demonstrate strong associations with endurance performance (Heck et al. 1985; Allen et al. 1985; Billat 1996). The lactate threshold appears to be a strong predictor of marathon performance (r = 0.88 – 0.93, Roecker et al. 1998), whilst the lactate turnpoint is a strong predictor of shorter endurance events (r = 0.89, Lorenzo et al. 2011).

A rightward shift of the lactate curve, and thresholds, whereby the blood lactate concentration is lower for a given exercise intensity, is considered to be an important adaptation representing enhanced endurance capacity, as it increases the fractional utilisation of $\dot{V}O_{2max}$ (Jones & Carter 2000). Such changes may occur from reduced lactate production owing to a reduced rate of glycogen utilisation, improved removal of blood lactate, or improved oxygen uptake kinetics than necessitates a reduced reliance on anaerobic metabolism as exercise intensity changes (Burnley & Jones 2007). In addition to being valid predictors of endurance performance, the importance of the lactate thresholds is heightened as they appear extremely sensitive to change following endurance training (Carter et al. 1999). Literature suggests the ability of skeletal muscle to oxidize pyruvate can double with training (Holloszy & Coyle, 1984). Consequently, interpreted alone, lactate thresholds represent a surrogate measure of an individual's metabolic status, which can be used to predict performance, asses training effects, demarcate training intensity zones and therefore, aid training prescription and monitoring. Interpreted alongside other determinants of endurance performance such as $\dot{V}O_{2max}$ and running economy, the lactate turnpoint will determine the percentage of $\dot{V}O_{2max}$ that can be sustained, and therefore the mean pace during a race.

Despite the apparent prominence of lactate thresholds in determining endurance performance, the precise methods through which to identify these thresholds remains contentious. There are three broad approaches that will be introduced; (1) fixed blood lactate concentrations, (2) individualised lactate thresholds and (3) maximal lactate steady-state.

2.1.3.1 Fixed Blood Lactate Concentrations

The respective exercise intensities that first elicit an increase in blood lactate concentration above steady-state levels, as well as the point at which an exponential increase occurs, can be identified by examining fixed blood lactate concentrations (FBLC) that are traditionally associated with these metabolic transitions. For example, through interpolation from visual plots of exercise intensity versus blood lactate, as illustrated in Figure 3, the first lactate

threshold may be calculated based on the intensity corresponding to 2 mMol.L^{-1} (Kindermann et al. 1979; Saunders & Green 2013), 2.2 mMol.L^{-1} (LaFontaine et al. 1981), 2.5 mMol.L^{-1} (Allen et al. 1985) or 3 mMol.L^{-1} (Borch et al. 1993). Most notably, a 4 mMol.L^{-1} concentration, termed the Onset of Blood Lactate Accumulation (OBLA), has been used to represent the maximal lactate steady-state and therefore also, the lactate turnpoint. Theoretically therefore, a blood lactate concentration of 4 mMol.L^{-1} represents the point at which an exponential increase in blood lactate accumulation follows (Sjödin et al. 1982; Heck et al. 1985; Weltman et al. 1990).

Figure 3: Hypothetical lactate threshold and lactate turnpoint identified in accordance with the recommendations of Saunders and Green (2013). Fixed blood lactate concentrations afford greater precision (<1 km.h^{-1}) than alternative methods.

Examining FBLC affords a simple and objective assessment (Davis et al. 2007), which also benefits from greater precision than alternative methods, which may be bound by the stage increments of the test, such as the 1 km.h^{-1} differences in running speeds as shown in Figure 3. At both amateur and elite levels, differences of less than 1 km.h^{-1} may be considered meaningful across a prolonged race (Hopkins & Hewson 2001; Tyler et al. 2015). Furthermore, this method does not discriminate between different protocols or the number of stages to develop a lactate curve, which typically varies between 6-8 stages (Spurway & Jones 2007). However, FBLC are strongly influenced by an athlete's nutritional and recovery status (Carter et al. 1999; Yoshida 1984), which may invalidate results if not appropriately controlled for, or if markedly different exercise protocols are used (Faude et al. 2009). Moreover, for aerobically trained athletes the 4 mMol.L^{-1} threshold may overestimate endurance capacity, or underestimate in anaerobically trained individuals (Stegmann et al. 1981; Stegmann & Kindermann 1982).

2.1.3.2 Individualised Lactate Thresholds

In order to mitigate against the influence of biological variation on FBLCs, Stegmann et al. (1981) proposed a complex model of individualised lactate thresholds. Based on the incremental exercise and recovery blood lactate response, a diffusion-elimination model was developed, from which the maximal rate of lactate elimination, individual anaerobic threshold, gradient between muscle and blood, muscle volume working above the threshold and whole body lactate could be obtained. However, underpinning assumptions of this model have since been questioned (Gladden 2000) and researchers have continued to seek simpler and more accurate methods, often based upon visual inspection of the lactate curve, alongside interpretation boundaries (Holloszy & Coyle 1984; Spurway & Jones 2007). For example, Spurway and Jones advocate the exercise intensity that elicits a 1 mMol.L^{-1} increase above steady-state levels to denote the lactate threshold, and the intensity that elicits a '*sudden and sustained increase*' as the lactate turnpoint, as shown in Figure 4.

Figure 4: Hypothetical lactate threshold and lactate turnpoint identified in accordance with the recommendations of Spurway and Jones (2007).

Such an approach is simple to adopt, but may be susceptible to fluctuation during the initial stages of an exercise test, as well as discrepancies between testers, particularly when some individuals demonstrate a curvilinear lactate curve, without discernible breakpoints (Yeh et al. 1983). Furthermore, unlike FBLC, individualised thresholds are typically only precise to the increment of the stage i.e. 1 km.h^{-1}, as shown in Figure 4.

To try to maintain an individualised approach, but yield an objective measure, Cheng et al. (1992) introduced the Dmax method, where the lactate threshold was denoted by the maximal perpendicular distance of the lactate curve from a line connecting the first and last stages of the curve. Despite early promise, Dmax may be liable to bias from the test starting speed, as

well as number of steady-state and higher intensity stages completed. To eliminate this bias, Bishop et al. (1998) introduced a modified Dmax, focussed primarily on the lactate turnpoint, whereby a line was drawn from first lactate threshold to the endpoint of lactate curve, with lactate turnpoint identified by the largest perpendicular distance. This approach has been shown to be highly correlated (r=0.84) with 1 hour TT performance in trained cyclists (Bishop et al. 1998), and the best predictor 10 km running performance in recreational runners (Nicholson & Sleivert 2001).

2.1.3.3 Maximal Lactate Steady State (MLSS)

The MLSS is the highest exercise intensity that can be maintained without a continual increase in blood lactate concentration (Beneke 2003), and therefore represents the highest exercise intensity that results in an equilibrium between lactate production and lactate elimination (Baron et al. 2008). Practically therefore, MLSS demarcates the upper limit for sustainable exercise intensity, above which leads to an accumulation of fatiguing metabolic by-products (Beneke 2003). This marker occurs at a similar exercise intensity to the LTP, but appears more ecologically valid for endurance exercise due to the need for repeated, prolonged periods of exercise at near to steady-state intensities, which closely replicates endurance events (Spurway & Jones 2007; Faude et al. 2009). Indeed, Jones and Carter (2000) suggest MLSS to be the best measure of endurance capacity, further evidenced by the high correlations with 5 and 8 km running reported by Haverty et al. (1988, $r = 0.87$) and Jones and Doust (1998, $r = 0.92$), respectively. However, the protocol to determine MLSS is inefficient, requiring multiple visits on different days, of at least 30 min. The protocol requires each test to be completed at a different steady-state intensity, typically within the range of 50-90% $\dot{V}O_{2max}$, until an increase in blood lactate of no more than 1 mMol.L^{-1} between 10-30 min is observed as shown below in Figure 5. The MLSS is identified as the highest exercise intensity that elicits a steady-state blood lactate response (<1 mMol.L^{-1}) between 10-30 min.

Figure 5: Theoretical representation of determining the maximum lactate steady state. Adapted from Faude et al. (2009). Athlete completes five treadmill runs at different running speeds on different days. MLSS is the speed that elicits a delayed but elevated steady-state blood lactate concentration.

Despite the apparent efficacy of MLSS for assessing endurance performance and affording the opportunity for simultaneous measurement of running economy, the number of repeated trials required precludes the widespread use of MLSS. Furthermore, the LTP derived from a traditional GXT has also been shown to be highly correlated with MLSS (Jones and Doust, 1998) and may be completed as part of a battery of tests in order to yield additional measures.

This section has summarised a variety of methods for estimating the same physiological markers. Whilst all methods appear to have advantages and disadvantages, when confounding influences of diet and prior exercise are controlled for (Yoshida et al. 1984), the exercise intensities at FBLC appear to afford the most objective and precise assessments of the lactate thresholds. Such calculations remove potential experimenter bias and the need for duplicate reviews of tests, whilst quantifying potentially meaningful differences that may be smaller than the stage increment. Finally, FBLC can be determined independently of the number of stages completed, which may be an important consideration in research when a participant cohort of heterogeneous performance level is used.

2.1.4   Running Economy

Running economy represents the metabolic energy required to cover a given distance and is traditionally expressed as the oxygen uptake ($\dot{V}O_2$) required to run at a given velocity (mL.kg⁻

$^{-1}$.min^{-1}), or as a mean across a number of velocities (mL.kg^{-1}.km^{-1}) (Bassett & Howley 2000). The $\dot{V}O_2$ at 16 km.h^{-1} (6 min per mile, 3 min 44 s per km) is the most common reference velocity, particularly amongst highly trained individuals, although velocities from 12 to 21 km.h^{-1} are observed across the literature (Daniels & Daniels 1992; Joyner 1991; Morgan et al. 1991; Pollock 1977; Conley & Krahenbuhl 1980; Saunders et al. 2004b). Mean running economy (RE) may be estimated from a range of running velocities by measuring the steady state $\dot{V}O_2$ during submaximal running, as shown below in Equation 2.

Equation 2: Calculation of mean running economy.

$$RE= \frac{\dot{V}O_2}{(S/60)} \quad mL\ O_2\ kg.^{-1}\ km.^{-1}$$

Where, $\dot{V}O_2$ is oxygen consumption in mL O_2 kg.$^{-1}$ min.$^{-1}$, and 'S' is velocity in km.h^{-1}, multiplied by 60 to convert km.h^{-1} to km.min^{-1} (Jones 2006a; Saunders & Green 2013).

At an elite level, a running economy value around 200 mL.kg.$^{-1}$ km.$^{-1}$ may be considered typical, with values above and below this value representing poor and good economy, respectively (Jones 2006b). An economical runner demonstrates a low $\dot{V}O_2$ for a given velocity, utilising a lower percentage of the $\dot{V}O_{2max}$ at that velocity, consequently preserving muscle glycogen and likely necessitating a lower anaerobic metabolism contribution, therein reducing metabolic acidosis (Saunders et al. 2004a; Jones 2006b). Due to the presence of the $\dot{V}O_2$ slow component, whereby $\dot{V}O_2$ may not reach a steady-state at higher exercise intensities, RE should be measured across velocities below an individual's LTP (Jones et al. 2003). The necessity for steady-state $\dot{V}O_2$ measurements also determines that stage increments must be of at least 3 min to account for $\dot{V}O_2$ kinetics (Burnley & Jones 2007), whilst a 1% gradient on a treadmill may be appropriate to compensate for the lack of air resistance and gait alterations when using a treadmill, compared with running outdoors (Jones & Doust 1996). Despite widespread use, $\dot{V}O_2$ measurement may be insensitive to subtle training improvements in fuel utilisation, compared with total energy turnover (Shaw et al. 2014). Notwithstanding this limitation, the traditional approach has shown great efficacy in quantifying improved running performance (Coyle 1999) and forms a critical component of an established model for predicting endurance running performance (Ingham et al. 2008; Di Prampero et al. 1993; Bassett & Howley 2000).

As RE represents the transfer of chemical energy into locomotion, it is influenced by both biomechanical and physiological variables, as shown in Figure 6. From a biomechanical perspective, body mass, anthropometry (i.e. muscle mass), flexibility (i.e. muscular elasticity),

and gait (i.e. stride length) are major contributing factors. From a physiological perspective, both muscle fibre type and temperature may influence $\dot{V}O_2$, as well as cardiovascular fitness, specifically pertaining to mitochondrial density, haemoglobin mass, aerobic enzyme activity, capillarisation and blood volume (Saunders et al. 2004b). With so many contributing factors, there is considerable individual variability in RE, with literature suggesting RE varies amongst runners of similar $\dot{V}O_{2max}$ by 30% (Daniels 1985). Broadly however, higher standard runners demonstrate a better RE than untrained runners (Pollock 1977), whilst long distance runners appear more economical than middle-distance runners at sub-maximal running speeds (Daniels & Daniels 1992), which may be a consequence of the larger training volumes typically completed by these athletes.

Figure 6: Factors that could determine RE (Saunders et al. 2004b).

Running economy appears to distinguish between runners of similar $\dot{V}O_{2max}$. Conley and Krahenbuhl (1980) identified a relatively strong correlation (r = 0.82) between running economy and performance in a 10 km run, with runners of similar $\dot{V}O_{2max}$, results which have been supported elsewhere (Costill et al. 1973; Morgan et al. 1989). This relationship is likely underpinned by subtle individual differences in the linear relationship between submaximal running velocity and $\dot{V}O_{2max}$. Individuals with a low RE will also benefit from a reduced metabolic heat production contributing towards body heat storage, an effect that will be especially important in hot and humid conditions (Jones 2006b). Expressing RE relative to

$\dot{V}O_{2max}$ appears to magnify differences in RE (Pollock 1977). More recently, the relative dominance of East African runners has been attributed to a markedly enhanced RE relative to their competitors, differences which were partially attributable to reduced body mass within this cohort (Lucia et al. 2006). Indeed, a relatively well-known case study containing data tracking the development of the Women's marathon world record holder, from the age of 18-29, highlights the importance of the relationship between RE and running performance (Jones 2006b). Performance improvements were observed in the absence of a consistent and observable change in $\dot{V}O_{2max}$, maintained around 70 mL.kg^{-1}.min^{-1}, or body mass (~ 54 kg^{-1}), but a 15% improvement in RE ($\dot{V}O_2$ at 16 km.h^{-1} in 1998; 205 mL kg^{-1}.km^{-1}, 2003; 175 mL.kg^{-1}.km^{-1}).

As highlighted by the multitude of contributing factors in Figure 6, interventions to improve RE are constantly sought by coaches and sport scientists alike. It has been postulated that chronic endurance training over many years may result in the transformation of type II fibres into type I fibres, which could have meaningful effects on RE given type I fibres consume less O_2 for a specific amount of muscular work (Coyle et al. 1992; Bottinelli & Reggiani 2000). Indeed, following endurance training, type II fibres take on many of the properties of type I fibres, including reduced myosin ATPase activity, increased mitochondrial density and oxidative enzyme activity, as well as greater capillarisation (Taylor & Bachman 1999). Changes that may occur alongside, or independently of, chronic endurance training, include changes in running gait, body mass and/or composition that also likely contribute to alterations in RE (Williams & Cavanagh 1987). Evidence also suggests strength and plyometric training may benefit RE, through improvements in strength and motor unit recruitment (Rønnestad & Mujika 2014). These training practices may also contribute towards the development of a 'stiffer' musculoskeletal unit that utilises more elastic energy during the stretch-shortening cycle for propulsion and thus improves mechanical efficiency (Saunders et al. 2004a). Accordingly, exposure to hypoxia through altitude training would also appear an appropriate intervention for RE, by provoking adaptations commensurate with oxygen delivery and uptake (Green et al. 2000). Ultimately, any intervention that can reduce $\dot{V}O_2$ across a range of running velocities will allow a runner to run faster over a given distance, or longer at a constant velocity.

2.1.5 Velocity at $\dot{V}O_{2max}$

The interaction between $\dot{V}O_{2max}$ and RE, and the consequential effect on running performance, is succinctly illustrated by the extrapolation of $\dot{V}O_2$ from submaximal intensities.

As shown in Figure 7, for two athletes of similar $\dot{V}O_{2max}$, the athlete with better economy (triangles) will be running at a greater velocity[3], as they approach maximal intensities.

Figure 7 :The athlete denoted by grey triangles demonstrates a higher running speed when $\dot{V}O_2$ is extrapolated to maximal intensity (Jones 2006b).

Consequently, the estimation of the running velocity associated with $\dot{V}O_{2max}$, termed $v\dot{V}O_{2max}$, provides a simple performance metric, which mitigates against the interpretation difficulties coaches and athletes may experience when $\dot{V}O_{2max}$ is expressed in units of $mL.kg^{-1}$ min^{-1}. An improvement in $v\dot{V}O_{2max}$ enables exercise at a given absolute, or relative intensity, to be performed at higher running speeds, which is important considering athletes operate at similar percentages of $\dot{V}O_{2max}$ within certain events (Leger et al. 1986). Daniels (1985) first advocated extrapolating the regression line of an individual's running velocity and $\dot{V}O_2$ relationship to the measured $\dot{V}O_{2max}$. Morgan et al. (1989) modified the approach of Daniels (1985), utilising an individual's mean RE and reported a strong relationship between $v\dot{V}O_{2max}$ and 10 km performance (r = -0.87), which provided similar predictive power as the running velocity at 4 $mMol.L^{-1}$ (r = -0.82). However, Morgan et al. (1989) highlighted a limitation of this estimate, as the accurate determination of RE may depend upon repeated trials to account for the reliability of measuring oxygen consumption. Notwithstanding, the strength of this relationship has subsequently been widely supported, with Jones and Doust (1998) demonstrating that $v\dot{V}O_{2max}$ correlated more strongly with 8 km running performance (r = 0.93)

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[3] Velocity is a vector quantity. This requires that direction, as well as magnitude, is stated or strongly implied. Conversely, 'speed' is a scalar quantity that indicates magnitude, but not direction (Winter & Fowler 2009). In accordance with recent recommendations (Winter & Fowler 2009), velocity will be predominantly used within this thesis when describing running performance, unless it is explicit that the performance refers to a treadmill protocol.

than other physiological measures, including $\dot{V}O_{2max}$ (r = 0.69) and RE (r = −0.16). Theoretically, the calculation of $v\dot{V}O_{2max}$ is most pertinent for events completed at, or around $\dot{V}O_{2max}$, such as the 3000 m. However, $v\dot{V}O_{2max}$ is also well supported for longer events, with McLaughlin et al. (2010) suggesting it to be the largest individual predictor of endurance performance, accounting for 94.4% of the variance in 16 km time trial performance.

Given RE is derived from steady-state $\dot{V}O_2$ measurements, hypothetically $v\dot{V}O_{2max}$ provides an estimate of the maximal velocity that can be maintained by oxidative phosphorylation. This value may be lower than the maximum running speed observed during a speed-incremented test, such as the peak treadmill velocity attained for 1 min (PTV), as the calculation of $v\dot{V}O_{2max}$ is independent of an anaerobic energy contribution (Noakes et al. 1990). Therefore, despite the intuitive similarity of $v\dot{V}O_{2max}$ and the PTV, a distinct difference between these measures should be acknowledged, given the potential for anaerobic capacity, muscle power and neuromuscular skill to contribute to the final performance, variables which will have a progressively smaller influence as event distance and duration increase (Jones & Carter 2000). Therefore, although PTV is highly correlated with endurance performance (r = - 0.88 - -0.94, Noakes et al. 1990), it may not be as good a marker as $v\dot{V}O_{2max}$ during prolonged endurance exercise, as shown by McLaughlin et al. (2010) during a 16 km time trial (PTV r = -0.892, $v\dot{V}O_{2max}$ r = -0.972). Furthermore, $v\dot{V}O_{2max}$ is widely applicable, given the ability to derive this measure from both speed and gradient incremented tests, whereby mean RE is utilised to represent the relationship between submaximal $\dot{V}O_2$ and running velocity for gradient protocols (Jones 1998; Jones 2006b). Therefore, for an athlete with a running economy of 210 mL.kg^{-1}.km^{-1} and a $\dot{V}O_{2max}$ of 70 mL.kg^{-1}.min^{-1}, $v\dot{V}O_{2max}$ would be 20 km.h^{-1} (70 x 60 / 210). In turn, this velocity may also represent an optimal individualised training pace to improve $\dot{V}O_{2max}$, as it theoretically describes the slowest speed which elicits $\dot{V}O_{2max}$ (Billat et al. 1999) and therefore, is the intensity that can be sustained for the longest possible duration while eliciting $\dot{V}O_{2max}$. In conclusion, $v\dot{V}O_{2max}$ is a widely supported performance estimation derived from two of the primary physiological determinants of performance that appears appropriate for use across a range of distances within endurance events.

2.1.6 Assessment of endurance exercise

Endurance athletes are routinely assessed in the laboratory to establish training status or highlight weaknesses for future training, with testing procedures also an effective tool for talent identification in endurance sports (Joyner & Coyle 2008; Jones 2006a). In accordance with the previously described model, a graded exercise test (GXT) can provide a measure of

each determinant of endurance performance. Many variations of GXT exist, across a range of endurance sports including running (Jones 1998), cycling (Hopkins & McKenzie 1994) and rowing (Ingham et al. 2013), whereby practitioners can manipulate stage duration and increment size to reflect the relevant event better. Thus, for an endurance runner, a generalised aim of the GXT is to assess blood lactate concentration, running economy and $\dot{V}O_{2max}$ while increasing exercise intensity until volitional exhaustion (Bentley et al. 2007).

Treadmill-based GXTs are considered highly reliable (Grant et al. 2002), with strong Pearson's correlation coefficients for LT (r = 0.89) and the velocity at the onset blood lactate accumulation (OBLA), demarked by 4 mMol.L^{-1} (r = 0.95), originally reported by Weltman et al. (1990). These results, and the use of three minute stages, have since been supported by Pfitzinger & Freedson (1998), who report very strong intraclass correlation coefficients (ICC) for LT (ICC = 0.98) and OBLA (ICC = 0.99) and Grant et al. (2002) (LT; r = 0.94, OBLA; r = 0.93). In a meta-analysis, Hopkins et al. (2001) stated the coefficient of variation (CV) for speed at LT to be 1.5%. $\dot{V}O_{2max}$ assessment during GXT also appears highly reliable, where a CV of 0.9% and standard error of the estimate of 0.24 l min^{-1} have been reported (Weltman et al. 1990). However, a more conservative estimate would indicate the typical error of $\dot{V}O_{2max}$ to be closer to 2% (Tanner & Gore 2013). Similarly, the sub-maximal assessment of running economy during a GXT appears to be highly reliable, with a typical error of 1.3 mL.kg^{-1}min^{-1} reported in trained runners (Saunders et al. 2004b). Table 2 displays published accepted levels of error for each of the determinants of endurance performance derived from a GXT through a range of absolute and relative reliability statistics.

Table 2: Reliability and sensitivity of the determinants of endurance performance. Data are from experiments conducted in predominantly temperate environments. Columns from left to right; respective determinant of endurance performance, a battery of relative and absolute reliability statistics that have previously been considered acceptable levels of error, previously observed change in the measure and whether this exceeds the typical variation (sufficient

Outcome	RELIABILITY MEASURES		SENSITIVITY	Δ for sig diff	Meaningful Δ

signal:noise ratio), typical change in the mean to elucidate a statistically significant difference ($p<0.05$) and the magnitude of change accepted as meaningful.

measure	Δ mean (±)	Pearson (r)	ICC	TEM (±SI unit)	TEM (±CV%)	LOA (±)	Typical Δ from intervention	S >N?	(SI unit)	
LT	<0.29 km.h^{-1} [5]	>0.89 [3]	>0.98 [4]	<0.2 mMol.L^{-1} [1, 2]	<10% [1]	0.47- 1.05 km.h^{-1} [5]	Aerobic training +1 km.h^{-1} [13]	Yes	0.96 km.h^{-1} [3]	>0.2 mMol.L^{-1} [2]
LTP	<0.33 km.h^{-1} [5]	>0.95 [3]	>0.99 [4]	<0.5 mMol.L^{-1} [2]	<3-6% [2]	0.85 -1.50 km.h^{-1} [5]	Aerobic training +1 km.h^{-1} [13]	Yes	1.18 km.h^{-1} [3]	>0.5 mMol.L^{-1} [2]
RE	<0.09 L.min^{-1} [8]	>0.95 [14]	>0.90 [8]	<0.09 L.min^{-1} [12] <1.3 mL.kg^{-1}min^{-1} [12]	<2.4% [12] 1.5-5% [7]	1.02-1.03 L.min^{-1} [3]	Altitude training +2.9% [9]	Moderate	2.8 mL.kg^{-1}min^{-1} [12]	>2.4% [12] (elite)
V̇O$_{2max}$	<0.04 L.min^{-1} [3]	>0.94 [10]	>0.97 [10]	<0.15 L.min^{-1} [2]	<2% [2]	-	Interval training +5% [11]	Yes	2 mL.kg^{-1}min^{-1} [15]	>0.15 L.min^{-1} [2]
vV̇O$_{2max}$	<0.5 km.h^{-1} [6]	>0.90 [6]	-	<14% [6]	<2.4% [2]	-	Interval training +0.6 km.h^{-1} [11]	Moderate	-	±0.5 km.h^{-1} [6]

Legend to Table 2; [1] Spurway & Jones 2007, [2] Tanner & Gore 2013, [3] Weltman et al. 1990, [4] Pfitzinger & Freedson 1998,-[5] Grant et al. 2002, [6] Billat & Koralsztein 1996, [7] Saunders et al. 2004a, [8] Doma et al. 2012, [9] Katayama et al. 2004, [10] Rivera-Brown et al. 1995, [11] Billat et al. 1999, [12] Saunders et al. 2004b, [13] Jones & Doust 1998, [14] Morgan et al. 1991, [15] Cunningham et al. 1977.

While the predominant measures taken during a GXT consist of physiological responses, there remains the potential for some measures of performance to be measured directly, or estimated. Depending upon the protocol chosen, the peak treadmill velocity may be recorded when an incremental speed protocol is used (Noakes et al. 1990), or estimate $v\dot{V}O_{2max}$ from the mean RE (Morgan et al. 1989). As such, $\dot{V}O_{2max}$ can be measured during an incremental gradient test, which may be more appropriate when testing runners not accustomed to running at maximal speeds, such as cross country runners, as well as minimising health and safety risks in the laboratory, when a treadmill safety harness is not available. $\dot{V}O_{2max}$ appears comparable (Kasch et al. 1976; McConnell & Clark 1988; Balducci et al. 2016), or slightly elevated (Morgan et al. 1989a), when adopting an incremental gradient rather than incremental speed protocol. Thus, during a GXT, at least one, if not both PTV and $v\dot{V}O_{2max}$ can be yielded, both of which may have a CV as low as 0.9% (Hopkins et al. 2001).

Laboratory investigations of endurance performance *per se*, typically adopt either self-paced time trials (TT), fixed-intensity time to exhaustion trial (TTE) or pre-loaded time trials (Currell & Jeukendrup 2008). Free-paced time trial protocols (TT) have been suggested as the most ecologically valid approach and display greater reliability than TTE, with the coefficient of variation (CV) below 5%, compared with 10-26% for TTE (Laursen et al. 2007; Currell & Jeukendrup 2008; Jeukendrup & Saris 1996). Furthermore, Currell and Jeukendrup (2008) argue *'pacing strategy is an inherent component of real performance rather than something that should be omitted from a performance test'*. This advocates avoiding fixed intensity protocols if the efficacy of an intervention is to be truly tested. However, the variation of exercise intensity that occurs during a TT as a consequence of free-pacing can impede interpretation of the physiological effects, and therefore the understanding of an intervention which can be achieved during a steady state TTE. This has led to calls for multiple TTE trials to be used as TT performance predictors (Hinckson & Hopkins 2005).

Consequently, the type of exercise test adopted remains a contentious issue. Schlader, Stannard, et al. (2011a) have suggested as a consequence of these differing experimental approaches the seemingly opposed theories of fatigue concerning endurance performance may have given undue prominence to measuring a different combination of physiological processes. For example, a TTE trial completed at a fixed intensity examines the participant's ability to exercise to exhaustion may emphasize physiological failures at the level of either the muscle (peripheral) or brain (central). This characterises fatigue as an 'all or nothing' phenomenon (Tucker 2008), despite most sporting events evidencing a level of fatigue and necessitating individuals pace themselves, slowing down to complete the event and avoid task failure. Thus,

a TT may better examine the regulation of exercise intensity and on-going fatigue, in accordance with the anticipatory regulation model of fatigue (Marino 2004). Such a contrast remains when considering laboratory endurance performance in hot environments, where exhaustion during TTE trials was associated with a T_{CORE} exceeding 40°C, providing the basis for the critical core temperature model (González-Alonso et al. 1999). However, this phenomenon is not supported by observations in the field during free-paced time trials, where T_{CORE} has been widely reported to exceed 40°C (Racinais et al. 2015b; Ely et al. 2009).

In light of these limitations, Jeukendrup & Saris (1996) attempted to combine these perspectives by developing a pre-loaded time trial, where a period of fixed-intensity work precedes a self-paced TT. They reported comparatively high reliability with a CV of 3.4% with respect to TT (3.4%) and TTE (26%) within their cohort. A pre-loaded time trial permits the assessment of the physiological response during the fixed intensity work before completing a more ecologically valid TT. This approach has been suggested to reflect long distance events in track athletics, which involve running as a group for the majority of a race before increasing pace in the final laps (Jeukendrup & Saris 1996). However, this typically only permits assessment at one prior speed during the pre-loaded part of the test and participants may complete a TT of an unusual length, once a pre-loaded period has been added to, or removed, from the total distance.

Despite the apparent greater inter-trial variation, a TTE may hold the same sensitivity for discerning differences between trials (Laursen et al. 2007, Amann et al. 2008). Currell & Jeukendrup (2008) have proposed a signal-to-noise ratio to assess sensitivity, where the effect size of any change in exercise performance following an intervention (signal) should be compared against the variation associated with the protocol used (noise). Thus the change in performance can be divided by the error of measurement. To illustrate, an ice slurry drink eliciting a 19% improvement in TTE (Siegel et al. 2010), where the CV may be 26% (Jeukendrup and Saris, 1996), may elicit a similar magnitude of change as a 1.3% improvement following ice slurry ingestion in a TT (Ross et al. 2011) where CV is 5% (Currell & Jeukendrup, 2008). Thus, whilst the CV for TT is generally low, so too appear changes in performance. Equally, whilst the CV for TTE is generally larger, so are observed changes (Laursen et al. 2007). This may be because small changes in exercise intensity result in larger changes in time-to-exhaustion tests (Hopkins et al. 2001).

Investigations pertaining to endurance performance under heat stress have primarily adopted TT and TTE trials to try to maximise external validity of investigations (Casa 1999;

Maughan & Shirreffs 2004). However, despite not allowing free-pacing, a GXT would still appear to provide meaningful information on exercise capability due to the determinants of endurance performance remaining valid markers of performance in hot and cold environments (Lorenzo et al. 2011). Moreover, the yield of measures from a GXT appear to permit a more holistic assessment of the physiological response during exercise than TT, pre-loaded time trial and TTE trials, as well as providing a valid and reliable estimated performance measurement in $v\dot{V}O_{2max}$.

2.2   Human thermoregulation, heat stress and heat strain

2.2.1   Heat exchange

Before considering how heat stress pertains to a decrement within the physiology of endurance performance, the principles of heat exchange and thermoregulation will be introduced. A measured temperature represents the mean kinetic energy of the movement of atoms within a substance (Parsons 2014). Within humans the mean measured temperature in the core of the body is 37±1°C, as humans are homeothermic mammals that maintain an internal body temperature (Cheung 2010). In the morning upon awakening, this is typically around 36.7°C, with temperature increasing during the day, potentially by 0.8°C in the evening, and declining again until the morning due to the circadian rhythm (Wenzel & Piekarsky 1984).

The resting body temperature principally arises as a net result of heat production from anaerobic and/or aerobic metabolism and ATP hydrolysis, when the total heat dissipated to the ambient environment has been accounted for (Parsons 2014). Thus, thermoregulation serves to balance internal and external heat sources, creating a normothermic homeostatic environment and thereby avoiding hypo- and hyperthermia (Cheung 2010). The processes of heat dissipation and heat gain are quantified most accurately through whole body calorimetry, but for most exercise science research, may also be estimated to an acceptable level of accuracy through indirect calorimetry, as measured from exhaled breath (Jay & Kenny 2007). The use of indirect calorimetry is pertinent given the limited accessibility and significant expense of whole body calorimeters (Kenny & Jay 2013).

Six fundamental parameters; air and radiant temperatures, humidity, air velocity, metabolic heat production and clothing insulation, determine human thermal interaction with an environment (Parsons 2014). Air temperature concerns the temperature of the air surrounding the human body, which in turn largely determines dry heat transfer. Radiant temperature is the temperature that is emitted from radiant sources such as the sun, or types of lighting. Environmental humidity can be expressed both relatively and in absolute terms; relative

humidity (%) is the percentage of saturated water vapour pressure, whilst absolute humidity is the total water content in the air, in mass per volume (g/m^3). Both of these will affect evaporative heat transfer from the skin to the environment. Air velocity is the movement of air across the human body ($m.s^{-1}$). Metabolic heat production (Watts) is the consequence of heat from energy production that is not stored within the body and lastly, clothing insulation (m^2 K/W), that is determined by clothing material and the consequential effect on dry and evaporative heat transfer from the skin to the environment. Based upon these fundamental parameters, human thermal interaction with an environment can be determined using the heat balance equation.

Equation 3: Heat balance equation.

$$S = M - W \pm R \pm C \pm K - E.$$

This equation dictates that at times of thermal balance, the heat storage in the body (S), is equal to metabolic heat production (M) minus external work performed (W) and dependent upon the four predominant pathways of heat exchange; radiation (R), conduction (C), convection (K) and evaporation (E). Elements of heat exchange are measured in Watts, unless made relative to the body mass ($W.kg^{-1}$) or body surface area ($W.m^2$). However, it is notable that variations in body mass or surface area do not systematically lead to differences in metabolic rate, therefore conventionally expressing M in absolute terms is appropriate (Sawka et al. 2011).

It should be noted that R, C and K may all be positive or negative, as they have the potential to contribute to heat gain, whilst E can only be negative. A positive value for S represents a gain in heat storage, whilst a negative value represents a net heat loss. Thus, both at rest and during exercise, such as running during experimental chapters of this thesis, an individual's steady-state body temperature is contingent upon an individual's ability to balance heat gain from metabolic heat production (MHP) and the environment, with the ability to dissipate heat. Furthermore, with reference to this thesis, as a positive S is typically observed during endurance running with a progressive and consistent increase in body temperature, thermal interventions must therefore aim to enhance heat dissipation through R, C, K and E, or indeed reduce M for a given running velocity. However, it is pertinent to note that a positive S may be a desirable outcome during heat acclimation training whereby the elevation of core temperature is a primary consideration, therefore under these circumstances, an objective may be to limit R, C, K and E, and/or increase W.

2.2.1.1 Heat production

As endotherms, humans produce heat during cellular metabolism, with metabolic energy generated during the liberation of energy from macronutrients such as carbohydrates, lipids and proteins. These ingested substrates may be converted into glucose, fatty and amino acids respectively, with glucose and fatty acids metabolised predominantly through an aerobic pathway for adenosine triphosphate (ATP) production. This energy production is relatively inefficient in terms of external work, with only ~20% of the produced metabolic energy used during physical exercise such as cycling. The remaining ~80% is released as heat to nearby tissues and travels through the body via the bloodstream (Brooks et al. 1996). Accordingly, anatomical locations where a large amount of energy is liberated, such as the musculature of the upper legs, are a large source of heat production. Based on the calorific value of the ingested substrates together with oxygen consumption to metabolise these for external work, a measure of metabolic energy expenditure (gross energy), and subsequently metabolic heat production (less external work), is possible. The energy yields per litre of oxygen consumed for carbohydrates, fats and protein are 21.13 kJ, 19.62 kJ and 19.48 kJ, respectively (Brooks et al. 1996). Based on the ratio of the volume of carbon dioxide produced to the volume of oxygen consumed, the predominant substrate being oxidised can be identified. Thus the respiratory quotient, or respiratory exchange ratio (RER) when measured via exhaled breath, indicates the proportion of carbohydrates (RER = 1.0), fat (RER = 0.71), protein (RER =0 .835) or mixed diet (RER = 0.85) being oxidised during a given task (Brooks et al. 1996). Therefore, the magnitude of MHP (M) can then be estimated through indirect calorimetry, incorporating oxygen uptake and the respiratory exchange ratio (RER) using the following formula where $\dot{V}O_2$ is the volume of oxygen (L.min^{-1}) and RER is the respiratory exchange ratio (Jay & Kenny 2007).

Equation 4: Calculation of metabolic heat production (Jay & Kenny 2007).

$$\dot{M} = \ \dot{V}O_2 \cdot \left[\frac{21166 \cdot (0.23RER + 0.77)}{60} \right] \text{Watts}$$

Resting levels of metabolic heat production may be 70 – 100 Watts (Parsons 2014), with this rising to 300-1200 Watts during most physical exercise (Sawka et al. 2011). As a consequence of the positive linear relationship between external work and oxygen consumption, as exercise intensity increases, a concomitant increase in the MHP associated with that activity is also observed. Therefore, as shown by the equation, despite the contribution of RER and irrespective of other descriptive characteristics, oxygen consumption is

the greatest determining factor for MHP and the prominent threat to homeothermy (Jay et al. 2011; Cramer and Jay 2014; Smoljanic et al. 2014).

2.2.1.2 Heat dissipation

In order to preserve a stable body temperature, increases in thermogenesis must be balanced by thermolysis, the rate at which heat is dissipated to the environment. The heat balance equation highlights the four pathways for heat exchange which will determine heat dissipation.

Radiation (R) is the electromagnetic energy transfer between a relatively cool and warm body. This occurs through a combination of solar radiation, reflection from the ground and diffuse radiation from atmospheric molecules colliding, with the magnitude of heat exchange dependent upon the gradient between the body and the environment (Cheung 2010, Parsons 2014). However, this relationship can be modified upon the composition of the ground and surrounding physical surfaces, cloud cover, time of year, altitude, exposed body surface area and clothing (Cheung 2010). When environmental temperature exceeds skin temperature, the thermal gradient between the body and the environment is reversed, with radiation presenting a source of net heat gain. At rest, radiation accounts for approximately 60% of total heat loss, reducing to 5% during exercise. However, when an individual is indoors radiative heat exchange is minimal and therefore, for experimental trials such as those in this thesis, assumed equal.

Conductive heat exchange (C) occurs through direct physical contact between objects. Specifically, as molecules of greater heat collide with cooler molecules, molecular agitation occurs, with a net transfer of heat to the cooler media (Cheung 2010). It is through this mechanism that heat production from a major muscle group increases temperature of the surrounding tissues. Under most circumstances, in terms of whole body heat exchange, the magnitude of conductive heat transfer is negligible. However, significant conductive cooling can be achieved through thermal interventions such as placing ice packs directly on the skin.

Convective heat exchange (K) occurs by mass motion of air or water, as the molecules of the warm body expand, become less dense and therefore move away in a convective current, thereby removing heat. Thus convection can occur in both the air and water and as with radiation, in humans, convection is dependent upon a temperature gradient between the skin and the air or water. This gradient can be modified by the rise in skin temperature that occurs during the enhanced cutaneous blood flow as an individual performs exercise, therefore enhancing convective cooling. Convective heat exchange is also dependent upon the net flow of the respective medium (air or water) over the surface of the skin, such as wind speed, as well as the exposed surface area, which may be determined by anthropometry, clothing and posture. Conduction and convection may be considered interconnected, due to their related

roles in heat exchange between media, such as solids, fluids and gases. Consequently, conduction and convection contribute approximately 20% to heat loss at rest, with this is reducing to 15% during exercise (Wendt et al. 2007).

Evaporative heat loss (E) occurs when there is a transfer of heat between a body and a medium, such as sweat, that requires an initial change in state from liquid to vapour at the skin surface (Parsons 2014). Human thermoregulation involves the secretion of sweat on to the skin surface via eccrine sweat glands, with an energy transfer occurring from the body to the environment as sweat evaporates from the skin surface. Evaporation can account for up to 80% of heat loss during exercise and is therefore considered the most effective method for losing heat and reducing core temperature in humans and thus offsetting a positive S, the rate of heat storage (Gagnon et al. 2013). Each litre of sweat evaporated from the skin liberates 2.4 MJ of latent heat, or 680 $W.hr^{-1}$ (Wenger 1972). Therefore, as MHP can reach 800 - 1500 W during high intensity exercise, a sweat rate exceeding 2.0 $L.hr^{-1}$ may be required to maintain heat balance (Gleeson 1998; Brotherhood 2008). Humans have a finite capacity for evaporative heat loss therefore, when combined with high heat stress, the evaporative requirement of a runner to maintain body temperature can exceed the evaporative potential of the environment. The rate of evaporative heat loss from the skin is primarily determined by the difference in the partial pressure of water vapour between the skin surface (sweat) and ambient air (humidity) (Kenney 1998). However, even under conditions where ambient air is fully saturated, such as 100% relative humidity, evaporation may still be possible if an absolute humidity gradient exists between the skin and air (Jay & Kenny 2013). In addition to the atmospheric conditions, evaporative heat loss will also be determined by maximum sweat rate, air velocity, clothing, body surface area and the fraction of this area saturated with sweat, known as skin wettedness (Jay & Kenny 2013). Evaporation also has a significant convective component since it is dependent upon the velocity at which ambient air passes across the skin.

By examining the relationship of heat exchange between the body and environment it is apparent that thermoregulation is a complex interrelated process that necessitates both dry heat exchange (R, C and K) and wet heat exchange (Cheung 2010). For example, the relative contribution of each may be determined by the magnitude of blood flow from the muscle and core delivering heat to the cutaneous anatomy, and then the ability to vaporise sudomotor output. Therefore, effective thermoregulation requires an integrated response to maximise the efficiency of these processes.

2.2.2 Human thermoregulation

Human thermoregulation is ultimately achieved through two collaborative processes; behavioural and physiological temperature regulation (Sawka et al. 2011). The effect of behavioural thermoregulation that serves to reduce MHP and body heat storage is significant, and manifests as an altered pacing strategy during endurance running performance (Flouris & Schlader 2015). Behavioural temperature regulation operates through conscious behavioural alterations to alter heat storage, and extends to clothing changes and seeking of shade. Physiological temperature regulation operates independently of conscious behaviour and can determine the rate of MHP, cutaneous vasodilatation and constriction and sweating.

The maintained internal body temperature has been termed a 'set-point' (Hammel et al. 1963; Hensel 1973), which is actively maintained by central and peripheral effectors when a 'load error' occurs between body and set-point temperature (Sawka & Young 2006). The 'set point' is a mathematical concept describing the thermal control of effector responses. The pre-optic anterior hypothalamus (POAH) acts as a central integrator of thermal stimuli, as shown in Figure 8, eliciting proportional responses for thermogenesis or thermolysis to maintain heat balance. In addition to the POAH, central thermoreceptors are located in the cortex and spinal cord, whilst peripheral thermoreceptors are found within the epidermis, blood vessels, and deep abdominal and thoracic tissue (Insler & Sessler 2006). Body temperature can be demarcated into central core and peripheral shell temperatures (Jay, Gariépy, et al. 2007). While T_{CORE} represents deep-tissue organs that possess high levels of basal metabolism, such as the brain, heart, and liver, the shell temperature is influenced by blood flow to the skin and environmental temperature. Shell temperature is usually around 4°C lower than core temperature, facilitating the transfer of heat away from the core to the periphery, which in turn is usually above environmental temperature (Tansey & Johnson 2015).

Lesion studies indicate neither central or peripheral areas independently and precisely control and coordinate both afferent and efferent signals as in the POAH (Boulant 2000). Afferent signals from both central and peripheral thermoreceptors are integrated at the POAH and initiate and elicit principal autonomic and behavioural thermoregulatory effector responses. The POAH appears sensitive to hypothalamic temperature to an accuracy of 0.01°C, due to predominance of localised warm sensitive neurons, that are characterised by thermally stimulated ion channels in the cell body (Nakamura 2011). Warm sensitive neurons effect a response by signalling to open ion channels which in turn create an electrical potential to evoke a proportional thermoregulatory response (Nakamura 2011).

Figure 8: Schematic of thermoregulatory control system. T_{sk} represents skin temperature and T_c represents core temperature. From Sawka & Young (2006).

Although the notion of a set-point, achieved through a central thermal command, is not wholly accepted (Hensel 1973; Romanovsky 2007), Sawka et al. (2011) suggest the unanimity of thermoregulatory responses from thermal variation in both the core and periphery is indicative of central, rather than peripheral control. For example, the primary autonomic effector responses of vasodilation and sweating appear to initiate simultaneously (Sessler 2008). Further, there appears to be cohesion in shifts of the set-point alongside variables such as biological rhythms, pyrogens, and heat acclimation. Such observations are suggestive of a form of holistic thermoregulatory control, rather than independent neural networks without a unified central integrator (Romanovsky 2007). Notwithstanding these considerations, a recent theory concerning each thermoregulatory effector response as part of an independent neural network, without a set point and with coordination achieved through body temperature, cannot be dismissed (Romanovsky 2007). Although afferent signals originate from both core and peripheral cutaneous receptors, the POAH appears more sensitive to changes in core temperature, in terms of effecting autonomic responses such as vasoconstriction. Nadel et al. (1971) suggested that a 1°C change in T_{CORE} equates to a thermoregulatory response nine times larger than would follow a 1°C change in T_{SKIN}. Indeed, Kurz (2008) has proposed the variation of T_{CORE} about the set-point before an autonomic response is evoked to be 0.2°C, while skin temperature generally varies over a far wider range of several degrees before a response is initiated. However, skin temperature is more sensitive to environmental changes than T_{CORE},

therefore the role of T_{SKIN} on thermoregulation should not be underestimated (Sawka et al. 2011). Moreover, it would seem peripheral thermoreceptors contribute to prompt behavioural changes, due to a strong relationship between peripheral temperature and thermal comfort (Frank et al. 1999).

As an individual begins to exercise, there is an immediate increase in MHP, however heat loss kinetics result in a temporary dissociation between heat gain and heat dissipation, thus resulting in an increase in body heat content and an increase in body temperature. The body initially responds to exercise through sympathetic vasoconstriction, directing blood flow to active muscles. Central and peripheral thermoreceptor afferent feedback will determine heat loss responses from the POAH as appropriate. Efferent output from the POAH subsequently promotes vasodilatation, resulting in a small transfer of heat to the periphery, proportional to the magnitude of blood redistribution. This increases convective and radiative heat loss as a consequence of an elevated T_{SKIN}, which has promoted an enhanced temperature gradient between the skin and environment. This vasodilation is achieved through a 1-2 $L.min^{-1}$ increase in cardiac output, mediated by an increased heart rate and decreased perfusion of splanchnic and renal tissue (Rowell 1973). Whilst the magnitude of skin blood flow is proportional to alterations in T_{CORE}, there appears an upper limit at a core temperature of ~38°C (Brengelmann et al. 1977; Smolander et al. 1987; Patterson et al. 1994). The skin blood flow response is covered in greater detail subsequently (Section 2.3.1). Alongside vasodilation, sympathetic cholinergic fibres stimulate sudomotor output across the body, enhancing evaporative heat loss to that from respiration. Again, the reader is referred to a subsequent section where the sweat response is discussed in greater detail (Section 2.3.3). However briefly, efferent output from the hypothalamus is relayed by sympathetic cholinergic fibres that innervate individual eccrine sweat glands (Shibasaki et al. 2006). Acetylcholine initiates a series of chemical reactions that ultimately releases isotonic fluid from the secretory cells of the sweat gland (Kellogg et al. 1995; Shibasaki & Crandall 2010). During exercise, the reliance on evaporative heat loss increases and becomes the predominant heat loss mechanism (Parsons 2014; Sawka et al. 2011; Kenney 1998). A litre of evaporated sweat has the potential to liberate 2.4 MJ or 680 $W.hr^{-1}$ of heat energy (Kenney 1998). Consequently, endurance runners are characterised by high sweat rates, such as 1.77 $L.hr^{-1}$ and higher (Godek et al. 2005), as sudomotor output attempts to balance MHP, which may exceed 1000 Watts during endurance running (Nielsen 1966).

Traditionally, it has been reported that fitter individuals have a greater thermoregulatory ability, as a consequence of a greater local and whole body sweat rates for a set relative

exercise intensity (Gisolfi & Cohen 1978; Greenhaff 1989; Saltin & Hermansen 1966). This follows the notion that individuals with a greater maximal aerobic capacity possessed a greater potential to dissipate heat, to balance the greater MHP for a given exercise intensity (Saltin & Hermansen 1966). Consequently, aerobic fitness has long been considered a prominent variable for determining changes in T_{CORE} (Saltin & Hermansen 1966; Havenith et al. 1998), which may be important to prescribing exercise during heat acclimation training, or identifying an individual's predisposition to heat illness. This theory has pervaded due to the implications concerning the prescription of exercise intensity relative to $\dot{V}O_{2max}$ in experimental trials. Accordingly, greater sweat rates have been observed in fitter individuals, compared with untrained, when exercising at the same percentage of $\dot{V}O_{2max}$ (Ichinose-Kuwahara et al. 2010). In recent years, this theory has been challenged (Jay et al. 2011; Cramer & Jay 2015; Smoljanic et al. 2014; Dervis et al. 2014), with the suggestion the lower sweat rates may be a consequence of a lower required sweat rate to maintain heat balance (E_{req}), due to exercising at a lower absolute MHP. Moreover, $\dot{V}O_{2max}$ does not have an independent effect on thermoregulation and rather the absolute rate of MHP appears to be the primary determinant for changes in T_{CORE}. Jay et al. (2011) compared two groups matched for body mass and body surface area, who displayed high (>60 mL.kg^{-1}.min^{-1}) or low (<40 mL.kg^{-1}.min^{-1}) $\dot{V}O_{2max}$ values. No difference in T_{CORE} change or whole body sweat loss were observed when individuals exercised at the same absolute rate of metabolic heat production (540 W) in a physiologically compensable environment. Conversely, when exercising based on %$\dot{V}O_{2max}$, large differences in T_{CORE} response (~0.6°C) were observed between groups during the 60 min exercise, as shown in Figure 9. Similarly, large differences were observed in whole body sweat rate (807 ± 155 ml trained vs 486 ± 59 ml untrained) when exercise was prescribed through %$\dot{V}O_{2max}$.

Relative intensity
60% VO$_{2max}$

Hi = >60 ml.kg.min = 10.7 w.kg
LO = <40 ml.kg.min = 7.7 w.kg

Fixed metabolic heat production
540 W (7.0 w.kg)

HI = >60 ml.kg.min = 40% VO$_{2max}$
LO = <40 ml.kg.min = 58% VO$_{2max}$

Figure 9: Modified from Jay et al. (2011). Left: Different change in T_{CORE} when exercising at %$\dot{V}O_{2max}$ in groups with high (HI) and low (LO) $\dot{V}O_{2max}$. Right: Similar T_{CORE} response when exercising at a fixed metabolic heat production irrespective of high (HI) or low (LO) $\dot{V}O_{2max}$.

Cramer et al. (2012) provided further evidence to emphasise the importance of controlling for MHP, with change in T_{CORE} and whole body sweat rate the same across high and low $\dot{V}O_{2max}$ groups during 60 min cycling at a MHP per unit of body surface area (275 W.m^{-2}), which produced the same E_{req} in all participants. Although interestingly, Cramer et al. (2012) did report differences in local sweat rate between trained and untrained individuals, with forehead sweating almost double that in trained participants. Thus Jay (2014) suggests previous studies may be confounded by methodological limitations and changes in T_{CORE} and sweating are primarily determined by heat production, body mass and BSA, not $\dot{V}O_{2max}$.

2.2.3 Interaction of heat stress and body temperature

Environmental heat stress may be defined as the environmental and metabolic conditions that may lead to elevations in body temperature (Sawka et al. 2011). The heat balance equation demonstrates how hot and humid conditions may exacerbate the challenge of homeothermy, during prolonged periods of elevated MHP, as occurs during endurance running. Increased ambient temperature reduces the potential for heat loss via convection and radiation, resulting in a greater reliance on the evaporation of sweat. Moreover, an increase in atmospheric humidity lowers the water pressure gradient from the skin to the air, potentially reducing the volume of sweat that evaporates and cools the skin. Therefore, whilst running in hot and humid conditions, a consistent rise in body temperature is observed, with the prominent thermoregulatory defence being a reduction in MHP, manifested through a behavioural alteration of reducing running speed (Tucker 2008; Schlader 2014).

Heat stress may be classified as compensable or uncompensable, determined by biophysics and notably, the ambient environmental conditions and the exercise intensity (Cheung et al. 2000). When heat stress is compensable, the heat balance equation is maintained and heat dissipation matches MHP such that body temperature remains at a steady state. Conversely, in an uncompensable thermal environment, core temperature continues to rise as long as work/exercise continues, at a given intensity, and steady-state core temperature is never attained. Whether a given set of conditions is uncompensable or not therefore depends on a combination of ambient temperature, water vapour pressure, exercise intensity and clothing insulation. This concept was proposed by Lind (1963), who identified a prescriptive zone for safe physical activity within compensable heat stress situations. This zone demarcates a range

of environmental temperatures where exercise at a given intensity results in thermal equilibrium and thus a steady-state body temperature. This is shown below in Figure 10.

Figure 10: The prescriptive zone (Lind, 1963), whereby a steady-state body temperature can be achieved under compensable heat stress (CHS), but not uncompensable heat stress (UCHS).

The model of Lind (1963) demonstrates how metabolic heat productions of 200, 350, 500 and 1000 W may elicit respectively elevated steady-state core temperatures within the prescriptive zone, when heat stress is compensable. Therefore, thermal equilibrium may be achieved throughout the prescriptive zone, producing a zero rate of heat storage, however absolute core temperature remains different at different rates of MHP. This is explained by a greater temporal dissociation between MHP and heat dissipation at these two workloads, caused by a greater delay to reach a steady state temperature at a higher MHP, due to heat loss kinetics. This delay reflects the time for sudomotor and circulatory responses to elicit an increase in heat loss that equates to heat production. As the upper limit of the prescriptive zones demarcates the transition to an uncompensable heat stress situation, alterations to exercise intensity, clothing and/or environmental conditions may all represent work completed beyond the prescriptive zone and therefore result in increased core temperature at the same heat production.

These data of Lind (1963) indicated that absolute metabolic rate determines core temperature within the prescriptive zone and beyond. However, these data originated from just three individuals and Lind (1970) subsequently showed large inter-individual variability in core temperature at fixed levels of heat productions. Cramer and Jay (201) have subsequently identified body mass as an important determinant of core temperature response and suggest

the rate of MHP in Watts per kilogram of body mass predicts the core temperature response better. This observation has practical consequences for prescribing exercise within heat acclimation training that necessitates rapid and repeated elevation of core temperature across individuals, over many days (Cramer & Jay 2015). Moreover, it facilitates a more controlled exercise prescription for those exercising in the heat who have different fitness levels, and therefore may exercise at different absolute exercise intensities (Jay et al. 2011), or may be obese (Leites et al. 2013) or experience limited thermoregulatory function (Goosey-Tolfrey et al. 2008; Price 2015).

2.2.4 Quantifying heat stress and heat strain

Accurately quantifying environmental heat stress is an important consideration for competitors, coaches and organisers of competitions in hot environments, in order to determine whether a competition will result in a compensable or uncompensable heat stress, and allow appropriate preparation. Furthermore, the heat balance equation alludes to the importance of incorporating a range of variables that will determine the efficiency of heat dissipation within any measure of heat stress. For example, dry ambient temperature and relative humidity will not account for heat from the sun, or indeed cooling from the wind. To address this, Yaglou & Minard (1957) developed the Wet Bulb Globe Temperature (WBGT) to help combat heat illness in American military personal in the late 1950s. This measurement has become established as both an International (ISO 7243) and British (BS EN 27243) standard for the assessment of heat stress (Budd 2008). The WBGT metric incorporates environmental temperature, humidity, wind, solar radiation and surface radiation through a dry bulb thermometer (DB), natural wet bulb thermometer (NWB) and a globe thermometer (GT). A DB indicates dry ambient temperature through a mercury-filled thermometer, which is also sensitive to cooling from the wind. The NWB represents the temperature when a wet wick surrounds the mercury bulb. The wick may provide an indication of evaporative cooling through difference between NWB and DB, with a minimal difference representing minimal evaporative cooling, due to a high relative humidity. Finally, GT provides a measure of radiant heat, through a black metal sphere surrounding the bulb. Therefore, the outdoor WBGT may be calculated from the following equation.

Equation 5: Calculation of outdoor wet bulb globe temperature (°C).

$$WBGT = 0.7NWB + 0.2GT + 0.1DB$$

This calculation has subsequently been modified to provide a holistic indoor heat stress assessment based on the assumption that GT matched DB in a sheltered environment, as shown below.

Equation 6: Calculation of indoor wet bulb globe temperature (°C).

$$\text{Indoor WBGT} = 0.7\text{NWB} + 0.3\text{DB}$$

The use of WBGT, within predefined heat stress guidelines, has been shown to be effective in reducing the incidences of heat illness in both military (Yaglou & Minard 1957) and sporting environments (Bergeron et al. 2012). For example, current guidelines recommend the suspension of play in soccer and tennis when WBGT is 32.2°C as the metabolic heat production associated with typical performances in these sports will elicit an uncompensable heat stress, that heightens the risk of heat illness (Mountjoy et al. 2012). A benefit of WBGT is that it incorporates heat stress of vastly different characteristics, such as hot-dry or hot-wet conditions, into one metric. Such integration appears valid for sporting performance, where no thermoregulatory differences have been observed between repeated cycle sprinting between hot-wet and hot-dry conditions, under matched WBGT (Hayes et al. 2014). However, the use of WBGT is not without criticism, based upon unreliable calibration procedures and how natural wet-bulb temperature should be assessed (Budd 2008). Furthermore, the relevance of WBGT for sporting performance has been questioned based on the inability to account for clothing accurately, and the measurement of air velocity not representative of that experienced by the individual during exercise (d'Ambrosio Alfano et al. 2014).

Whilst useful, the inability of such a measure of environmental heat stress to predict or monitor individual responses validly, is a consequence of the independent effect of factors such as metabolic heat production according to exercise intensity and efficiency/economy (Cramer & Jay 2015; Smoljanic et al. 2014), athlete morphology (Dervis et al. 2014), acclimation state (Willmott et al. 2015), and clothing (Havenith 1999). Therefore, a need to consider heat stress in combination with measures of individual heat strain is apparent (Racinais et al. 2015a). Moran et al. (1998) developed a Physiological Strain Index (PSI) based around exercising heart rate (HR) and core temperature (T_{CORE}), relative to resting levels, that reliably reflects heat strain across different types of heat stress (see Equation 11, General Methods Section 3.7.2). Such an index has the benefit of being individualised, accounts for diurnal variation by incorporating resting measurements, and should be sensitive to thermal changes arising from clothing that is worn. However, PSI adopts arbitrary end-points for both HR (185 b.min^{-1}) and T_{CORE} (38.5°C) that are routinely exceeded during endurance running in the heat, and therefore PSI may not be appropriate for comparing between individuals or between exercise modes.

Through a series of experiments by Moran and colleagues, the PSI has been shown to be valid across a range of environmental conditions including; hot-dry, hot-wet, and normal conditions, with data primarily based upon steady-state measurements (Moran et al. 1998; Moran, Horowitz, et al. 1999; Moran, Shapiro, et al. 1999). Subsequently, PSI has been approved for use during intermittent exercise, based on a study involving three repetitions of 15 min : 5 min work : rest cycles (Gotshall et al. 2001).

Alternative approaches to quantifying thermal strain at rest and during exercise include combining core and skin temperature to provide an estimates of mean body temperature (Burton 1935; Colin et al. 1971; Jay & Kenny 2007) from which can be calculated body heat content (BHC) and body heat storage (BHS) (Jay & Kenny 2007). The inclusion of T_{SKIN} in a measure of thermal status appears appropriate given it's responsiveness to changes in environmental or body temperature (Roberts et al. 1977), as it is through the skin that heat is gained or lost. Additionally, Sawka et al. (2011) has highlighted how the extremely high T_{CORE} associated with fatigue and exercise cessation is routinely observed in association with a high T_{SKIN} and there is emerging evidence that T_{SKIN} has a large and direct influence on behavioural thermoregulation and therefore exercise intensity during free paced trials (Schlader, Simmons, et al. 2011a; Flouris & Schlader 2015). Although the accuracy of mean body temperature measurements has been called into question when whole body calorimetry is not used (Jay, Reardon, et al. 2007), such an estimation may remain useful for interpreting alongside other measures such as PSI and alone may remain appropriate for determining treatment effects in repeated measures designs (Sawka et al. 2011). However, many methods of skin temperature measurement are cumbersome, and require long, trailing wires and the investigation of less intrusive, portable, wireless and non-contact methods of T_{SKIN} measurement would therefore appear warranted for both practitioners and within research. This would enhance the ability to better quantify and predict thermal status during exercise in the field, individually, or in combination with other measures such as WBGT and PSI.

2.3   Physiological effects of heat strain

2.3.1   Skin blood flow

The anatomy of human skin affords the potential for a large temperature gradient between circulating blood and the external environment. The skin is dual layered, with an exterior epidermis containing epithelial cells located above a dermis layer that contains blood vessels, nerves, eccrine sweat glands and hair follicles. Modulation of dermis microcirculation facilitates heat loss through radiation and convection by enhancing this temperature gradient to the

environment with an increase in warm blood from the core. Thus, the body can control dry heat loss by varying skin blood flow and skin temperature. Furthermore, when sweating occurs, skin blood flow serves to deliver heat that can subsequently be removed by sweat evaporation, therein sweating and skin blood flow complement each other in dissipating heat.

Large regional differences in skin blood flow exist, in part due to anatomical differences in the microstructure of the skin (Roddie 1983). Glabrous skin, which contains no hair, can be found on the palms, ventral region of fingers, soles, forehead and lips. Conversely, nonglabrous skin contains hair, and is more widely found across the body (Johnson et al. 2014). It has been suggested that between 25% and 27% of total skin blood flow is directed to the head and hands, despite these areas only comprising about 13% of the total skin surface (Tikuisis et al. 2001). Consequently, extrapolation of localised blood flow data into whole body estimations may be inaccurate. Regional differences in skin blood flow and heat transport can be partially attributed to the presence of arterio-venous anastomoses (AVAs). AVAs are only present in glabrous skin and once opened, allow blood to bypass high resistance capillaries at the skin surface, connecting arterioles to venules. Therefore, the opening of AVAs greatly increases the volume of blood, and thus heat, transported to the skin (Roddie 1983, Johnson et al. 2014). Although the area of glabrous skin is small relative to the entire body surface, the contribution to thermoregulation is significant, as a consequence of not only AVAs, but because glabrous skin is routinely and directly exposed to the environment, and not covered by clothing.

Skin blood flow is controlled by sympathetic nervous system activity, in response to changes in T_{SKIN} and T_{CORE}, with vascular smooth muscle acting as effectors to central commands (Johnson & Kellogg 2010). Human skin is under dual vasomotor control (Johnson et al. 2011), with nonglabrous skin is innervated by both noradrenergic vasoconstrictor and cholinergic vasodilator nerves, whereas glabrous skin, is innervated solely by vasoconstrictor nerve fibres, with vasodilatation achieved in these regions by withdrawing vasoconstrictor activity. The precise mechanisms that determine skin blood flood remain a topic of debate (Johnson et al. 2014), however acetylcholine appears to mediate initial vasodilation (Kellogg et al. 1995), with an, as yet unidentified cholinergic vasodilator determining the overall response (Charkoudian 2010). This could be nitric oxide (Kellogg et al. 1998; Lorenzo & Minson 2010), or indeed a collective response to adenosine, prostaglandins and nitric oxide (Mortensen et al. 2009). Moreover, a number of other substances have been implicated as contributors, including vasoactive intestinal peptide, histamine and transient receptor channels, however their precise roles remain to be fully elucidated (Tansey & Johnson 2015; Sawka et al. 2011). Finally, in contrast to a model of independent control of skin blood flow, it has been suggested that there

is a shared response with that of sudomotor output, given the concurrence of these responses (Charkoudian 2010).

Initially, during exercise in the heat, skin blood flow is characterised by vasoconstriction, as active muscle is supplied with blood at the expense of cutaneous circulation (González-Alonso et al. 2008). However, as T_{CORE} rises, afferent feedback from central thermoreceptors increases and peripheral vasodilation is facilitated, manifested by an increase in heart rate to maintain cardiac output (González-Alonso et al. 2008). Although, maximal whole-body skin blood flow measurements are not possible, it has been estimated that skin blood flow can be as high as 7.8 L.min^{-1} in a hot environment (Taylor et al. 1984; Rowell 1986). Despite the potential conflict for cardiac output from haematic requirements for skeletal muscle metabolism and thermoregulation in the periphery, muscle blood flow is maintained at sub-maximal intensities at the expense of skin blood flow (Gonzalez-Alonso et al. 1998). Thus as exercise continues, the magnitude of skin blood flow is proportional to the elevation in T_{CORE} (Rowell 1974), until an upper limit at a core temperature of ~ 38°C, despite this equating to approximately 50% of the potential maximal blood flow (Brengelmann et al. 1977; Smolander et al. 1987; Patterson et al. 1994), as shown in Figure 11. Compromised skin blood flow during exercise is also a consequence of increased vasoconstrictor tone, elevating the T_{CORE} threshold for the increase of skin blood flow to aid thermoregulation during exercise, compared to rest (Bevegard & Shepherd 1966; Kenney & Johnson 1992).

Figure 11: Schematic description of the thermoregulatory control of skin blood flow as modified by moderately intense exercise. The relation of skin blood flow to internal temperature is

affected, relative to resting conditions, in at least three ways by exercise: a vasoconstrictor response at the onset of dynamic exercise (A), an increase in the internal temperature threshold at which skin blood flow begins to increase (B), and a levelling off, or plateau, in skin blood flow above an internal temperature of 38°C at a level well below maximal (C) (Gonzalez-Alonso et al. 2008).

As ambient temperature increases, so too does the importance skin blood flow to maintain the core-to-skin gradient and in turn, heat balance (Kenefick et al. 2010). The skin blood flow requirement to maintain heat balance can be estimated, based on an individual exercising at a known exercise intensity, T_{CORE} and T_{SKIN}, as shown in Table 3.

Table 3: Marathon runner's skin blood flow requirements for several core (T_c) and skin (T_{sk}) temperatures during heat stress (Kenefick et al. 2010). Estimated using 60 kg body mass and 325 m.min^{-1} running velocity, after subtracting for work (20% efficiency) and 50% dry and evaporative heat losses.

Heat production (watts/kcals/min)	T_c (°C)	T_{sk} (°C)	Core-to-skin gradient (°C)	Skin blood flow[b] (L/min)
540/7.7	38	30	8	1.1
540/7.7	38	34	4	2.2
540/7.7	38	36	2	4.4
540/7.7	39	36	3	3

a   Calculations assume the metabolic energy to be dissipated is ≈540W (50% of 1080W), with 50% coming from evaporation. Calculations assume a constant sweat rate independent of skin temperature.

b   Equation for skin blood flow: $Q_S = 1/C \times h/(T_c - T_{sk})$.

C = specific heat of blood ≈0.87 kcal/°C/L; **h** = heat production; **Q_S** = skin blood flow.

For example, based on the assumption that blood entering and leaving the cutaneous vasculature is equal to T_{CORE} and T_{SKIN} respectively, a net MHP of 540 Watts with T_{CORE} of 38°C and T_{SKIN} of 30°C necessitates a skin blood flow of 1.1 L.min^{-1} (Sawka et al. 2011). Table 3 demonstrates how the minimal skin blood flow requirement to preserve the same heat transfer rate increases in accordance with increased skin temperature and a reduced core-to-skin gradient. The interaction of T_{CORE} and T_{SKIN} also alludes to the potential role of an elevated T_{CORE} in maintaining heat dissipation, without enhancing the skin blood flow requirement, which is not in keeping with typical interventions that habitually seek to minimise increases in T_{CORE} (Sawka et al. 2011).

2.3.2   Cardiovascular system

To meet the concomitant demands of muscular and skin blood flow, cardiac output is increased through an increase in heart rate, with prolonged endurance exercise characterised

by a cardiovascular drift as blood flow demands become more pronounced with increasing body temperature (Wingo et al. 2005). A classic study by Rowell et al. (1966) demonstrates how uphill walking, in a euhydrated state, elicits significantly greater cardiovascular strain in hot (43°C) vs control (26°C) conditions, with a 150 – 200 ml reduction in central blood volume, 19 – 23 ml reduction in stroke volume, 21 – 29 b.min^{-1} elevation in heart rate and 1.1 – 1.2 L.min^{-1} reduction in cardiac output.

Importantly, muscular blood flow is maintained at submaximal intensities (Gonzalez-Alonso et al. 1998), achieved in part, through a redistribution of blood, with visceral circulation markedly reduced during heat strain (Rowell et al. 1966). As exercise approaches maximal intensities, González-Alonso & Calbet (2003) have demonstrated that cardiac output and mean arterial pressure reduce, leading to an impairment to skeletal muscle blood flow. Consequently, a reduction in oxygen delivery, oxygen uptake at the muscle, and expedited fatigue compared with normothermic conditions is observed (González-Alonso & Calbet 2003). The reduced stroke volume occurs as per the Frank-Starling mechanisms, with increased cutaneous resistance impairing venous return, thereby decreasing central venous pressure and central blood volume (Gonzalez-Alonso et al. 1999; Crandall et al. 2008). This is in contrast to lower exercise intensities whereby despite reductions in cardiac filling pressure, stoke volume is maintained as a consequence of increased cardiac contractility (Brothers et al. 2009). Indeed, cardiac output may reach up to ~13 L.min^{-1} under heat stress as the heart attempts to meet convergent demands of muscular (2.5 – 4 L.kg^{-}1.min^{-1}) and cutaneous flow (~8 L.min^{-1}) (Rowell 1974). González-Alonso et al. (2008) highlights how haematic insufficiency may be the same mechanism limiting maximal aerobic exercise performance under normothermic conditions, as these demands combined would require a cardiac output of almost 40 L.min^{-1} to sustain maximal exercise involving the recruitment of a large proportion of muscle mass.

This negative relationship between blood availability and exercise tolerance may be exacerbated by dehydration, with trained runners demonstrating sweat rates of 3 L.hr^{-1}, which exceeds the maximal gastric emptying rate of 600 – 800 mL.hr^{-1} (Montain & Coyle, 1992; Gonzalez-Alonso et al. 2000). The reduction in plasma volume that facilitates sudomotor output reduces the central blood volume, consequently impairing stroke volume, cardiac output and blood pressure (Gonzalez-Alonso et al. 1998). Gonzalez-Alonso et al. (2000) demonstrated a 6.4 ± 1.3 % decrease in stroke volume per percent of dehydration, necessitating a 11 - 14 b.min^{-1} higher heart rate and decreased mean arterial blood pressure during 30 minutes of cycling at 72% $\dot{V}O_{2max}$ at 35°C. Furthermore, the attenuation of exercise intensity is likely inevitable during

exercise that continues for many hours, such as marathon running, given the associated sweat rates and maximal gastric emptying rate.

2.3.3 Sudomotor function

Alongside alterations in peripheral circulation and radiative and convective heat loss, afferent feedback concerning elevations in T_{CORE} and T_{SKIN} are integrated within the POAH and stimulate concomitant sudomotor activity to facilitate evaporative cooling. The primary effectors of this response are the eccrine sweat glands, of which there are thought to be 1.6 – 4.0 million in the human body (Shibasaki et al. 2006). Efferent signals innervated the ducts and coils of glands by releasing acetylcholine from cholinergic fibres. In turn, acetylcholine binds to muscarine receptors, increasing calcium concentration and enhancing the permeability of K^+ and Cl^- channels, which initiates the release of an isotonic precursor fluid from the secretory cells (Sato et al. 1989). It is thought that as the fluid travels up the duct toward the surface of the skin, sodium and chloride are reabsorbed, resulting in sweat on the skin's surface being hypotonic relative to plasma. However, during periods of high sweat rates, such as running in the heat, ion reabsorption mechanisms may be overwhelmed by the volume of sweat secreted into the duct, resulting in higher ion losses and making sweat sodium content dependent on sweat rate (Bulmer & Forwell 1956; Cage & Dobson 1965). The torso appears to demonstrate a sweat response in advance of peripheral limbs, with the localised sweat volume dependent on the density and secretion rate of sweat glands (Nadel et al. 1971). Fitter individuals do not independently possess a greater whole body sweat rate, but may demonstrate localised differences, such as on the forehead (Cramer et al. 2012).

The evaporative heat loss to the environment will be determined by the moisture content of the air, quantified through the ratio of ambient vapour pressure to the saturated water vapour pressure, the relative humidity (Kenney 1998). Thus, the rate of sweat evaporation is a product of the gradient between skin and air water vapour pressures, and the coefficient of evaporative heat transfer (Gagge & Gonzalez 2011). Based on a known exercise intensity, the evaporate requirement (E_{req}) of an individual can be estimated *a priori*. For example, an individual performing exercise at a 600 W metabolic rate, liberates approximately 80% of the energy consumed as heat. Consequently, 480 W (28.8 kJ·min^{-1}) must be dissipated to maintain heat balance. As the specific heat of body tissue is 3.5 kJ.°C^{-1}, a 70 kg individual has a heat capacity of 245 kJ·°C^{-1}. Therefore, based on the latent heat of sweat evaporation of 2.43 kJ.g^{-1}, approximately 12 g.min^{-1} or 0.72 L.h^{-1} of sweat must be secreted and evaporate to prevent an elevation of body temperature (Sawka et al. 2011). Consequently, activities such as running

that require a high MHP likely result in net dehydration when sweat loss exceeds 2 L.hr^{-1}, irrespective of hydration strategies when gastric emptying may be only 600 − 800 ml.hr^{-1} (Montain & Coyle 1992; Gonzalez-Alonso et al. 2000). As previously detailed, the consequences of sweat-induced dehydration on the cardiovascular system are profound, and may contribute to an impaired $\dot{V}O_{2max}$ in the heat. It may however, be possible to predict such situations and implement strategies to mitigate against dehydration. For example, as shown below, Shapiro et al. (1995) developed a sweat rate prediction equation, which may permit informed decisions to be taken concerning an athlete's readiness to compete at a given location.

Equation 7: Sweat rate prediction equation (Shapiro et al. 1981).

$$\text{Sweat rate (g·m}^{-2}\text{·h}^{-1}) = 147 + 1.527 \cdot E_{req} - 0.87 \cdot E_{max}$$

Consequently, it may be possible to individualise thermal interventions to ensure that adaptation is sufficient for the intended environment so long as the required evaporative cooling (E_{req}) and evaporative cooling capacity of the environment (E_{max}) are known.

2.3.4 Skeletal muscle metabolism

Exercising in hot environments elicits a range of effects on metabolism, although the precise mechanisms underpinning such alterations remain to be fully elucidated (Febbraio 2001). Skeletal muscles temperature can often vary by 2 to 4°C during exercise-heat stress (Gonzalez-Alonso et al. 1999; Jay, Gariépy, et al. 2007) and elevated muscle temperature is associated with an increased rate of glycogenolysis (Fink et al. 1975; Parkin et al. 1999), and earlier increases in plasma blood lactate concentration (Hargreaves 2008). Hepatic glucose production is also increased during exercise and heat stress, although this change is not accompanied by an increase peripheral glucose uptake (Angus et al. 2001). Although these metabolic changes are not considered to limit prolonged endurance exercise in the heat directly (Nybo et al. 2014), they may contribute towards the reduced LT and LTP in the heat (Lorenzo et al. 2010), as well as increasing the likelihood of chronic glycogen depletion for those consistently training in the heat (Hargreaves 2008). Furthermore, changes in metabolism may elicit a more direct effect on fatigue during endurance performance in the heat as they occur in association with cardiovascular and neuromuscular impairments (Nybo et al. 2014). The enhanced contribution of glycogenolysis and glycolysis would appear to lead to an imbalance in the production of lactate and ensuing conversion to pyruvate (Robergs et al. 2004). This imbalance may be partly attributable to compromised blood flow arising from the visceral vasoconstriction (Rowell et al. 1971). Limiting splanchnic circulation in order to facilitate peripheral vasodilation for heat dissipation, likely leads to a reduction in lactate

buffering in the liver. Concurrently, an increased lactate concentration may occur as a consequence of the Q10 effect, whereby the increase in body temperature leads to an overall increase of substrate metabolism (Nadel 1985). Finally, Febbraio et al. (1994) has previously highlighted a strong relationship between reduced plasma adrenaline levels in explaining metabolic changes following heat acclimation, notably a reduced RER, indicating greater fatty acid or ketone oxidation and less glycogenolysis. These findings were in the apparent absence of changes in fibre type recruitment and with changes far exceeding that from a Q10 effect. This investigation built on the apparent relationship between an elevated T_{CORE} and exertional heat stress, that have been shown to increase plasma adrenaline levels (Febbraio et al. 1994; Gonzalez-Alonso et al. 1999; Hargreaves, Angus, et al. 1996; Hargreaves, Dillo, et al. 1996), and for which there is evidence of an associated increase in glycogenolysis during exercise (Greenhaff et al. 1991; Spriet et al. 1988). Since glycogen phosphorylase activity is enhanced by β-adrenergic receptor stimulation (Richter et al. 1982) it seems plausible that an increase in circulating adrenaline drives a concomitant increase in intramuscular glycogen utilisation. Notwithstanding this apparent relationship, such a finding has not been universally observed, and the relationship between adrenaline and glycogenolysis, as well as more broadly conferencing metabolic alterations in the heat, remains a somewhat mixed picture (Febbraio 2001).

2.4 Endurance performance in the heat

The aforementioned physiological changes arising from heat stress are associated with an increased percentage of non-finishers during prolonged running races (Vihma 2010; Wegelin and Hoffman 2011), reflecting an inverse relationship between ambient temperature and endurance performance (Ely et al. 2007; Ely et al. 2008; Vihma 2010; Wegelin & Hoffman 2011). Therefore, it is apparent that endogenous and exogenous thermal strain combine to produce a relative exercise intolerance. Such intolerance is underpinned by the absolute level of MHP required in different endurance distance events. To identify events that are most susceptible to heat stress, Taylor and Cotter (2006) modelled the theoretical BHS for world record performances in events from 100m through to the marathon, using normative morphometric characteristics and the predicted MHP, assuming a net heat loss of zero (Figure 12). After accounting for the specific heat capacity of human tissue, whereby 3.47 kJ of heat energy is required to elevate 1 kg of tissue by 1°C, the resultant heat production curve demonstrates that world record pace in the 1500 m and 3000 m races would increase body temperature by 2 and 4°C respectively.

Commensurate with the model of Taylor and Cotter (2006), larger impairments to performance have been reported in longer duration events than 3000 m. Consequently, in a meta-analysis of marathon running performance, El Helou et al. (2012) calculated that an increase in ambient temperature 20°C above the optimal temperature increased running time by 17.7% for males and 12.4% for females, equating to 0.03% per 1°C increase. El Helou et al (2012) went on to identify median optimum environmental temperatures of 6.2°C for men and 6.8°C for women from 1,791,792 marathon finishers.

Figure 12: Estimated external work and metabolic heat production during world record track performances over distances from 100m through to the marathon, using the morphometric characteristics of each record holder, with no heat loss (Taylor & Cotter 2006).

This model of Taylor and Cotter (2006) provides a theoretical underpinning of the recent results of Guy et al. (2014) who demonstrated a statistically significant, mean reduction of 3% in performance across endurance events in hot (>25°C), compared with cool (<25°C) conditions. Guy et al. (2014) also observed a *'medium'* negative effect on World Championship Athletics performance from 1500 m through to 10,000 m. As shown in Table 4, typical impairments from heat stress within experimental studies are equally pronounced, with a mean reduction of -13.4% in TT and -29.6% in TTE (Nybo et al. 2014). Whilst athlete fitness levels, event duration/distance and the specific environmental conditions prevent clear synthesis both between and within the studies selected by Guy et al. (2014) and Nybo et al. (2014)

respectively, it is apparent that the magnitude of impairment from heat strain is both significant and meaningful for a competitive athlete.

Table 4: Comparison of normothermic (control) and hot (heat) environments on submaximal aerobic performance during time trial and time to exhaustion tests (modified from Nybo et al. 2014). '-' denotes data not provided by respective study. 'Bike' = cycle ergometer.

Study	n	Protocol	Exercise mode	Distance/ Duration	Control condition				Hot condition				Impairment (%)
					Temp (°C)	T_{CORE} (°C)	T_{SKIN} (°C)	HR (b.min^{-1})	Temp (°C)	T_{CORE} (°C)	T_{SKIN} (°C)	HR (b.min^{-1})	
Bannister & Cotes 1959	3	TTE	-	-	17	39.6	-	161	25	39.2	-	-	>-12
Dill et al. 1931	5	TTE	Bike	37-60 min	12	38.2	-	139	34	39.0	-	175	-25
Galloway & Maughan 1997	8	TTE	Bike	~50-105 min	21	~39.3	~31	~164	31	~40.1	~35.0	~174	-36
Gonzalez-Alonso et al. 1999	7	TTE	Bike	~26-66 min	36	40.1	37.2	198	40	40.1	37.0	196	-39
MacDougall et al. 1974	6	TTE	Treadmill	-	23	~39.4	~31.5	178	39	~39.4	~35.7	181	-36
													Mean = -29.6%
Altareki et al. 2009	9	TT	Bike	4 km	13	37.9	36.4	-	35	38.3	38.0	169	-2
Ely et al. 2010	8	TT	Bike	15 min (30 min pre-load)	21	38.1	31.1	95%	40	38.2	36.2	96%	-17
Lorenzo et al. 2010	12	TT	Bike	1 hour	13	38.7	25.1	130	38	38.9	34.6	165	-17
Lorenzo et al. 2010	8	TT	Bike	1 hour	13	38.8	23	127	38	34.4	33.9	150	-16
Périard et al. 2011	8	TT	Bike	40 km	20	38.9	~27	~179	35	39.8	~35.8	~184	-7
Roelands et al. 2008	8	TT	Bike	~40 min (1 hour pre-load)	18	39.5	-	~185	30	40.0	-	~190	-25
Tatterson et al. 2000	11	TT	Bike	30 min	23	39.0	~26	195	32	39.2	~32.0	195	-7
Tucker et al. 2004	10	TT	Bike	20 km	15	38.8	~28	181	35	39.2	~35.0	184	-6
Tyler & Sunderland 2008	9	TT	Treadmill	15 min (75 min pre-load)	14	~38.5	-	~178	30	~39.0	-	~180	-7
Watson et al. 2005	9	TT	Bike	~40 min (1 hour pre-load)	18	39.2	-	-	30	39.7	-	178	-30
													Mean = -13.4 %

The precise manner in which the physiological effects of heat stress combine and manifest as impaired performance and/or capacity remain a topic of fierce debate (see reviews by; Périard [2013], Nybo et al. [2014], Hargreaves et al. [2008], Febbraio [2001], Cheung [2007], Tucker [2008], Ely et al. [2007]). Nevertheless, it is commonly accepted that athletes demonstrate a reduction, or regulation, of exercise intensity, in order to optimise performance, with such adjustments termed '*pacing*' (Tucker 2008). The following sections will outline the current state of knowledge concerning exercise performance and hyperthermia. Initially this will address the effect of heat stress on the determinants of endurance performance, before considering alternative, currently advocated models that specifically concern self-paced endurance performance in the heat.

2.4.1 Effect of heat stress on the determinants of endurance performance

The determinants of endurance performance model dictates that an individual's $\dot{V}O_{2max}$ defines the upper limit of aerobic metabolism, beneath which the interaction of the LT and RE will determine running velocity (Coyle 1995). In turn, the formula of Joyner (1991) (Equation 1, Section 2.1.1) demonstrates how the determinants integrate, when confounding individual factors such as motivation, dehydration, hyperthermia and carbohydrate depletion are excluded. Potentially, heat stress may influence each of these factors, and furthermore, heat appears to impair both $\dot{V}O_{2max}$ and LT, whilst the effects on RE are equivocal, so it is unclear whether the decrement in performance is proportional to the impairments within the model.

The most notable reduction appears to be in $\dot{V}O_{2max}$, although the uniformity of heat stress induced impairments to $\dot{V}O_{2max}$ is not without contention (Williams et al. 1962; Rowell et al. 1965; Pirnay et al. 1970). However, when methodological factors such as heat stress duration, training status and a concomitant elevated T_{CORE} are controlled for, there is greater unanimity with $\dot{V}O_{2max}$ reductions apparently proportionate to the T_{SKIN} elevation (Arngrimsson et al. 2004). The $\dot{V}O_{2max}$ reduction is likely mediated by an inability to achieve equivalent maximal cardiac output as in normothermic conditions (Rowell 1966), as demand for both cutaneous and muscular circulations compounds venous return (Gonzalez-Alonso & Calbet 2003). This is supported by recent data, whereby despite apparent alterations to the Frank–Starling relationship and cardiac contractility arising during passive heat stress (Wilson et al. 2009), no differences in myocardial systolic or diastolic function were observed during maximal exercise in the heat, compared with normothermic (Smith et al. 2015). The magnitude of $\dot{V}O_{2max}$ decline is typically between 5-20% as shown in Table 5, which is closely associated with reduced exercise capacity ($r = 0.82 - 0.84$, Arngrimsson et al. 2004) as shown in Figure 13.

Table 5: Effect of environmental heat stress (Heat) on VO_{2max} and Physical Work Capacity (PWC) during incremental intensity protocols, compared with temperate (Control) conditions. Modified from Nybo et al. (2014).

Study	n	Control	Heat	$\dot{V}O_{2max}$ change	PWC change
Arngrímsson et al. 2003	22	25°C	35°C	-4%	-8%
Arngrímsson et al. 2003			40°C	-9%	-14%
Arngrímsson et al. 2003			45°C	-17%	-32%
González-Alonso & Calbet 2003	8	14-16°C	44°C (perfused suit)	-8%	-28%
Klausen et al. 1967	6	25°C	40°C	-4%	
Lafrenz et al. 2008	10	22°C	35°C	-9%	-52%
Lorenzo et al. 2010	12	13°C	38°C	-16%	
Lorenzo et al. 2010	8	13°C	38°C	-18%	
Pirnay et al. 1970	18	23°C	46°C	-7%	
Pirnay et al. 1970	8	23°C	46°C	-27%	
Rowell et al. 1969	27	26°C	49°C	-3%	
Sawka et al. 1985	13	21°C	49°C	-8%	
Wingo et al. 2005	7	22°C	35°C	-7%	-9%

Figure 13: Relationship between reduction in exercise capacity (P_{TIME}) and reduction in $\dot{V}O_{2max}$ from 25 men (filled symbols; r = 0.84) and women (open symbols; r = 0.82). Data derived from seven repeat trials. Solid line indicates the mean for men and dashed line for women (Arngrimsson et al. 2004).

As previously discussed, there is an observable increase in blood lactate concentration under heat stress (see Section 2.3.4). Consequently, a reduction in the exercise intensity and fractional utilisation at the LT and LTP, compared with normothermic environments, is well-established (Powers et al. 1985; Young et al. 1985; Smolander et al. 1986; Tyka et al. 2000; Papadopoulos et al. 2008; Tyka et al. 2009; Lorenzo et al. 2010; De Barros et al. 2011). Consistent with the determinants model, an increased reliance on glycogen consumption and higher blood lactate concentration is likely associated with a decrease in endurance performance (Bassett & Howley 2000). Although decrements to the LT and LTP under heat stress are widely reported, different methodologies for defining and reporting LT precludes synthesis of reported effects across the literature, although notably, Lorenzo et al. (2010) has reported a ~5% reduction in LT in the heat from a range of methodologies during an incremental cycling test in the heat. Similarly, Tyka et al. (2009) reported an 8% reduction in LTP during incremental cycling at 31°C versus 23°C. De Barros et al. (2011) report a 17% reduction in maximum lactate steady state at 40°C compared with 22°C, however the larger decrement may reflect the prolonged nature of an MLSS protocol that likely results in greater heat strain. Furthermore, the majority of literature pertains to cycling, so the decrement may differ between exercise modes, due to a greater MHP when running, as well as participant fitness. Consequently, the precise anticipated reduction in LT and LTP for trained runners, exercising under heat stress, has yet to be established.

The effect of heat stress on RE is less well defined. Historically, elevated T_{CORE} has been associated with small increases in metabolic rate and therefore $\dot{V}O_2$ during prolonged submaximal exercise (Sawka et al. 1983; Shvartz et al. 1977; MacDougall et al. 1974). This likely reflects an increased metabolic demand from augmented circulation, sweating, ventilation and impaired efficiency of oxidative phosphorylation within the mitochondria during heat strain (Brooks et al. 1971, MacDougall et al. 1974). Consequently, Grimby (1962) has detailed a 5.5% increase in $\dot{V}O_2$ when T_{CORE} was elevated by 1.3°C, whilst Thomas (1999) observed a 6.2% increase, when T_{CORE} was elevated by 1.0°C. However, results appear equivocal, with both Maron et al. (1976) and Rowell et al. (1969) reporting no changes in $\dot{V}O_2$ during exercise in the heat. It has been suggested the increased metabolic demand arising from thermoregulatory responses and reduced mitochondrial efficiency may be compensated for by increased biomechanical efficiency from a warmer muscle (Saunders et al. 2004a).

In summary, evidence indicates the determinants of performance are impaired in the heat. However, it is currently unclear to what extent these impairments explain the reduction in endurance performance in the heat and whether these remain the primary determinants of endurance performance under heat stress. Such information will be relevant to those predicting and monitoring performance of those routinely training and competing in the heat in the heat.

2.4.2 Effect of heat stress during free-paced exercise

This section will introduce common models pertaining to endurance performance and pacing in the heat. Specifically, the influence of the additional thermal and perceptual strain under heat stress will be discussed, with regard to whether this acts independently to, or in combination with impairments to the determinants of endurance performance, in influencing performance in the heat.

Traditionally, literature suggested exercise ceases in the heat at a '*critical*' core (brain) temperature of 40°C irrespective of starting T_{CORE} (González-Alonso et al. 1999, Walters et al. 2000). When T_{CORE} exceeds 40°C, symptoms of exertional heat illness typically present, resulting in exercise cessation and potentially severe exertional heat illness (Armstrong et al. 1996). The apparent independence of the critical T_{CORE} from both acclimation and hydration status (Nielsen et al. 1993), implicated a high T_{CORE} as a direct contributing factor to fatigue. Whilst the precise mechanisms behind this apparent internal threshold temperature remained unclear (Siegel & Laursen 2012), a high T_{CORE} may independently reflect a central nervous system protective mechanism, which in turn limits motor-output (Cheung 2007). More recently, attention has

been drawn to the associated high T_{SKIN} and significant cardiovascular strain at the point of exercise cessation in many studies demonstrating an apparent critical T_{CORE}, suggesting fatigue was not a consequence of solely the absolute body temperature, but rather coinciding with a combination of cardiovascular and sensory impairments (Ely et al. 2009; Sawka et al. 2011). Furthermore, a critical T_{CORE} has been proposed to be a laboratory phenomenon, particularly during fixed intensity exercise (Tucker & Noakes 2009; Marino 2011), as athletes may exceed this internal temperature during free-paced trials in the field (Ely et al. 2009; Lee et al. 2010; Racinais et al. 2015b). In contrast to a critical T_{CORE} hypothesis, an anticipatory regulation of exercise intensity model has been advocated, suggesting most self-paced exercise is voluntarily controlled in advance of an excessive rise in T_{CORE} (Tucker & Noakes 2009). Accordingly, anticipatory regulation states that an individual's brain regulates exercise intensity to control, and typically lower MHP, thereby ensuring exercise is completed without a catastrophic failure of homeostasis as with a critical T_{CORE}. The ability of runners to pace themselves to throughout hot, long distance events such as marathons, before demonstrating an end-spurt, supports the premise of central anticipation, rather than localised fatigue in the muscle (Noakes et al. 2009; Tucker & Noakes 2009).

However, whilst more widely accepted than a critical T_{CORE} hypothesis to explain fatigue in the heat, anticipatory regulation is not without criticism (Jay & Kenny 2009; Ravanelli et al. 2014). Notably, Jay and Kenny (2009) have questioned the supposition that regulation can be based upon BHS (Tucker et al. 2006), as there can be no feed-forward mechanism for heat storage, given it represents the instantaneous difference between the MHP and net heat loss. Indeed, recent evidence demonstrates reductions in self-paced exercise intensity in the heat were not mediated by early differences in BHS (Ravanelli et al. 2014). However, the premise that exercise intensity is the consequence of some form of internal regulation remains.

To mediate between the apparently contrasting models of anticipatory regulation and critical T_{CORE}, Schlader, Stannard, et al. (2011a) have suggested these models may in fact complement each other, existing on a continuum. At one end of the continuum, fatigue manifests through a progression of processes that develop into voluntary exhaustion, before the loss of behavioural thermoregulation and '*catastrophic'* failure at the other end of the continuum. Schlader, Stannard, et al. (2011a) provided evidence from a retrospective analysis of the literature to demonstrate alterations in work rate, at submaximal intensities in accordance with sensations of fatigue, to ensure the safe completion of the exercise bout in hand. Such an observation is underpinned by the principle of anticipatory regulation. However, under certain circumstances, such as in a competition or when a highly motivated athlete is

exercising, Schlader, Stannard, et al. (2011a) suggest these fatigue signals can be over-ridden and exercise performance is limited by volitional exhaustion i.e. a critical core temperature.

In addition to these prominent models, it has been suggested that the role of cardiovascular stress in determining exercise performance in the heat may have been overlooked (Périard 2013). Endurance performance in the heat has been shown to be associated with a relative exercise intensity, as a percentage of $\dot{V}O_{2max}$, which appears to be consistent despite the reduction in $\dot{V}O_{2max}$ in the heat (Cheuvront et al. 2010; Périard et al. 2011; Périard 2013; Racinais et al. 2015b). This assertion is supported by the data of Lorenzo et al. (2010) who reported an 8% increase in time trial performance alongside an 8% increase in $\dot{V}O_{2max}$. Similarly, the recent study by Racinais et al. (2015b) reported a progressive reduction in exercise intensity during a time trial in the heat, compared to cool conditions, which the authors suggest reflects a progressive reduction in $\dot{V}O_{2max}$ as hyperthermia develops, and therefore the maintenance of a physiological threshold or relative exercise intensity. In this study, athletes began a 43 km cycling time trial at the same intensity in both cool and hot conditions, with a progressive reduction in power output occurring after 10 km, however heart rate remained elevated and stable throughout the time trial. In accordance with the progressive reduction in $\dot{V}O_{2max}$, power output reduced. Unlike the anticipatory regulation model, a cardiovascular model does not immediately explain how an end-spurt may be achieved in the heat, given individuals appear to be operating at a physiological threshold. However, these authors have suggested this may be achieved through anaerobic energy provision (Racinais et al. 2015b).

The evidence of Racinais et al. (2015b) demonstrates $\dot{V}O_{2max}$ to be an important determinant of endurance performance in the heat, however $\dot{V}O_{2max}$ represents the integrative ability of a selection of physiological processes, therefore in itself, it may not be the single best determinant. Consequently, other research has highlighted the influence of high T_{SKIN}, alongside a high T_{CORE}, in determining exercise performance under heat stress (Sawka et al. 2013; Lee et al. 2015; Flouris & Schlader 2015). A high T_{SKIN} has direct cardiovascular consequences, due to the associated skin blood flow requirements, but also heavily influences thermal perception and therefore self-selected exercise intensity (Taylor et al. 2009). From a cardiovascular perspective, a high T_{SKIN} that occurs independently of an elevated T_{CORE}, does not result in the typical reduction in left ventricular end diastolic volume and in turn stroke volume (Lee et al. 2015) and therefore $\dot{V}O_{2max}$ (Arngrimsson et al. 2004). Therefore, Flouris and Schlader (2015) recently proposed that when only T_{SKIN} is elevated, reductions in self-selected exercise intensity may be mediated by alterations to thermal perception and perceived exertion. However, when

both elevated T_{CORE} and T_{SKIN} present, cardiovascular factors likely determine voluntary reductions in exercise work rate.

In order to model these apparently interrelated and progressive perturbations during exercise in the heat, Nybo et al. (2014) recently proposed a model of endurance performance under hyperthermia (Figure 14). This model suggests fatigue to be multifactorial, highlighting specific roles for cardiovascular, metabolic, neurological, respiratory, psychological and sensory alterations.

Figure 14: Integrative model proposed by Nybo et al. (2014) with the potential cardiovascular, respiratory, central nervous system and peripheral factors that may influence fatigue during prolonged exercise in the heat. Central nervous system alterations arise from changes in perceptual and psychological factors. Elevated tissue temperature or impaired oxygen delivery may directly result in peripheral muscular fatigue, depending on exercise intensity. The associated afferent feedback from elevated T_{SKIN} and cardiovascular strain also influences the central nervous system response. Event characteristics including absolute metabolic heat production, duration, environmental conditions and training status will determine the relative influence of each factor in a given situation.

The model states hyperthermia-induced changes may influence muscle function and therefore exercise performance if CO becomes inadequate to support muscle blood flow and oxygen delivery declines, such as at $\dot{V}O_{2max}$. However, at submaximal intensities, muscle blood flow and $\dot{V}O_2$ are maintained, regardless of cardiovascular strain and/or high skin temperature. As hyperthermia progresses, a combination of central and peripheral fatigue presents, with central fatigue apparently related to the dopaminergic system. Conversely, afferent feedback from core, muscle and skin temperatures, increased ventilation rate, cardiovascular strain and metabolic alterations within the muscle appear to determine peripheral feedback and in turn exercise intensity. However, the authors stress the model is situation specific, representing a theoretical integration of factors, with the precise role of each factor depending on the degree of hyperthermia and the exercise intensity (Nybo et al. 2014).

2.4.3 Determinants of fatigue in endurance running

The model of Nybo et al. (2014) demonstrates the interrelation of different physiological impairments under heat stress, but does not make these relationships directional as the specifics of an exercise bout may alter the importance of specific factors. For example, cardiovascular strain and the subsequent impairment in $\dot{V}O_{2max}$, may be greater in endurance running, compared with cycling, due to the greater MHP and reduced convective cooling arising from a lower maintained velocity during running (Chan et al. 2008, Millet et al. 2009). Recent evidence indicates $\dot{V}O_{2max}$ to be a primary regulator of exercise intensity in the heat (Periard & Racinais 2015), therefore mediating the decline in $\dot{V}O_{2max}$ in the heat would appear to be a priority for an endurance runner. However concomitantly, an elevated T_{SKIN} influences both cardiovascular and perceived thermal and exertional strain (Schlader, Simmons et al. 2011a). Therefore, the importance of T_{SKIN} as a regulator of exercise intensity in the heat is being realised (Schlader, Simmons et al. 2011a). However, independently elevating T_{SKIN} does not impair $\dot{V}O_{2max}$ (Arngrimsson et al. 2004), therefore T_{SKIN} alone would not appear to be a primary determinant of fatigue during endurance running in the heat. Rather, when an elevated T_{SKIN} presents alongside an elevated T_{CORE}, then the T_{SKIN} appears to determine the magnitude of $\dot{V}O_{2max}$ reduction (Arngrimsson et al. 2004) due to the increasing skin blood flow demands for heat dissipation (Cuddy et al. 2015; Cheuvront et al. 2010). Therefore, it would appear important to maintain the core:skin gradient, which otherwise narrows as T_{CORE} and T_{SKIN} increase (Cheuvront et al. 2010). This trend may be exacerbated by skin blood flow reaching maximal levels at an approximate T_{CORE} of 38°C (Gonzalez-Alonso et al. 2008), impairing heat dissipation at the skin. As the core:skin gradient narrows, increased skin blood flow reduces stroke volume and in turn impairs $\dot{V}O_{2max}$ (Périard et al. 2011; Lee et al. 2015). Therefore, an

individual with a lower RE would appear to benefit from a reduced MHP and therefore better maintaining the core:skin gradient.

From a perceptual perspective, elevated T_{SKIN} influences thermal sensation, thermal comfort and ultimately RPE, all of which are associated with the voluntary reduction of exercise intensity in the heat (Flouris & Schlader 2015). Indeed, the interrelation of $T_{SKIN,}$ $\dot{V}O_{2max}$ and RPE has previously been highlighted by Schlader, Simmons et al. (2011b), reinforcing the potential for fatigue during endurance running to be determined by a combination of these variables, and ultimately multifactorial in nature (Nybo et al. 2014). Consequently, the role of the LTP, another determinant of endurance performance in cool conditions, may also be significant, as this marker represents changes in metabolism and fuel utilisation under heat stress, that result in both increased glycogenolysis (Febbraio et al. 1994) and metabolic acidosis (Jones & Carter 2000). However, the combined model of $\dot{V}O_{2max}$, LTP and RE has yet to be assessed during endurance running in the heat.

Having considered underpinning mechanisms of endurance running performance, both in and out of the heat, this literature review will now focus on interventions that can be used to enhanced performance in the heat. For the applied physiologist, models such as that of Nybo et al. (2014) highlight not only the multitude of mechanisms through which performance in the heat is impaired, but equally, potential avenues that interventions may target to ameliorate decrement to endurance performance. The available evidence indicates interventions should prioritise alleviation of $\dot{V}O_{2max}$ and perceptual strain, whilst maintaining the core:skin gradient. Accordingly, exercise tests for endurance performance in the heat should encapsulate as many of the physiological responses as possible, in order to understand and subsequently improve how interventions can be used. This will begin with the traditional, chronic approach that facilitates heat adaptation.

2.5 Chronic strategies for exercise in the heat

In preparation for sporting competitions that occur under heat stress, such as the summer Olympic Games, and to mitigate against the thermal challenges previously detailed, coaches and sport scientists may have to balance opposing conditioning goals. Specifically, a training taper phase would appear in direct conflict with the need to undertake repeated bouts of exercise in a hot environment to provide habituation to the competitive environment. Such habituation may occur through heat acclimatisation, within a naturally hot environment during the weeks prior to competition. Alternatively, athletes may complete repeated bouts of exercise under artificial heat stress, termed heat acclimation (HA). Heat acclimation is

habitually classified as either short term (STHA, <7 days), medium term (MTHA, 8-14 days) or long term (LTHA, >15 days) (Garrett et al. 2011). Acclimation typically involves a total daily heat exposure of about 2 hours, accompanied by exercise (Chalmers et al. 2014; Racinais et al. 2015a; Périard et al. 2015). Such long term, chronic interventions, have traditionally been the preparation undertaken by those who will compete in the heat.

Heat acclimation affords a potentially more convenient alternative for those who do not reside in a hot and humid environment to remotely, and artificially, promote thermoregulatory adaptations (Armstrong & Maresh 1991). Therefore, the opportunity to undertake HA, rather than acclimatization, may reduce the logistical and financial burden of arriving in a hot environment in the weeks prior to competing. Notwithstanding, HA remains a chronic intervention that necessitates a lot of an athlete's time, which alludes to why STHA appears the most widely adopted approach within sport (Périard et al. 2015).

Thus, the need for a time-efficient HA protocol that complements a taper phase is apparent, which may be reflected by the recent prominence of alternative acute thermal interventions such as precooling. This need has also focussed attention towards remodelling the traditional chronic HA approach, with HA through relatively higher intensity exercise of shorter duration gaining prominence, rather than long duration, low intensity exercise (Houmard et al. 1990; Sunderland et al. 2008), whereby HA may in fact complement, rather than compromise a taper. This section will first introduce the physiological adaptations conferred by heat acclimation strategies and the associated effects on endurance performance, before examining the different models of heat acclimation training.

2.5.1 Physiological adaptations to heat acclimation

The heat acclimated phenotype arises from adaptation across multiple systems. Before exploring the individual adaptations and exploring the underpinning mechanism(s), an overview of the physiological adaptations and functional consequences arising from heat acclimation is provided below in Table 6.

Table 6: Physiological adaptations and functional consequences associated with the heat acclimation phenotype that lead to improved thermal comfort and submaximal aerobic performance, and increased maximal aerobic capacity (Périard et al. 2015).

Adaptation	Consequence	Adaptation	Consequence
Core temperature	Reduced	Cardiovascular stability	Improved
Rest (temperate) – decreased		Heart rate – lowered	
Exercise – decreased		Stroke volume – increased	
Sweating	Improved	Cardiac output – better sustained	
Onset threshold – decreased		Blood pressure – better defended	
Rate – increased		Myocardial compliance – increased	
Sensitivity – increased		Myocardial efficiency – increased	
Skin temperature	Reduced	Cardioprotection – improved	
Skin blood flow	Improved	Skeletal muscle metabolism	Improved
Onset threshold – decreased		Muscle glycogen – spared	
Sensitivity – increased		Lactate threshold – increased	
Rate (tropical) – increased		Muscle and plasma lactate – lowered	
Fluid balance	Improved	Muscle force production – increased	
Thirst – improved		Whole-body metabolic rate	Lowered
Electrolyte losses – reduced		Acquired thermal tolerance	Increased
Total body water – increased		Heat shock proteins expression – increased	
Plasma volume – Increased		Cytoprotection – improved	

2.5.1.1 Thermoregulation

Heat acclimation is characterised by a myriad of physiological adaptations, many of which independently contribute towards improved thermoregulation, with a predominant central thermoregulatory adaptation concerning a decreased resting and exercise T_{CORE} (Armstrong & Maresh 1991). The reduction is typically between 0.3-0.5°C, depending upon the method and duration of acclimation (Buono et al. 1998), as well as time of day that acclimation occurs (Shido et al. 1999). This alteration would appear to follow a reduction of the POAH set point, and possibly improved heat dissipation efficiency (Armstrong 1998). The work of Kampmann et al. (2008) alludes to the importance of the exercise stimulus during HA training to elicit this adaptation, having demonstrated a small reduction in T_{CORE} (~0.2°C) following matched intensity normothermic training. A reduced T_{CORE} affords individuals an enlarged heat storage capacity before heat strain becomes significant and heat illness may present (Casa et al. 2015). Therefore, the potential for a cooler body following HA may help to maintain $\dot{V}O_{2max}$, as demonstrated by the maintenance of skeletal muscle blood flow and $\dot{V}O_{2max}$ in normothermic conditions, compared with hot conditions (Périard et al. 2011).

2.5.1.2 Cardiovascular

A primary adaptation of HA is increased cardiovascular stability, characterised by a reduction in resting and exercising heart rate (Rowell et al. 1967), and hypervolaemia (Sawka et al. 2000). Collectively, these adaptations may partially, or completely at lower exercise intensities, attenuate the elevated heart rate observed during submaximal endurance exercise in the heat. Hypervolaemia is manifested through an increased plasma volume (PV), without an

increase in cell mass, which is usually (Shapiro et al. 1981; Sawka et al. 2000; Corbett et al. 2014) but not always (Dill et al. 1966; Young et al. 1993; Neal et al. 2015), present following heat acclimation. An enhanced central blood volume serves to increase venous return, thus maintaining stroke volume and cardiac output through an increased cardiovascular reserve (Fortney et al. 1981). A large reduction in resting and exercising heart rate is observed within 5 days, with no further reduction evident beyond 7 days (Pandolf 1998), which is in keeping with the time-course of plasma volume expansion during heat acclimation (Armstrong & Maresh 1991). As such, it has been suggested that a 1% increase in plasma volume elicits a 1% reduction in exercising heart rate (Convertino 1983) and that exercising heart rate is 'moderately-to-largely' related to plasma volume expansion following heat acclimation (Buchheit et al. 2011). Accordingly, following a 10 day MTHA programme which resulted in a 6.5% increase in PV, Lorenzo et al. (2010) details a 15 b.min^{-1} reduction in exercising heart rate on day 10 of training in the heat, alongside increased maximal cardiac output and stroke volume in the cool, but not in the heat as shown in Figure 15. The reported increase in PV appears typical following HA, and is in agreement with other literature (Nielsen et al. 1993; Patterson et al. 2004a). However, it should be noted that some of the reduction in exercising heart rate on day 10 may be attributable to a reduced relative exercise intensity arising from the prescription of exercise based on a percentage of $\dot{V}O_{2max}$, given that $\dot{V}O_{2max}$ was increased by 8% following HA.

Figure 15: Effect of heat acclimation on maximal cardiac output (A), stroke volume (B), and heart rate (C) during a $\dot{V}O_{2max}$ test in cool (13°C) and hot (38°C) environments. Data are means ± standard error for both heat acclimation (*n* = 12) and temperate training control (*n* = 8).* denotes significant (*p*<0.05) difference versus pre-acclimation within the same environmental condition (Lorenzo et al. 2010).

Plasma volume expansion is thought to be primarily mediated through the release of plasma aldosterone (Garrett et al. 2014). Aldosterone is produced by the adrenal cortex to facilitate reabsorption of sodium ions in the kidneys and sweat glands, thereby controlling extracellular fluid volume and blood volume. The release of aldosterone is mediated by plasma

potassium (K$^+$) concentration, activity of the renin-angiotensin system, as well as plasma Na$^+$ concentration (Garrett et al. 2011). In addition to aldosterone, Patterson et al. (2004b) suggested a contributing role for the movement of total plasma protein to the interstitial fluid in the vascular space, either independently or through interacting with aldosterone. Increased plasma protein serves to exert colloidal pressure within the plasma, thereby drawing water into the circulatory system (Patterson et al. 2004b). Garrett et al. (2014) highlighted a significant role for aldosterone, in response to HA training using a controlled hyperthermia model and permissive dehydration, to the magnitude of 1.8% of body mass. The authors suggest permissive dehydration presents an additional, independent stressor, supported by the heightened response of the fluid and stress-regulatory hormones such as aldosterone, arginine vasopressin and cortisol (Sawka et al. 1987). Therefore, the type of HA training and dehydration level may determine a heightened plasma volume and cardiovascular adaption (Garrett et al. 2014; Racinais et al. 2015b; Mee et al. 2015b) and *ab-libitum* drinking during HA may help explain the failure of some HA protocols to elicit PV expansion. Although it should be acknowledged that support for plasma volume expansion following permissive dehydration controlled hyperthermia is not unanimous (Neal et al. 2015).

From a thermoregulatory perspective, an increased PV would also permit a greater volume of blood for cutaneous circulation, facilitating enhanced heat dissipation via radiation and convection. However, studies that have artificially and acutely enhanced PV have not observed conclusive differences in thermoregulatory variables such as T_{CORE}, T_{SKIN} and sweat response (Sawka et al. 1983; Fortney et al. 1981). Thus, whether increased PV directly improves thermoregulatory function remains equivocal (Sawka et al. 2000).

Acute plasma volume expansion has been highlighted as a likely mechanism leading to improved endurance exercise performance in temperate (Corbett et al. 2014) and hot (Sims et al. 2007) conditions. Hypervolaemia may proffer the greatest benefit at maximal exercise intensities in the heat, where failure to sustain cardiac output and mean arterial pressure is most pronounced (González-Alonso & Calbet 2003). As such, Lorenzo et al. (2010) reported improved $\dot{V}O_{2max}$ in hot and cool conditions, as well as time trial performance in hot and cool conditions, following PV expansion of 6.5%. Similarly, Coyle et al. (1990) has demonstrated an enhanced $\dot{V}O_{2max}$ in normothermic conditions following acute PV expansion via infusion of a dextran solution 24 hours prior to exercise. However, the improvements in the study of Lorenzo and colleagues (2010) followed a HA programme, so improvements cannot be solely attributed to PV expansion, as other centrally mediated aerobic adaptations, such as improved contractile efficiency of the heart (Sawka et al. 2000; Horowitz 2002) and reduced sympathetic

nervous activity (Berlyne et al. 1974; Hodge et al. 2013) will likely have contributed. Indeed, Horowitz (2002) highlights the importance of an improved excitation contraction coupling cascade following HA which facilitates an improved ejection fraction and, in turn, stroke volume. Such changes may occur through myocardial adaptation, whereby increased compliance and distribution of myosin isoenzymes reduce the myocardial energy cost (Horowitz et al. 1986). In summary, cardiovascular adaptions from HA are significant and wide-ranging, with these changes likely underpinning improved exercise performance.

2.5.1.3 Skin blood flow

Skin blood flow appears to be increased whilst exercising at a given T_{CORE} following HA, likely mediated through an enhanced blood plasma volume (Nadel et al. 1974; Roberts et al. 1977; Lorenzo & Minson 2010), as shown below in Figure 16. Roberts & Wenger (1979) suggested HA may also lower the T_{CORE} threshold for cutaneous vasodilation, which is typically elevated during exercise compared with rest (Kenney & Johnson 1992). This has the effect of presenting a lowered T_{SKIN} whilst exercising in both hot and normothermic conditions (Nielsen et al. 1993; Buono et al. 2009; Lorenzo & Minson, 2010).

Figure 16: Effect of 10 days fixed intensity HA versus normothermic control training on cutaneous vascular conductance (CVC) in response to 1 , 10 and 100 mMol.L^{-1} acetylcholine infusion. * denotes a significant difference between acclimation and control group ($p<0.05$) (Lorenzo & Minson 2010).

Whilst these adaptations could be suggestive of central alterations in the POAH, equally peripheral vascular adaptations may be responsible for improved vasodilatory efficiency, possibly through an increase in the number of muscarinic receptors, or the responsiveness and sensitivity to localised acetylcholine (Lorenzo & Minson, 2010). Interestingly, maximal forearm skin blood flow does not appear to change following a period of heat acclimation, indicating the observed changes are attributable to improvement in cutaneous vascular function and not to structural changes that limit maximal vasodilator capacity (Lorenzo & Minson, 2010). Peripheral vasodilatory adaptations likely contribute towards the reduced cardiovascular strain during exercise following HA by enhancing the potential for radiative and convective heat loss, and alongside sudomotor alterations, more effectively maintaining the core-to-skin gradient. In turn, such adaptations aid heat dissipation and contribute towards an alleviation of heat strain, minimising the competition for cardiac output whilst exercising in the heat.

2.5.1.4 Skeletal muscle metabolism

Heat acclimation would appear to ameliorate many of the alterations in muscle metabolism observed under heat stress. Notably, HA reduces the enhanced submaximal circulating lactate concentration and reliance on carbohydrate metabolism, relative to cooler conditions (Febbraio et al. 1994; Young et al. 1985). These alterations likely occur as a consequence of a reducing resting and exercising body temperature, but there may be other contributing mechanisms (Febbraio 2001). For example, a reduced body temperature would promote a reduction in plasma adrenaline, thereby reducing glycogenolysis following HA (Febbraio et al. 1994). Indeed, there may even be a reduction in the overall aerobic metabolic rate following HA (Sawka et al. 1983; Young et al. 1985), as has been reported in hot climates during warmer months (Hori 1995). A greater contribution to energy supply from lipolysis is supported by reductions in lactate accumulation, the respiratory exchange ratio, blood glucose concentration and glycogen depletion at given exercise intensities following STHA (Sunderland et al. 2008; Febbraio et al. 2004). The changes in lactate from HA appear to be most pronounced during submaximal exercise, with lactate removal kinetics unchanged across the 60 min following maximal exercise (DiLeo et al. 2014). Finally, a contributing factor may also be an increased blood plasma volume, by mediating against the compromised visceral vasoconstriction in the heat, thus maintaining lactate conversion in the liver (Rowell et al. 1968).

2.5.1.5 Sudomotor function

Adaptations to sudomotor function appear to require a greater thermal stimulus than cardiovascular adaptations, typically requiring between seven and ten days of HA (Patterson et

al. 2004b). Sudomotor adaptations are characterised by an enhanced sweat rate and sensitivity at a given T_{CORE} (Sato et al. 1990; Roberts et al. 1977a; Yamazaki & Hamasaki 2003; Mee et al. 2015b; Gibson et al. 2015a), but likely not an increased maximum sweat rate (Lorenzo and Minson, 2010). Furthermore, there is likely not a redistribution of sweat secretion towards the limbs (Patterson et al. 2004b), despite early evidence arguing for such an alteration (Hofler 1968). Complete adaptation is thought to occur after 7-10 days of heat exposure (Patterson 2004), but 75% of adaptations are evidenced from 4-6 days (Pandolf 1998). The findings of Patterson et al. (2004b) suggest sudomotor adaptation may be accelerated during controlled hyperthermia acclimation training, with respect to the traditional timescale of 10-14 days (Pandolf 1998). A principle objective of HA protocols must apparently include maintaining an elevated T_{SKIN}, as sudomotor function adaptation appears insensitive to heat strain without elevated T_{SKIN} (Chen & Elizondo 1974). Augmented sudomotor function lowers T_{SKIN} through evaporative heat loss, potentially reducing cardiovascular strain arising from peripheral vasodilation and appears to make an important contribution towards an improved thermal sensation (Schlader, Simmons, et al. 2011b). Despite the unanimity of evidence supporting an enhanced sweat loss following HA, recently Poirer et al. (2014) reported an improvement in evaporative heat loss of 11% can be achieved following 14 days of heat acclimation (90 min cycling at 50% $\dot{V}O_{2peak}$ in 40°C and 20% RH.

The enhanced sweat rate is likely attributable to sweat gland hypertrophy (Sato & Sato 1983; Chen & Elizondo 1974), whilst an earlier internal temperature threshold or set point, represents increased cholinergic sensitivity of the eccrine sweat gland (Nadel et al. 1974; Lorenzo & Minson 2010). The exogenous administration of acetylcholine after HA supports this assertion (Collins et al. 1965; Lorenzo & Minson 2010). The combination of a lowered sweat onset and reduced resting T_{CORE} represents an improved centrally mediated response to heat stress (Nadel et al. 1974; Roberts & Wenger 1979). As well as improvements concerning the volume and onset of sweating, it is suggested that the composition of sweat may be altered following HA to protect against the loss of electrolytes (Garrett et al. 2014). Specifically, when fluid homeostasis is stressed during HA sessions, increased fluid-electrolyte retention may be facilitated by PV expansion, which is in turn attributable to increased aldosterone and arginine vasopressin and a cardiovascular response to heat stress (Garrett et al. 2014). Such changes are seen through reduced sweat sodium (Kirby & Convertino 1986) and mineral (Chinevre et al. 2008) concentrations following HA. Despite the apparent benefits of an enhanced sweat rate, it should be noted that sudomotor adaptations may also lead to an earlier onset of dehydration and associated cardiovascular consequences (Gonzalez-Alonso et al. 1998). Thus, the event

length and hydration strategies should be considered when planning a HA protocol and thermal adaptations for a specific event.

2.5.1.6 Cytoprotection

Adaptation to HA is also apparent at a cellular level, with cytoprotection necessary to mitigate against the cellular abnormalities associated with severe heat stress. These abnormalities include protein denaturation, translational inhibition, ribosomal biogenesis attenuation, and cytoskeletal damage (Mizzen & Welch 1988), which collectively can be mediated through acquired thermal tolerance (Horowitz 2014). An increased extracellular concentration of stress response proteins, most notably heat shock proteins (HSP), is observed during a heat exposure (Gibson et al. 2014) and may be maintained through repeated subsequent exposures (Sandström et al. 2008). This response is not unique to heat stress, with elevations in HSP also observed following stresses that include hypoxia, substrate depletion and dehydration (Gething & Sambrook 1992). Thus any protection afforded through repeated heat exposures may also have efficacy across a variety of environmental situations (Kim et al. 2004; Gibson et al. 2015b). Specifically, HSPs function as molecular chaperones assisting with protein folding and refolding after denaturation and therefore assist in maintaining protein homeostasis (Kresfelder et al. 2006; Schmitt et al. 2007).

The most inducible HSP is the 70 kDa family (HSPA1A), categorised based on the molecular weight of the proteins (Moseley 1997). HSP70 is a stress protein family whose inducible form (HSP72), rather than the extracellular expression, promotes thermal tolerance by performing a chaperone function (Horowitz 2002). Consequently, basal HSP concentration has been proposed as a marker of HA status (Kim et al. 2004), with Kresfelder et al. (2006) having reported a positive relationship between HA criteria and levels of serum HSP70. Despite increased in vitro HSP70 expression being associated with elevated internal temperatures, the HSP70 response may in fact be independent of T_{CORE} (Skidmore et al. 1995) and may better reflect metabolic stress such as glycogen depletion (Febbraio et al. 2002) or hypohydration (Kim et al. 2004). However, whilst both heat exposure and high intensity exercise elicit a HSP response, the combined metabolic and thermal stress elicits a greater HSP response than either stressor independently (Skidmore et al. 1995). It would appear that a stimulus whereby T_{CORE} is >38.5°C is required (Morton et al. 2009; Gibson et al. 2014) and that adaptation is not sensitive to the type of HA protocol (Gibson et al. 2015a), so long as sufficient endogenous strain is achieved. Sandström et al. (2008) has reported an initial increase in HSP70 levels during exercise under heat stress, reflecting enhanced thermotolerance, as well as upregulation of

HSP70 above basal levels following 15 days of HA. The greatest elevation in HSP levels occurred after 5 days, with the greatest response during exercise after 15 days. This is supported by the findings of McClung et al. (2008) who analysed baseline and ex vivo heat-induced expression of HSP72 and HSP90 in peripheral blood mononuclear cells (PBMCs) on day 1 and 10 of a HA protocol. Alongside classical physiological responses to HA, baseline levels of HSP72 and HSP90 increased by $17.7 \pm 6.1\%$ and $21.1 \pm 6.5\%$, respectively. Furthermore, the ex vivo heat inducibility of HSP72 was blunted after HA, and individuals demonstrating the adaptations to HA exhibited the greatest blunting of ex vivo HSP induction during heat stress. These data demonstrated that physiological adaptations in humans undergoing HA may be accompanied by both increases in baseline levels and changes in the regulation of cytoprotection. In addition to having a role for cellular thermotolerance, there is evidence to suggest a shared mechanism between the adaptation of cellular thermotolerance and traditional markers of heat acclimation in humans. Through the inhibition of HSP72 via quercetin supplementation, Kuennen and colleagues (2011) highlighted impaired cellular and traditional systemic heat adaptations. Thus, it is apparent the ability of humans to adapt and develop thermotolerance is multifactorial, of which the heat shock response is an important, acute and transient component.

2.5.2 Endurance performance following heat acclimation

The combined effect of the previously detailed physiological adaptations that enhance heat dissipation is succinctly illustrated by the theoretical model from Taylor and Cotter (2006) of body temperature across a range of endurance distance events, pre and post HA, as shown in Figure 17.

The predictive model of Taylor and Cotter (2006) demonstrates how an acclimated athlete may display a smaller increase in body temperature than when non-acclimated, which will delay, or possibly prevent reaching a T_{CORE} that has been associated with fatigue and exercise cessation (Cheung 2007), in events of 5 km and above. Notably, the reduced BHS likely reflects acclimation-induced changes in sweating, skin blood flow and skin temperatures (Taylor & Cotter, 2006). Consensus across experimental studies supports this model, with a recent review by Guy et al. (2014) identifying beneficial effects of heat acclimation on endurance performance using both STHA (2.4 ± 3.5 %) and MTHA protocols (10.2 ± 14.0 %).

Figure 17: Predicted changes in mean body temperature for heat-acclimated and non-acclimated athletes at world record pace over distances from 100 m to marathon, in ambient and hot environments (Taylor & Cotter 2006).

Racinais et al. (2015b) recently demonstrated considerable changes in 43 km field-based cycling time trials (~37 °C) following heat acclimatisation. Although starting exercise intensity was similar in the heat, to a cold, control condition (~8 °C), from ~9 km a progressive and marked decrease in power output was observed in the heat, which was partly recovered after 1 week of heat acclimatisation and almost fully restored after 2 weeks. The authors attributed the initial reduction in performance in the heat, and subsequent improvement, to the maintenance of a relative exercise intensity, reflecting the impairment to $\dot{V}O_{2max}$ in the heat (Nybo et al. 2014), which may be partially mediated by an enhanced $\dot{V}O_{2max}$ following HA (Lorenzo et al. 2010). It has been reported however, that HA may not entirely abate the decline in $\dot{V}O_{2max}$ in the heat, with Sawka et al. (1985) reporting a 3.5% increase in $\dot{V}O_{2max}$ in the heat, compared with the initial ~8% reduction. Similarly, Lorenzo et al. (2010) reported an 8% improvement in $\dot{V}O_{2max}$ following LTHA, compared with an initial ~20% reduction. Interestingly, an 8% improvement in 1 hour cycling time trial performance was also observed, in accordance with relative exercise intensity determining endurance performance (Périard et al. 2011; Périard, 2013). It should also be noted however, that the improvements reported by Lorenzo et al. (2010) occurred alongside increases in LT, PV expansion, lower skin temperatures, and a larger core-to-skin gradient, each of which have the potential to contribute to performance in the heat (Nybo et al. 2014). The authors attributed changes in $\dot{V}O_{2max}$ to the enhanced PV, maintaining cardiac output for longer in the heat. Similarly, the increased LT was attributed to

reduced splanchnic vasoconstriction arising from a reduced heat strain, and muscle metabolism adaptation. The observed changes from Lorenzo et al. (2010) for $\dot{V}O_{2max}$, LTP, maximal cardiac output and time trial performance are shown below in Figure 18.

Figure 18: Percentage change for cardiorespiratory variables (Q_{cmax} = maximal cardiac output) and time trial performance in hot and cool conditions, following HA. *$p < 0.05$ vs. pre acclimation within the same environmental condition (Lorenzo et al. 2010).

While changes in LT arising from heat acclimation appear supported elsewhere in the literature (Chalmers et al. 2014; Neal et al. 2015), any changes in RE are less clear. Saunders et al. (2004a) highlighted the potential for acclimation to improve RE, as a consequence of PV expansion and reduced cardiovascular strain, however this question does not appear to have been directly addressed within the literature. In conclusion, chronic heat acclimation/acclimatization strategies appear to have great efficacy for improving endurance performance in hot environments. However, specific questions remain, with a relative dearth of research pertaining to running time trials, and a wide variety of HA protocols adopted. Given the variation in length of HA training programme, as well as the ability to adopt different models, as discussed in the following section, it is unclear to what extent the determinants of endurance performance and time trial performance can be enhanced in trained runners.

2.5.3 Models of heat acclimation

The overall training stimulus during HA is determined by the environmental heat stress, length and regularity of exposure and exercise intensity (Garrett et al. 2011). Accordingly, HA models may manipulate different components of this stimulus to meet specific physical conditioning goals for different sports. As well as distinctions between short, medium and long-term acclimation, further distinctions between programmes can be made based on exercise prescription. Garrett et al. (2011) classifies HA protocols as self-regulated exercise, constant work or controlled hyperthermia, also known as isothermic. Self-regulated protocols involve participants self-selecting exercise intensity based on thermal comfort and aerobic conditioning (Garrett et al. 2011). However, lack of experimenter control over exercise intensity leads to large variations in MHP, which may preclude the use of such protocols for answering research questions where training intensity must be closely controlled and matched between individuals (Taylor 2014).

2.5.3.1 Constant work

Constant work protocols, involving a consistent exercise intensity that is often fixed at a percentage of $\dot{V}O_{2max}$, are considered the 'traditional' HA approach and as a result, considerable evidence documents the response to such protocols (Garrett et al. 2011; Taylor 2014). From this evidence, it would appear the optimal protocol involves 60-100 minutes of exercise per day, at intensities greater than 40% $\dot{V}O_{2max}$ for 10 - 14 days, completed under heat stress that exceeds the environment being prepared for (Sawka et al. 2011; Taylor 2014). Constant work protocols benefit from being simple to administer as they typically adopt a fixed intensity for the duration of the training session. However, they do not provide a homogenous thermal strain across participants because of differences in MHP at individual's relative proportion of $\dot{V}O_{2max}$, and thus do not always elicit the same adaptive response across individuals (Jay et al. 2011). Further, when exercise intensity is determined from pre acclimation testing, and heat stress is consistent throughout the protocol, the relative heat strain diminishes as adaptation occurs, such that it may become sub-optimal for adaptation (Taylor & Cotter 2006; Taylor 2014). During LTHA the reduction in relative strain has the potential to lead to the decay of cardiovascular adaptations before the end of the protocol (Patterson et al. 2004a). Thus, constant work protocols appear inefficient and require progression in exercise intensity or heat stress to maintain the thermal strain throughout the duration of the programme and maximise the potential for adaptation (Taylor 2014). Consequently, progressive protocols have been developed to incrementally increase heat stress across the training period (Daanen et al. 2011; Costa et al. 2014). This approach was successfully used by Costa et al. (2014) in preparation for

a 6-day ultra-marathon. Following three, 2-hour treadmill running sessions (60% $\dot{V}O_{2max}$) in 30°C, participants completed three sessions in 35°C and observed meaningful improvements in exercising T_{CORE} (~-0.2°C), heart rate (~-7 b.min^{-1}) and thermal comfort during subsequent heat stress tests. However, comparing these improvements to existing literature is difficult as this study did not include a control group who completed traditional fixed HA.

2.5.3.2 Controlled hyperthermia

An alternative approach targets the maintenance of a consistently high T_{CORE} throughout the HA programme, such that the relative strain becomes progressively harder with adaptations eliciting a reduced resting T_{CORE} and improved heat dissipation capability (Patterson et al. 2004a; Garrett et al. 2014). The controlled hyperthermia, or isothermic, model (ISO) is underpinned by the principle that a sustained high T_{CORE} for approximately 60 min is a primary effector to adaption. This follows the seminal work of Fox et al. (1963) who investigated the response to controlled increases in body temperature in 18 men across 12 days, and reported reductions in oral temperature and increased sweat rate during subsequent heat stress tests. Maintaining an elevated T_{CORE} may offer a more complete adaptation, as well as being more time efficient than the traditional approach (Gisolfi & Cohen 1978; Taylor 2000). A comparison of the T_{CORE} response to ISO and fixed-intensity HA models are shown below in Figure 19.

Figure 19: Core temperature changes on days 1, 4, 8 and 12 of two, 12-day heat acclimation regimens using either the fixed intensity (A– Steady-state work rate and thermal load) or isothermic (B - controlled hyperthermia methods). Insets show integrated core temperature for each day. A diminished thermal strain is evident across day 1-12 in the fixed intensity group (Taylor & Cotter 2006).

During ISO, exercise can be used discontinuously, to generate MHP to quickly and raise T_{CORE}, and then to maintain T_{CORE} at the desired level. Therefore, ISO often permits higher intensity work than in fixed intensity HA, which may not only result in a more representative and sport-specific exercise profile, but may also complement a traditional competition taper phase characterised by higher intensity and reduced exercise volume (Spilsbury et al. 2015). Sunderland et al. (2008) has advocated higher intensity exercise during acclimation, suggesting adaptive mechanisms may be enhanced when a greater level of stress is experienced, resulting in faster acclimation. This followed the work of Houmard et al. (2000), who applied short duration, high intensity exercise (75% $\dot{V}O_{2peak}$ daily, 30–35 min per day), and observed identical adaptation to a longer duration, lower intensity method (50% $\dot{V}O_{2peak}$ daily, 60 min per day). Subsequently, Garrett et al. (2009) has demonstrated the effectiveness of ISO in a short-term heat acclimation programme, requiring participants to exercise and ensure T_{CORE} was consistently above 38.5°C during each 90 min session for five consecutive days. After five days, reductions in T_{CORE} and heart rate were observed at the end of exercise, although not at rest. The lack of a reduction in resting T_{CORE} was not thought to be a limitation of the protocol, but rather an artefact of the time of day acclimation took place, as time of day of habitual heat exposures appears to determine resting T_{CORE} response (Shido et al. 1999). Garrett et al. (2009) also reported improved exercise capacity, a reduction in thermal and cardiovascular strain, and cellular thermo-protection through elevated resting plasma HSP72 concentration.

An internal temperature of 38.5°C would appear to represent a minimum endogenous threshold in order to induce cellular adaptation to thermal stress (Gibson et al. 2014). Furthermore, Patterson et al. (2004a) adopted an ISO model across 7 days of training, involving 90 min daily bouts, with much of the physiological and performance benefits conferred during this period, as after 21 days of traditional acclimation. In a direct comparison of ISO and fixed intensity methods across 10 days of HA, Gibson et al. (2015a) did not observe differences in physiological adaptation between methods, with all inducing HA, however this was achieved with a reduced total work (-9%) during ISO and higher mean exercise intensity (1.6 W.kg^{-1} fixed vs 2.0 W.kg^{-1} ISO). Therefore, despite the theoretical benefits of maintaining the potentiating stimuli of elevated T_{CORE} throughout a HA programme, recent evidence suggests the benefits of ISO may be most apparent in terms of maintaining sport-specific training intensity and efficiency of time, through a reduced training volume, rather than providing a greater physiological adaptation *per se* (Patterson et al. 2004a; Gibson et al. 2015a).

2.5.4 Induction and decay of heat acclimation

Awareness of the time-course for HA adaptation induction and decay is paramount not only for those looking to apply HA strategies with athletes, but also to ensure the valid assessment of exercise performance and capacity following a HA intervention. Complete physiological adaptation typically occurs after ~7–10 days (Pandolf 1998; Shapiro et al. 1998; Garrett et al. 2009). However, there is a rapid initial induction (75%) after ~4–6 days, characterised by heart rate and plasma volume changes (Armstrong & Maresh 1991). The reduction of T_{CORE} likely follows after ~7 days (Weller et al. 2007), with the adjustments in sweat response and composition adapting last (Cotter et al. 1997). An overview of this process is provided below in Figure 20. Commensurate with this timescale, larger effects of endurance performance have been reported following MTHA, compared with STHA, notably including larger reductions in endpoint exercising heart rate (STHA: -3.5 ± 1.8 % vs MTHA: -7.0 ± 1.9 %) and endpoint T_{CORE} (STHA: -0.7 ± 0.7 % vs -0.8 ± 0.3 %) (Guy et al. 2015).

Figure 20: Simplification of the induction of heat acclimation adaptation. NB. The magnitude and maintenance of these adaptations is dependent on the initial acclimation status, environmental conditions (i.e., dry or humid), exercise intensity, and acclimation model (Périard et al. 2015).

Cardiovascular stability appears to be compromised within days of ceasing HA (Horowitz 2014) and the HA phenotype is no longer observed after 3 weeks (Pandolf 1998). Therefore, whilst the induction of cardiovascular adaptations occurs within days, they also appear to be lost relatively quickly. This was demonstrated by Saat et al. (2005) who reported a greater relative loss of heart rate adaptation compared with changes in T_{CORE}, when assessed 4 days

after HA. These results are supported by Flouris et al. (2014) who observed changes in T_{CORE} persisting for at least 2 weeks, while changes in heart rate and heart rate variability were only partially evident after 2 weeks. A prominent adaptation, plasma volume (PV) expansion, appears to be lost very quickly and it has been proposed some LTHA strategies may demonstrate reductions in PV within the HA programme in the absence of a stepwise increase, or maintenance of intensity (Patterson et al. 2004a). Both Convertino et al. (1980) and Garrett et al. (2009) reported a return to baseline levels of PV within 1 week of the cessation of exertional heat exposures. Ultimately, this suggests changes that take longer to present, last longer (Pandolf 1998; Garrett et al. 2011). Of particular relevance for competition preparation is the notion for every two days spent not working in the heat, one day of adaptation is lost (Givoni & Goldman 1972).

As the HA phenotype decays, Taylor (2000) suggests one heat exposure for every 5 days away, may be sufficient to maintain adaptations. Consequently, Weller et al. (2007) has reported the re-induction of HA adaptation after 2-4 days of training, 12 days following a LTHA programme of controlled hyperthermia at 38.5°C. Therefore, in the month after LTHA, relatively few heat exposures may be required for the re-induction of HA. An expedited re-induction potential may be underpinned by a physiological acclimation memory at a cellular level (Horowitz 2014), with specific gene clusters remaining in an active transcriptional state throughout the entire decay and re-induction period (Tetievsky et al. 2008). In conclusion, whilst HA affords improved thermo-tolerance through a myriad of adaptations, the state is transient whereby induction and decay may occur within 3 weeks, necessitating re-exposure to maintain benefits. Therefore, it would seem appropriate that experimental trials following a STHA protocol should occur within 10 days and within 20 days following LTHA.

2.6 Acute strategies for exercise in the heat

2.6.1 Precooling

The time and expense associated with chronic heat acclimation strategies, as well as the relatively fast rate of decay, has led to the development of simple and acute manoeuvres as alternative preparation strategies for competitions in the heat (Booth et al. 1997). Meta analyses and systematic reviews provide substantial evidence that both chronic strategies such as heat acclimation (Garrett et al. 2011; Chalmers et al. 2014) and acute strategies such as precooling (Jones et al. 2012; Tyler et al. 2013; Ross et al. 2013; Bongers et al 2015), provide meaningful benefits to endurance exercise, although there currently does not appear to be a direct comparison between techniques. Pre-cooling occurs in the hour prior to exercise

performance and may provide benefit to competitors for the following hours (Siegel & Laursen 2012) by increasing heat storage capacity (Wendt et al. 2007) and delaying, or preventing individuals reaching core temperatures associated with fatigue and task cessation (González-Alonso et al. 1999). Consequently, such techniques may increase heat storage reserve and improve the perceived thermal strain, permitting greater power output in self-paced exercise (Cotter et al. 2001; Duffield et al. 2010), or extending the time to exhaustion (TTE) at a set intensity by as much as 25 minutes (González-Alonso et al. 1999), with a reported overall mean improvement of 5.7 % (d = 0.44, Bongers et al. 2015).

The precooling literature is divergent, with reviews considering both precooling and cooling during exercise, whilst differences in methodologies concerning time of cooling, inclusion of appropriate warm-up and type of exercise test (i.e. open/closed loop, intensity, duration) can confound systematic interpretation. Precooling studies can be further differentiated into internal or external precooling, depending upon where the cooling impulse is delivered. Both internal techniques (+6.3%, Cohen's d=0.40, Bongers et al. 2015) and external techniques (+7.3%, d =0.72 Bongers et al. 2015, d=1.91, Tyler et al. 2013) are supported for use prior to endurance performance, with an overview of relevant experimental studies provided in Figure 21 below.

Study	Effect size (95% CI)	
Cooling Vest		
Arngrímsson et al. 2007	0.21 [0.03, 0.39]	
Castle et al. 2006 *	0.15 [0.01, 0.29]	
Duffield et al. 2003	0.14 [0.02, 0.26]	
Duffield et al. 2007 *	0.14 [0.00, 0.28]	
Quod et al. 2008 *	0.37 [-0.34, 1.08]	
Ückert et al. 2007	0.51 [0.26, 0.76]	
Cold water immersion		
Castle et al. 2006 *	-0.05 [-0.09, -0.01]	
Duffield et al. 2010	0.93 [0.17, 1.69]	
Kay et al. 1999	1.13 [0.23, 2.03]	
Siegel et al. 2012 *	0.83 [0.38, 1.28]	
Skein et al. 2012	0.27 [0.03, 0.51]	
Cold water/ice slurry ingestion		
Burdon et al. 2013	1.09 [0.15, 2.03]	
Byrne et al. 2011	0.21 [0.07, 0.35]	
Ihsan et al. 2010	0.42 [0.09, 0.75]	
Siegel et al. 2012 *	1.40 [0.71, 2.09]	
Stanley et al. 2010	0.18 [-0.11, 0.47]	
Stevens et al. 2013	0.30 [0.08, 0.52]	
Cooling packs		
Castle et al. 2006 *	0.44 [0.05, 0.83]	
Minett et al. 2011 *	0.35 [-0.14, 0.84]	
Mixed method cooling		
Cotter et al. 2000	1.35 [0.64, 2.06]	
Duffield et al. 2007 *	0.92 [-0.02, 1.86]	
Duffield et al. 2009	1.85 [0.38, 3.32]	
Duffield et al. 2013	0.49 [-0.27, 1.25]	
Minett et al. 2012	0.53 [0.24, 0.82]	
Minett et al. 2011 *	0.42 [0.24, 0.60]	
Minett et al. 2011 *	0.77 [0.46, 1.08]	
Quod et al. 2008 *	0.98 [0.51, 1.45]	
Subtotal (95%)	**0.44 [0.31, 0.56]**	

Figure 21: Modified from Bongers et al. (2015). Forest plot summarising the effects of different cooling techniques on exercise performance for the precooling studies. The black rectangles represent the respective weighted effect size, with grey lines representing the 95% CIs. Studies that used multiple cooling intervention trials were included more than once, as denoted by *. NB Quod et al. (2008) 'mixed methods' involved ice vest and cold water immersion, whereas all other mixed methods studies involved multiple garments.

2.6.1.1   External precooling

The majority of precooling investigations have traditionally utilised external modalities including ice packs applied to major muscle groups (Castle et al. 2006), ice jackets (Hunter et al. 2006; Price et al. 2009), or whole-body cold water immersion (Vaile et al. 2008). Cold water immersion is considered the gold standard cooling method for the treatment of exertional heatstroke (Casa et al. 2005), as shown in Figure 22. This reflects the rate at which it cools T_{CORE}, as a consequence of the high volume-specific heat capacity of water and skin surface area in contact with the water, thereby affording a significantly enhanced the core-to-skin ratio.

Figure 22: Cooling rates associated with different external modalities with healthy hyperthermic athletes and heatstroke casualties (Casa et al. 2005).

In contrast to the successful application as a treatment for heat illness, Stanley et al. (2010) suggested the aggressive lowering of T_{SKIN}, as proffered by cold water immersion, may provoke concomitant vasoconstriction when individuals are not experiencing heatstroke. This has the potential to reduce skeletal muscle blood flow, and in turn impair force generation capacity and locomotion (Stanley et al. 2010) and likely reflects the apparent impairment to sprint performance afforded by precooling (Tyler et al. 2013). Furthermore, the practical application of this technique may be limited, as it would likely necessitate athletes to get wet and change prior to competing. Finally, it should be noted opportunities for cold water immersion in the field will vary, or may not exist. Reflecting these concerns, a meta-analysis concerning precooling techniques for sports performance did not include the manoeuvre in their analysis, citing impracticality (Wegmann et al. 2012).

Consequently, athletes and coaches have continued to seek out alternative, simple and practical approaches to cooling the body before competition, often adopting individual cooling garments. Despite initial perceived benefits and support from athletes (Martin et al. 1998), ice jackets have fallen in popularity in favour of more aggressive cooling, with Jones et al. (2012) stating there was moderate evidence to suggest ice jackets were an ineffective precooling method. Tyler et al. (2010) pioneered the use of a neck cooling collar before and during exercise in order to deliver a cooling impulse in close proximity to the hypothalamus. A 5.9% improvement during a 15 min running time trial was observed, despite the absence of a reduced T_{CORE} or effect across a range of biomarkers including cortisol, prolactin, adrenaline, noradrenaline or dopamine. Tyler et al. (2010) suggested neck cooling may improve thermal sensation which in turn alters an anticipatory regulation response, permitting greater pace. Although the cooling collar was worn during exercise, subsequent repeat applications provided no further benefit, so there would appear efficacy in including neck cooling as a component of a precooling intervention (Tyler & Sunderland 2011).

Duffield et al. (2009) suggested a lack of cooling volume may explain the limited effectiveness of individual cooling garments on endurance performance and subsequently combined garments to develop a mixed methods approach in order to deliver a greater cooling impulse. The approach of Duffield et al. (2009) was intended to be practical, such that it could be applied across a range of situations and whilst ensuring regions of large MHP, such as the quadriceps, were targeted directly. Minett et al. (2012) and Duffield et al. (2009) combined four cooling techniques; wet, iced towels (3°C) covering the head, neck and trapezius muscles, forearm and hand immersion in cold water (9°C), an ice vest on the torso and plastic bags of ice on the quadriceps. Interestingly, Duffield (2009) did not observe a reduction in T_{CORE} before

beginning exercise, but demonstrated a blunted rise in T_{CORE} during exercise compared with no precooling, and greater total distance covered during 30 minutes of intermittent sprinting (3.35 ± 0.20 vs. 3.11 ± 0.13 km). Therefore, it has been speculated that external cooling may not always elicit a reduction in T_{CORE} prior to exercise, but appears to permit a reduced rate of heat storage during exercise, by enhancing heat dissipation through an increased core-to-skin gradient (Kay et al. 2010). Furthermore, an 'after-drop' may occur following external cooling, whereby T_{CORE} falls following the onset of exercise as vasoconstriction dissipates and warm blood is subsequently cooled in the periphery (Webb 1986). Direct cooling of the skin also likely reduces the cutaneous blood flow demand, mediating cardiovascular strain (Price & Maley 2015). Minett et al. (2012) subsequently identified a dose-dependent response, citing cooling surface area coverage as the critical factor for exercise capacity. This was evidenced by a step-wise interaction between exercise performance and cooling volume between no cooling control, individual neck cooling, combined neck and hand cooling and whole body mixed methods cooling respectively. Thus, a mixed methods approach was shown to improve work completed during intermittent sprinting by 12% compared with no cooling (Minett et al. 2011). The recent meta-analysis of Bongers et al. (2015) supports the concept of applying a greater cooling volume interaction with mixed method cooling (7.3%, d = 0.72), displaying the largest effect size compared with individual cooling packs (4.3%, d=0.40) and cooling vests (3.4%, d = 0.19) for improving exercise performance. However, this effect of mixed methods may be slightly inflated due to the inclusion of a mixed methods technique involving cold water immersion.

Despite these promising results, the more aggressive, practical, whole-body, mixed methods technique of Duffield et al. (2009) has yet to be evaluated prior to endurance exercise, where precooling techniques appear to provide the greatest benefit (Wegmann et al. 2012; Tyler et al. 2013). In conclusion, external cooling techniques are well supported for use prior to endurance performance, with Tyler et al. (2013) reporting an overall effect size of d = 1.91 from 13 investigations, which included a total of 16 cooling interventions/performance/capacity measure combinations. The reported effects appear unanimous across both TTE (n = 6, d = 2.88) and TT protocols (n = 7, d = 1.06).

2.6.1.2 Internal precooling

Considerable growing evidence supports the use of internal cooling through ice slurry ingestion (ICE) as an ergogen for endurance exercise in the heat (Wegmann et al. 2012; Siegel & Laursen 2012; Jones et al. 2012), by a likely magnitude of 6.3% (d = 0.40, Bongers et al. 2015).

Indeed, as an alternative to cold water immersion, cold drinks and ICE have also been proposed as the most effective form of pre-cooling in a meta-analysis by Wegmann et al. (2012) and systematic review by Jones et al. (2013). Internal cooling through cold drinks or ICE lowers T_{CORE} by delivering the cooling impulse closer to the core organs, whilst maintaining muscle temperature for high intensity efforts (Stanley et al. 2010). The mechanisms underpinning internal precooling remain equivocal, but may include limiting gastrointestinal epithelia permeability to limit the degree of endotoxemia occurring under heat stress (Moseley et al. 1994), and thus preventing an apparently increased perception of effort (Cheung & Sleivert 2004). Furthermore, aggressive cooling via the mouth and gut may positively influence the anticipatory regulation of exercise due to improved thermal comfort (Marino 2004), as a consequence of the relative prominence of thermoreceptors in these regions (Flouris 2011; Villanova et al. 1997). This mechanism is supported by the observation of greater power output following ICE during exercise, versus a combined internal and external technique, which elicited a greater reduction in T_{CORE} (0.4 vs. 0.6°C, Ross et al. 2011).

Despite the demonstrable efficacy of cold drink ingestion, questions remain concerning the optimal quantity and ingestion time before performance in order to avoid gastro-intestinal complaints (Ross et al. 2011). Addressing this, Siegel et al. (2010), proposed the ingestion of a smaller dosage of ice slurry (7.5 g kg^{-1}). A smaller dosage is appropriate because a larger transfer of heat energy is required to complete the phase change of ice into water (H_2O), than to heat cold water. Through precooling with 7.5 g kg^{-1} of ICE for 30 minutes prior to exercise, Siegel et al. (2010) demonstrated a greater reduction in T_{CORE} and thermal sensation than following 7.5 g.kg^{-1} of cold drinks (-0.66 ± 0.14°C ICE vs. -0.25 ± 0.09°C cold drinks, p=0.001), which resulted in an increased running TTE of 19 ± 6% at an intensity corresponding to the ventilatory threshold. A summary of the reported improvements following ICE in open and closed loop trials is displayed below in Figure 23.

Study or Subgroup	Std. Mean Difference IV, Fixed, 95% CI	Std. Mean Difference IV, Fixed, 95% CI
Ihsan (2010)	0.40 [-0.66, 1.46]	
Ross (2011)	0.35 [-0.49, 1.20]	
Siegel (2010)	1.16 [0.19, 2.12]	
Siegel (2012)	0.73 [-0.30, 1.75]	
Ihsan (2010)	0.45 [-0.62, 1.51]	
Ross (2011)	0.30 [-0.54, 1.14]	

-4 -2   0   2   4
Diminished Improved

Figure 23: Effect sizes (Cohen's *d*) for ice slurry ingestion versus control (Jones et al. 2012). Graph represents effect of intervention on exercise performance. Ihsan et al. (2010) = 40 km cycling TT. Ross et al. (2011) = 23 km cycling TT. Siegel et al. (2010, 2012) running TTE at ventilatory threshold.

Furthermore, when directly compared with cold water immersion, the same volume of ICE cooled T_{CORE} by a greater magnitude (0.43 ± 0.14°C ICE vs. no change from rest) and resulted in similar TTE (52.7 ± 8.4 min ICE vs. 56.8 ± 5.6 min, *p* = 0.335, Siegel et al. 2012). However, as previously discussed with external cooling modalities, an after-drop in the water immersion condition was observed and T_{CORE} continued to reduce until 5 minutes into exercise such that the mean total reduction was -0.25 ± 0.20°C. Whilst these data are not representative of the total cooling potential of water immersion due to the onset of exercise, it is apparent that ice slurry cools T_{CORE} faster than water immersion.

Free-paced trials also demonstrate improved endurance performance following ICE, with Ihsan et al. (2010) reporting a 6.5% improvement following ICE versus a warm water control (26.8 ± 1.3°C) during a 40 km cycling time trial. However, the failure to include a cold water trial control group may over-estimate the magnitude of the effect, as may the lack of representative air-flow whilst using a cycling ergometer in the heat (Morrison et al. 2014). Furthermore, caution should be taken interpreting the T_{CORE} response from this trial as the validity of telemetric gastro-intestinal capsules as measures of T_{CORE}, when subsequently providing a cooling impulse to the gut has been criticised due to the significant inter-individual variation in gut transit time (Lee 2011; Byrne & Lim 2007). A subsequent time trial experiment by Ross et al. (2011) helped reaffirm the results of Ihsan et al. (2010), using three conditions of cold water immersion, cold water drink and a mixed-methods slurry and iced towels technique prior to a 46 km cycle. The mixed methods slurry and iced towels technique resulted in a 1.3 % improvement in finishing time and 3% (~8 W) increase in mean power output. These effects were observed despite Ross et al. (2011) delivering the intervention in hot conditions (32-35°C 50-60% relative humidity), rather than thermo-neutral locations reported by Siegel et al. (2010; Siegel et al. 2012) and Ihsan et al. (2010). Moreover, Ross et al. (2011) included representative air-flow within the laboratory (Morrison et al. 2014).

Currently no study has investigated the effect of precooling on the determinants of endurance performance in the heat. Furthermore, there remains a lack of research concerning internal precooling and endurance running performance. Given the differing absolute energy requirements and potential for greater MHP during running versus cycling (Millet et al. 2009),

caution should be taken before extrapolating the magnitude of performance improvements following ICE from cycling to running. Ultimately, athletes and coaches require a simple, time-efficient intervention for use across a number of competition venues. Internal cooling modalities are practical for field delivery and demonstrate a greater reduction in T_{CORE} than whole body water immersion across 20-30 min. Endurance performance in the heat following ICE would appear to be similar to whole body immersion, and improved relative to cold drinks or no cooling. Further benefits to this approach may arise from the ability to combine ICE with an existing pre-race hydration or carbohydrate supplementation strategy (Rollo & Williams 2011).

2.6.2 Ischemic Preconditioning

As with precooling, ischaemic preconditioning may be an acute intervention that can benefit endurance performance in the heat independently, or in combination with existing strategies such as heat acclimation. Ischaemic preconditioning (IP), that may be achieved from repeated bouts of occlusion and reperfusion of a peripheral limb, has been shown to aid exercise performance across a range of different sports and environmental conditions (Sharma et al. 2015), and proffers transient protection that has the potential to alleviate physiological consequences of heat strain including the increased contribution of anaerobic energy provision (Febbraio 2001) and heightened perceived exertional strain (Flouris & Schlader 2015).

A recent meta-analysis by Marocolo et al. (2015) details anecdotal information concerning the origins of the IP technique, suggesting the practice originated from South American indigenous populations who ligated the thighs before long journeys. In Europe It would seem that the technique emerged in the mid-20th century, with a prominent study by E.A. Muller reporting increased exercising time being associated with prolonged prior application of tourniquets, to achieve occlusion of the lower extremities (Muller 1958). The difference in exercise performance was attributed to an increased blood flow to the working muscles during the reactive hyperaemia during reperfusion that follows occlusion. IP may be considered an example of hormesis, an adaptive response of cellular mechanisms to intermittent stress (Mattson 2008). Since these early experiments, IP has become established clinical practice as an intervention to treat those at risk of coronary infarction, or to prepare cardiac muscle to withstand infarction that may occur during surgery. From a clinical perspective, this practice follows the discovery of a markedly reduced infarct size during occlusion of the left anterior descending coronary artery, when cardiac muscle had been preconditioned through intermittently clamping the circumflex coronary artery (Przyklenk et al. 1993). Crucially, this

study highlighted a systemic component to protection, as the heart was preconditioned through a different vessel to which the subsequent occlusion was made. This introduced the concept of 'remote ischaemic preconditioning', and stimulated other, successful investigations concerning the effect on other organs. Among the wide ranging responses to IP, the IPC phenomenon has provided protection against reperfusion injury during clinical studies concerning smooth muscle of the lungs (Chen et al. 1999) and liver (Clavien et al. 2003), as well as a range of effects on skeletal muscle which may include ATP sparing (Pang et al. 1995), greater muscle oxygenation (Andreas et al. 2011) and as well as vasodilation, potentially elevating muscle blood flow (Bailey, et al. 2012b). As with cardiac protection, because the effects are not localised to the occluded muscles, such a technique may be of benefit across a wide range of sports when skeletal muscle is preconditioned. Skeletal muscle IP typically involves three or four, 5 min bouts of occlusion, separated by 5 min of reperfusion. Some protection is conferred within minutes, lasting 1-2 hours, followed by a second window of protection which may last for up to 48 hours (Hausenloy & Yellon 2008).

Early investigations into the mechanisms underpinning the protective effects of IP before surgery suggested that the artificially induced metabolic stress may result in cellular protection through a heat shock protein response (Marber 1994). However, this is no longer thought to be the case, with Bushell et al. (2002) observing no change in heat-shock protein content following IP, as well as highlighting the need for a relatively acute and transient stress to elicit protection, which is not in keeping with stimulating the expression of a transcribed protection factor. Subsequent research suggests adenosine, bradykinin, opioids and oxygen radicals have roles in the protective response induced by IP, combining to signal the metabolic cascade which provides the ischemia/reperfusion protection (Cohen et al. 2000; Hausenloy & Yellon 2008). The role of adenosine in particular appears significant, with Bushell et al. (2002) replicating the protective benefits of IP and as well as demonstrating the maintenance of ATP levels, following adenosine infusion into skeletal muscle of rats. Adenosine is produced rapidly by the endothelium during ischemia (Minamino et al. 1995) and mediates reactive hyperaemia during reperfusion (Tóth et al. 2007), following occlusion. This serves to protect organs from future ischaemic events and likely contributes to enhanced exercise performance, through vasodilation of the peripheral vasculature during the windows of protection. Adenosine is likely released from the vasculature, rather than the skeletal muscle, due to the relatively small amounts of adenosine stored in muscle (Bushell et al. 2002). Such a mechanism helps explain the rapid benefits from IP, as well as why IP is effective across a range of locations including cardiac, smooth and skeletal muscle.

As well as acting as a vasodilator (Gustafsson et al. 1993), the presence of adenosine also opens mitochondrial potassium (K_{ATP}) channels, which may reduce mitochondrial oxygen consumption (Cooper & Brown 2008). The results of Hopper et al. (2000) allude to the importance of the roles played by adenosine and K_{ATP} channels, with reduced preconditioning effects when adenosine or K_{ATP} channel blockers were administered, and increased effects when the respective agonists were administered. K_{ATP} channels are closed at rest, but open during hyperthermia, as well as during hypoxic events, which could include normothermic exercise, leading to increased plasma potassium levels and in turn, nitric oxide release (Marshall 2000). In turn, the release of endothelial nitric oxide effects haemodynamic changes by attenuating sympathetic vasoconstriction (Thomas & Victor 1998) and appears to maintain vascular function that may be impaired following high intensity exercise (Bailey et al. 2012b). Specifically, nitric oxide maintains the flow-mediated dilation of blood vessels, and the results of Bailey et al. (2012b) suggest IP can mediate against the impairment to endothelial function that occurs following high intensity exercise. The authors suggested nitric oxide release may effect this change, with the likely additional benefit of helping maintain the supply of oxygen and energy substrates to the skeletal muscles during intense exercise, when muscles may be subject to hypoxia, therefore sustaining contractile activity. Indeed, under heat stress, nitric oxide has an established role for inducing smooth muscle relaxation as part of the secondary plateau phase of the bimodal cutaneous response to local hyperthermia (Kellogg et al. 1998). Kimura et al. (2007) has also demonstrated a direct effect of IP in promoting endothelial nitric oxide biosynthesis, suggesting this will increase shunting of blood flow to the local muscle beds at the IP site and away from less active muscle tissue. Under heat stress, such a redistribution of blood towards active skeletal muscle may help to maintain the delivery of oxygenated blood that ultimately impairs $\dot{V}O_{2max}$ (Gonzalez Alonso et al. 2008), whilst reducing the $\%\dot{V}O_{2max}$ at submaximal intensities as a consequence of reducing anaerobic metabolism. The study by Andreas et al. (2011) utilised functional magnetic resonance imaging to characterize metabolism changes to IP in vivo, providing novel evidence of the metabolic cascade likely mediated through adenosine and nitric oxide, highlighting greater energy replenishment and a higher oxygen consumption at rest during reperfusion, mechanisms that may transfer to exercise in the heat. However, given the vasodilation that already occurs during heat strain, were IP to promote a greater redistribution of blood to the active muscles, this could be to the detriment of skin blood flow and thermoregulation. Therefore, when investigating the performance effects of IP in a hot environment, the on T_{SKIN} and T_{CORE} during exercise in the heat should also be quantified to better understand the consequences of the intervention.

de Groot et al (2010) reported a 3% increase in $\dot{V}O_{2max}$ alongside a 1.3% increase in maximal power output during incremental cycling following IP, relative to a control condition. This increase in $\dot{V}O_{2max}$ is comparable to a month of altitude (Stray-Gundersen et al. 2001) or high intensity interval training (Milanović et al. 2015). To explain their findings, de Groot et al. (2010) recognised the potential for enhanced maximal exercise-induced blood flow following IP, but also suggested an alternative mechanism, because increased maximal blood flow does not always unanimously increase $\dot{V}O_{2max}$, as oxygen extraction can be impaired from a less efficient flow distribution (Calbet et al. 2006). Alternatively, De Groot et al. (2010) suggested an increase in muscle oxygenation may have occurred, as has been reported in rats that have undergone IP (Saito et al. 2004) and in humans at rest (Andreas et al. 2011). De Groot et al. (2010) also proposed that any changes in muscle oxygenation may be complemented by a small, unobserved, energy sparing effect (Pang et al. 1995) alongside augmented mitochondrial function reducing oxygen consumption, as a consequence of increased nitric oxide availability following IP (Kimura et al. 2007). Such a mechanism would appear to be independent of body temperature, therefore would be maintained during exercise in the heat, and may help explain how IP can benefit both short and longer duration exercise, as well as performance in hypoxia. However, the failure to observe any differences in oxygen consumption at sub-maximal exercise intensities is not in support of these mechanisms, and it remains difficult to validate given the lack of muscle oxygenation or muscle biopsies to establish muscle ATP levels following maximal exercise.

In a subsequent study, the improved incremental cycling performance of de Groot et al. has been supported by Crisafulli et al. (2011) who reported a 4% increase in maximal power output and a ~40 s increase in total exercise time to exhaustion, following IP. However, IP did not improve $\dot{V}O_{2max}$, nor maximal stroke volume or cardiac output. Therefore, Crisafulli et al. (2011) suggested IP may benefit performance through a perpetual mechanism, such as an altered fatigue perception, potentially utilising a greater proportion of muscular functional reserve, as per an end-spurt (Noakes 2011) or indeed a 'placebo' effect (Tocco et al. 2014). Such a mechanism has been suggested to be mediated by desensitised group III and group IV afferents (Sharma et al. 2015). Such a mechanism may proffer particular benefit when exercising in the heat, due to the heightened perceived exertional strain that exists as a result of thermal discomfort (Flouris and Schlader 2015). A sub-question of the Crusifulli et al. (2011) study compared the effect of IP following an exercise bout, versus IP following a period of rest. This built on the suggestion that in order for IP to provide benefit, the metabolic stress must exceed a minimum threshold (Przyklenk & Kloner, 1995), therefore a greater metabolite build-

up may yield a greater benefit. However, no difference was observed between conditions, suggesting an IP protocol alone is already sufficient to achieve a metabolite threshold, and exceeding this does not provide a further benefit.

Jean-St-Michel et al. (2011) suggested IP may provide particular benefit for events such as swimming whereby an enhanced physiological strain occurs as a result of specified breathing patterns, leading to a decreased arterial partial pressure of oxygen and decreased blood pH. Accordingly, Jean-St-Michel et al. (2011) reported a meaningful and statistically significant 0.7 s improvement in 100m swim time (-1.1%, p=0.02) following IP, improving personal best times within a highly trained cohort. Likewise, IP appears to be effective when additional environmental stressors are present, improving exercise performance in hypoxia (Foster et al. 2011) and preventing illness (Sikri & Chawla 2015; Berger et al. 2015) at altitude, but it is unclear if these benefits extend to heat stress. Like swimming, endurance running is a whole-body exercise modality, which elicits different $\dot{V}O_{2max}$ and oxygen kinetics responses to cycling (Hill et al. 2002; Millet et al. 2009). This may in part explain why Bailey et al. (2012a) went on to report lower blood lactate concentration (mean difference -1.07 mMol.L^{-1}) throughout an incremental submaximal running test, followed by improved 5 km running time with a mean improvement of-34 s, although again, no effect on $\dot{V}O_{2max}$ was observed. The authors suggested the improved time trial performance could be explained by the decreased lactate accumulation at sub-maximal speeds, based on the association of reduced blood lactate concentration and cycling time trial performance (Jacobs et al. 2011) and accurate predictive ability of the lactate turnpoint (Lorenzo et al. 2011). Bailey et al. (2012a) suggested IP mediated improvements in vascular function facilitated enhanced blood flow to transport and remove lactate for uptake and use away from the working muscles. In a more recent study, and only the second concerning endurance running, Tocco et al. (2015) recruited national standard athletes and investigated the effect of IP on 5 km time trial performance on an outdoor running track. Self-paced running was not enhanced by IP, and no differences were observed within metabolic data collected by a portable metabolic system worn by participants during all trials. The authors suggested that despite habituation with the testing conditions, the established pacing strategies of the participants may have been stronger than the influence of IP. This suggests there may be a need for individuals to familiarise with IP pacing strategies prior to self-paced trials, whilst the authors also highlighted a difference in training status of participants in the respective studies, speculating that the better trained participants may be less sensitive to IP because they may already be more resistant to ischaemia than sedentary subjects (Crisafulli 2006).

In conclusion, considerable, recent debate concerns the precise mechanisms underpinning IP (Salvador et al. 2015, Sharma et al. 2014, Marocolo et al. 2015). Research has demonstrated beneficial effects of IP under normothermic conditions, in longer, aerobic based events (de Groot et al. 2010; Crisafulli et al. 2011; Bailey et al. 2012a; Kjeld et al. 2014) as well as shorter, anaerobic events (Patterson et al. 2015; Kido et al. 2015; Beaven et al. 2012). However, findings are not unanimous and recent meta-analyses conducted on a relatively heterogeneous field of IP and exercise performance studies both support (Salvador et al. 2015) and dispute (Marocolo et al. 2015) the benefits of IP under normothermic conditions. Thus it is apparent the effect of IP on exercise performance remains a new and evolving area, with such ambiguity reflecting the large variation in methodologies when conducting IP research (Sharma et al. 2015), as well as the small number of studies. Notwithstanding this recent apparent disagreement, where IP has shown benefit, results are highly meaningful for both amateur and elite populations, including trained endurance runners, warranting further investigation. Moreover, there is emerging evidence that IP may be highly effective when additional environmental stresses are present, which is highly relevant, given the physiological alterations that occur when exercising under heat stress. Based on the limited, existing literature in this area it is plausible that IP could enhance endurance performance in the heat through haemodynamic, metabolic and/or perceptual mechanisms, with the large previously reported effects indicating highly meaningful performance improvements if benefits transfer to a hot environment.

2.7 Conclusion

The purpose of this literature review was to identify limiting factors to endurance performance in the heat in order to identify approaches that may be adopted to enhance performance. Therefore, it began by introducing the mechanisms that underpin endurance performance, principally through the determinants of endurance performance model. This included defining the lactate threshold, lactate turnpoint, running economy and $\dot{V}O_{2max}$, and introducing controversy surrounding the measurement and limiting factors of each variable. For example, there are a number of different approaches to defining indices of the blood lactate response (Faude et al. 2009) and it is common for exercising individuals not to demonstrate a $\dot{V}O_2$ plateau during maximal aerobic exercise (Edvardsen et al. 2014). The efficacy of these determinants for predicting endurance performance, both individually and collectively, was then discussed, as well as how this approach corresponds to traditional endurance time trials or time to exhaustion protocols. The determinants of endurance performance appear to provide a multi-faceted model through which to assess and interpret

endurance performance, combining to produce a valid performance calculation of velocity at $\dot{V}O_{2max}$. Having detailed the fundamentals of endurance performance, the review then introduced how hot and humid conditions influence human thermoregulation, and exacerbate physiological strain that is commensurate with exercise. This included identifying the predominant physiological stresses that arise from exertional heat stress, and discussing the individual, and collective effects of these changes. It is apparent that there are numerous hypotheses of fatigue, (Gonzalez-Alonso et al. 1999, Cheung 2007, Tucker et al. 2008, Nybo et al. 2014), however there is consensus that cardiovascular, perceptual and metabolic alterations appear prominent in determining the voluntary control of intensity during self-paced endurance exercise in the heat. Therefore, it is apparent that interventions must mediate many of these effects to maintain performance in the heat better. Accordingly, recent integrated models of endurance performance in the heat such as that of Nybo et al. (2014) were explained, in order to provide a basis for how interventions such as heat acclimation, precooling and ischaemic preconditioning may provide a benefit to performance in the heat. The mechanisms underpinning these widely evidenced interventions were then explained, in terms of their time course i.e. chronic (heat acclimation) and acute (precooling and ischaemic preconditioning). Despite evidence advocating the use of each intervention individually, specific questions were identified that should be answered in order to develop an optimised strategy for endurance performance in the heat. Regarding precooling, the recent development of modified cooling techniques such as mixed-methods external cooling appear beneficial, but have yet to be examined on endurance performance, and it is unclear whether this is a preferential approach to internal precooling strategies. Ischaemic preconditioning has yet to be investigated under heat stress, and has the potential for benefits from normothermic conditions to transfer through effecting a change in the peripheral vasculature and blood availability, thereby mediating heat stress-induced metabolic changes. From a chronic perspective, LTHA strategies appear beneficial for endurance performance and have been shown to enhance the determinants of endurance performance (Lorenzo et al. 2010). However, these notable results warrant replication in a more widely utilised STHA model and during running experiments, due to the greater MHP that may result in an enhanced thermal strain in running versus cycling (Millet et al. 2009). Furthermore, despite the inability for HA strategies alone to restore $\dot{V}O_{2max}$ (Sawka et al.1985) and endurance performance (Lorenzo et al. 2010) to levels observed under normothermic conditions, remarkably little research has investigated the combined effect of acute and chronic strategies. Finally, the extent to which the determinants of endurance performance model explains performance in the heat has yet to be investigated.

Therefore, the following series of experiments were designed in order to address these gaps in the research and optimise preparation for endurance runners competing in a hot environment.

2.8   Research Questions and Hypotheses

Study 1 research question:

Are telemetry thermistors and a thermal camera valid and reliable for measuring skin temperature during exercise in the heat?

Study 1 hypothesis:

Telemetry thermistors and a thermal camera will provide acceptable levels of error for both reliability and validity when compared against hard-wired thermistors during exercise in the heat.

Study 2 research question:

Do practical and evidenced internal and external precooling techniques enhance the determinants of endurance performance in the heat?

Study 2 hypothesis:

Internal and external precooling will increase blood lactate indices, improve running economy and increase $\dot{V}O_{2max}$, relative to no cooling, during incremental exercise in the heat. Internal cooling will elicit a greater improvement relative to EXT.

Study 3 research question:

Does ischaemic preconditioning enhance the determinants of endurance performance in the heat?

Study 3 hypothesis:

Ischaemic preconditioning will increase blood lactate indices, improve running economy and increase $\dot{V}O_{2max}$, relative to a sham control condition, during incremental exercise in the heat.

Study 4 research question:

Does short term heat acclimation improve the determinants of endurance performance and 5 km time trial performance in endurance runners?

Study 4 hypothesis:

Short term heat acclimation will elicit a larger improvement in the physiological determinants of endurance performance and 5 km time trial performance than normothermic training.

Study 5 research question:

Does combining short term heat acclimation and mixed methods external precooling technique yield an additive effect, above that of heat acclimation alone?

Does precooling or heat acclimation result in the largest improvement in 5 km time trial performance?

Study 5 hypothesis:

Combining short term heat acclimation and precooling will enhance time trial performance relative to short term heat acclimation.

Short term heat acclimation will improve time trial performance more than precooling.

Study 6 research question:

What percentage of the variation of 5 km running performance in the heat amongst heterogeneous runners can be explained by the determinants of endurance performance when measured in the heat?

What percentage of the variation of 5 km running performance in the heat amongst heterogeneous runners can be explained by the determinants of endurance performance when measured in the cool?

Study 6 hypothesis:

The determinants of endurance performance will explain less of the variation in endurance performance than previously reported from endurance performance in cool conditions. Measuring the determinants of endurance performance in the heat will better predict endurance performance than when measured in the cool.

3   General Methods

This chapter describes the common methods used in the experimental chapters within this thesis. Each of these methods were conducted in British Association of Sport and Exercise Sciences (BASES) accredited laboratories (Welkin Human Performance Laboratories, Eastbourne). Where experimental chapters used alternative methods, appropriate descriptions are detailed within the methods section of that specific chapter. Data collection was combined for Studies 1 and 2 (Chapters 4 and 5), as well as Studies 4 and 5 (Chapters 7 and 8). Therefore, participants, methods and equipment used in these chapters were the same.

3.1   Health and safety

The studies that form this thesis were each individually approved by the University of Brighton Research Ethics and Governance Committee and conducted in accordance with the Declaration of Helsinki 1964, as revised in 2013. Written informed consent and recent medical history was obtained from all participants prior to the start of each study. Biological waste and hazardous materials were handled and disposed of in accordance with University of Brighton standard operating procedures.

During each data collection session at least two experimenters were present throughout, with one located within the environmental chamber monitoring the participant, and one outside of the chamber remotely controlling the treadmill where necessary.

Termination of testing occurred at the request of the participant, the attainment of the University of Brighton Ethics and Governance Committee approved maximum permissible core temperature of 39.7°C or evidence of signs of heat illness that included syncope, exhaustion, disorientation, nausea and vomiting. Following the cessation of exercise participants were routinely chaperoned until T_{CORE} had returned to within 0.5°C of the baseline measure obtained prior to testing. Drinks were also provided and active cooling through ice packs and fans were utilised where appropriate.

3.2   Participants

As the investigations in this thesis were focussed towards improving endurance running, trained endurance runners were the cohort recruited. Participants who volunteered to participate in the studies were all healthy, predominantly male and between the ages of 22-60. Prior to attending the laboratory, participants were provided with an information sheet

detailing the requirements, risks and benefits of the study, as well as explicitly stating their right to withdraw at any time and without reason.

Any participant displaying a medical condition that contraindicated participation in maximal exercise was excluded from the investigation. Participants were excluded from participation if they stated a history of having any blood carried infections (Hepatitis, HIV), were diabetic, or presented with a known history of haematological, cardiac, respiratory, or renal disease. Additionally, individuals with known conditions such haemorrhoids, fissures and anal bleeding or those with a history of symptoms of nausea or light-headedness resulting from needles, probes or other medical-type equipment were not recruited.

Participants were recruited if they met specific criteria to demarcate 'trained' status. These criteria were derived from running performance equivalent to a good club runner, a standard which necessitates individuals to train regularly, typically at least 3 times per week. Therefore, inclusion criteria of having completed a sub-22 min 5km or sub 45 min 10km race in the previous 2 months were adopted across the thesis, alongside a requirement that individuals undertook running training at least three times per week for the previous 2 months.

3.3 Pre-trial diet and standardisation

During Studies 1-3 (Chapters 4-6) trials were separated by 7-10 days to prevent an acclimation effect from repeated heat exposures (Barnett & Maughan 1993), and occurred at similar time of the day to minimise fluctuation in thermoregulatory responses as a result of circadian variation (Reilly & Waterhouse 2009; Winget 1985). During Studies 4 and 5 (Chapters 7 and 8), due to the need for four pre and three post experimental trials, visits were typically separated by 48 hours with the order of trials counterbalanced to control for acclimation effects arising from repeated thermal exposures.

Throughout each study participants were encouraged not to change their physical activity or dietary patterns. Furthermore, participants were asked to write a food diary covering the 24 hours before each trial such that they could match this before subsequent sessions. This was reinforced, through verbal reminders. Prior to the first session, participants were asked to maintain hydration from at least 24 hours prior to testing, through regular drinking, ensuring that ~500ml was consumed in the 2 hours before arrival. A urine sample was requested upon arrival so that hydration status could be verified, which is detailed in Section 3.6.2.

The following guidelines were also given prior to each testing session:

- No eating in 2 hours prior

- No strenuous exercise in 48 hours prior (weightlifting, sprint training & exercise >30min)

- No caffeine in 12 hours prior & no alcohol in 24 hours prior.

Participants were also instructed to abstain from prolonged thermal exposures (baths, saunas, steam rooms) and refrain from participating in vigorous physical activity or exhaustive exercise and alcohol consumption 2 days prior too, and throughout the duration of each experiment. Finally, participants were asked to prepare for each trial as they would a competition. Adherence to these guidelines was verified verbally upon arrival for each trial.

Studies 4 and 5 (Chapters 7 and 8) necessitated a total of 7 experimental trials to be completed, which were predominantly separated by 48 hours, rather than 7 days. To assess readiness to exercise, upon arrival participants were required to note hours of sleep for the previous night, as well as complete 5-point Likert scales based on:

- Muscle soreness

- Motivation

- Stress

3.4 Exercise protocol

All studies involved serial graded exercise tests, to which participants were familiarised, and instrumented during familiarisation. An overview of each trial is provided in Figure 24. Trials were comprised of four phases; 10 min rest, 20-40 min for the relevant acute intervention (precooling or ischemic preconditioning), 5 min warm-up and then the graded exercise tests (GXT 1 and GXT 2), with the entire trial within the hot and humid environment. All resting measurements occurred at the end of 10 min rest period within the hot environment. In order to better replicate a competition schedule, the exercise test began 15 min after the acute intervention.

Figure 24: Protocol overview. Entire protocol completed in hot environment. 'GXT 1' denotes 3 min exercise stages with increments of 1km.h^{-1}. 'GXT 2' denotes gradient based test to exhaustion incorporating 1 min stages with increments of 1%.

Following rest and intervention phases, a five min warm-up was completed on a motorised treadmill (Woodway ELG2, Weil am Rhein, Germany). The treadmill gradient was calibrated by the manufacturer annually, using a certified spirit level. The treadmill speed and distance functions were verified every 6 months through marking the treadmill belt and counting the revolutions across of a range of speeds from 6-15 km.h^{-1} in accordance with the manufacturer guidelines. Warm-up speed was determined from discussion with the participant about their recent running performances.

GXT 1 was similar to that described by Jones (2006), initially a submaximal incremental speed protocol totalling 6-8 stages, using speed increments of 1 km.h^{-1}. This was followed by GXT 2, an incremental gradient protocol to volitional exhaustion. Starting speeds were between 8–11 km.h^{-1} (1% gradient), again dependent upon recent running performance, such that each participant would complete 6-8 stages without reaching volitional exhaustion. Each stage was 4 min, consisting of 3 min running and 1 min for capillary blood sampling. These times were fixed across all trials, with the treadmill following a predefined protocol. Exercise continued until an exponential increase in blood lactate was observed, blood lactate concentration exceeded 4 mMol.L^{-1} or the participant felt unable to complete the subsequent stage. The same number of exercise stages and running speeds completed were replicated during all subsequent trials within each Chapter.

Following a short rest, GXT 2 began at a speed 2 km.h^{-1} below the previous final speed. This rest period was fixed at 2 min for Studies 1 and 2 (Chapters 4 and 5), and 10 min for Studies 3-5 (Chapters 6-8). The shorter rest period was adopted to maintain the effects of precooling prior

to GXT 2, as per the investigation in Study 2. During GXT 2, the gradient increased by 1% each min and the test continued until volitional exhaustion (Jones, 2006). Participants were verbally exhorted to continue running for as long as they possibly could, whilst ensuring that they could still safely terminate the test by pressing the stop button or jumping off and straddling the treadmill belt. An experimenter was in close proximity to the treadmill and participant at all times during GXT 2 for safety. Standardised instructions were given to participants immediately prior to GXT 2 during each trial to emphasize the importance of running to maximum. No feedback on running time or number of stages completed was provided at any time.

3.5   Ambient Environment

All trials were completed in a large purpose built environmental chamber (Figure 25) with an available range of -20 to +50°C and 20-95% relative humidity chamber (TISS, Hampshire, UK). The targeted conditions for this thesis was 32°C and 60% relative humidity. These conditions represent the expected environment across many of the sites at the 2016 Rio de Janeiro Olympics, as detailed earlier (see Chapter 1, Introduction).

Within the environmental chamber, conditions were thermostatically controlled (WatFlow control system, TISS, Hampshire, UK), as well as being continuously monitored throughout the trial using a heat stress meter (HT30, Extech Instruments, USA). Environmental conditions were logged at the beginning, middle and end of the trial to accurately describe the environment experienced by participants.

Figure 25 Environmental chamber within Welkin Laboratories, Eastbourne, UK.

3.6 Physiological measures

Details of the techniques and equipment adopted for measuring physiological variables are detailed in this section. In the following section, reliability data for outcome variables that are prominent in experimental chapters are presented. Data is presented on physiological measures and procedures having been collected from participants of experimental studies within this thesis, all of whom have followed the same standardisation procedures as for experimental trials. The observed variation between trials is the sum of technical, biological and random error (Weir 2005). Therefore, when appropriate standardisation procedures have been followed and potential confounding factors are accounted for, a quantitative assessment of the total variation within a dependent variable can facilitate accurate interpretation of interventional change, by distinguishing meaningful change from inherent variation of the measure. Where possible, and appropriate given the different experimental designs, data from different chapters are combined to enhance the robustness of reliability calculations.

Atkinson and Nevill (1998) recommend a battery of both relative and absolute reliability statistics to provide a more robust analysis than significance testing and a singular test such as the 95% limits of agreement (LOA), previously advocated by British Standards Institution (1987). Therefore, a range of statistics are presented, with absolute reliability assessed through the typical error of the measure (TE), calculated from the standard deviation of the mean difference for each pair of trials using the formula TE = SD(diff)/$\sqrt{2}$ and expressed as a mean coefficient of variation (CV%) (Hopkins 2000, Hopkins et al 2009). The 95% limits of agreement are also presented, in accordance with Bland and Altman (1986), reflecting the range within which the scores of 95% of the given population would be expected to fall within if this measure or technique was replicated. Relative reliability is presented as Pearson's correlation coefficient (r), as data concerns no more than 2 trials, in accordance with Hopkins (2000). Finally, paired samples T-tests are used to identify mean bias between samples. From these statistics, a more informed assessment of a variable is possible, whereby low typical error, narrow 95% LOA, high correlation and no statistically significant differences between tests, demonstrates good reliability of the measure.

3.6.1 Anthropometry

Anthropometric data were collected for stature, body mass and a four site skin fold calliper assessment (Harpenden, Burgess Hill, UK) across iliac crest, subscapular, triceps and biceps. Body mass was measured using AE Adam GFK150 scales, precise to 0.01 kg (AE Adam, Milton

Keynes, UK). From these measures of skinfold thickness, body density was calculated in accordance with the formula of Durnin and Womersley (1974).

Equation 8: Calculation of body density (Durnin & Womersley 1974).

$$\text{Body density} = 1.1610 - 0.0632 \text{ Log} \sum \text{Iliac crest, Subscapular, Triceps, Biceps}$$

Following determination of body density, percentage body fat could be calculated.

Equation 9: Calculation of percentage body fat (Siri 1956):

$$\% \text{ Body fat} = ([4.95 / \text{body density}] - 4.5) \times 100$$

3.6.2 Hydration

During all trials a urine sample was requested upon arrival. Euhydration was achieved when urine osmolality and urine specific gravity were below 700 $mOsmol.kg^{-1}$ H_2O and 1.020, respectively (Sawka et al. 2007).

Urine specific gravity (USG) was measured using a refractometer (Specific Gravity Refractometer Model 32, Atago; USA). The refractometer was calibrated prior to each test using water (USG = 1.000). A small volume (~2 ml) of urine was placed into the surface reader and the plastic cover closed. The refractometer was then held towards the light, with the value obtained from the scale within the eye piece.

Urine osmolality was measured using a hand held osmometer (Osmocheck™ Pocket, Vitech Scientific Ltd, UK.). The osmometer was calibrated prior to each measurement using water (Osmolality = 0). A small volume of urine (~2 ml) was placed on the aperture. The result was displayed to within ±10 mOsmols kg H_2O. The measurement surface was then cleaned immediately.

In the event that a participant arrived dehydrated, they would initially be provided with 500 ml of water to drink across 30 min, before another urine sample was requested. If this was not possible or the individual did not yet meet the hydration criteria, then rehydration would continue for another 30 min or the trial would be rearranged.

3.6.3 Determinants of endurance performance

3.6.3.1 Lactate thresholds

Blood lactate was determined from arterialised fingertip capillary samples. The fingertip was cleaned with an alcohol wipe and punctured using a Softclix Pro lancet (Roche Diagnostics, Lewes, UK). To prevent contamination or erythrolysis, the first drop of blood was discarded. Approximately 25 µL of blood was collected into lithium heparin coated microvette tubes (CB300 µL, Sarstedt, Germany) before being presented to the analyser sipper. Blood samples were analysed for lactate concentration using an automated lactate and glucose analyser (YSI 2300, YSI, Ohio USA). The analyser contains an enzyme specific for the substrate of interest within the three layered membrane. The substrate is oxidized as it enters the enzyme layer, producing hydrogen peroxide, which passes through the bottom of the membrane containing cellulose acetate, where it meets a platinum electrode and the hydrogen peroxide is oxidized. The resulting current is proportional to the concentration of the substrate (YSI, Ohio USA). Specifically, lactate is oxidised in the presence of lactate oxidase, producing hydrogen peroxide as per the following reaction.

$$Lactate + O_2 \xrightarrow{\text{Lactate Oxidase}} Pyruvate + H_2O_2$$

Reliability data for resting blood lactate concentration from all experimental chapters is presented below in Table 7. Samples taken upon arrival at the laboratory, following a 10 min seated rest.

Table 7: Reliability of resting blood lactate concentration.

	Trial 1	Trial 2	
n	40	40	
Mean	0.81	0.76	mMol.L^{-1}
SD	*0.21*	*0.29*	mMol.L^{-1}
TE		0.22	mMol.L^{-1}
TE (CV%)		27.9	%
95% LOA		0.61	mMol.L^{-1}
Lower/Upper	-0.56	0.66	mMol.L^{-1}
r		0.25	
p		0.347	

During graded exercise tests, blood samples were taken immediately after each 3 min stage was completed, with participants momentarily jumping astride the treadmill belt. In Study 2

(Chapter 5), fixed blood lactate concentrations of 2 and 3.5 mMol.L^{-1} were adopted to denote the lactate threshold and lactate turnpoint respectively by solving the polynomial regression equation for blood lactate versus speed at 2 and 3.5 mMol.L^{-1} as per Saunders and Green (2013). This was modified from Saunders and Green (2013) in response to not all participants displaying a blood lactate value exceeding 4 mMol.L^{-1} across all trials. Through Studies 3-5 (Chapters 6-8), fixed blood lactate concentrations of 2 and 4 mMol.L^{-1} were adopted.

Fixed blood lactate concentrations afforded precision below 1 km.h^{-1} in identifying the running speeds associated with the respective thresholds. Such precision is appropriate for the participant cohort within this thesis where changes of less than 1 km.h^{-1} are meaningful, and this level of precision cannot be objectively achieved through visual inspection when stage increments of 1 km.h^{-1} are used. This approach also eliminated the possibility of experimenter bias in identifying thresholds, and individualised the analysis, allowing comparison across participants irrespective of the number of stages the individual completed. To control for the influence of nutritional status and resting blood lactate on the absolute blood lactate response (Jacobs 1981; Yoshida 1984), pre-exercise standardisation guidelines and diet replication were routinely reinforced to participants the evening prior to every trial.

The reliability of running speeds at fixed blood lactate concentrations are presented below, calculated from the control group in Studies 4 and 5 (Chapter 7 and 8). The heat acclimation group of Studies 4 and 5 were excluded based on the effect of acclimation on the lactate threshold (Lorenzo et al. 2011).

Table 8: Reliability of 2 mMol.L^{-1}.

2 mMol.L^{-1}			
	Trial 1	Trial 2	
n	7	7	
Mean	12.20	12.40	Km.h^{-1}
SD	*1.92*	*1.85*	Km.h^{-1}
TE		0.25	Km.h^{-1}
TE (CV%)		2.0	%
95% LOA		0.69	Km.h^{-1}
Lower/Upper	-0.89	0.49	Km.h^{-1}
r		0.98	
p		0.858	

Table 9: Reliability of 4 mMol.L^{-1}.

4 mMol.L^{-1}			
	Trial 1	Trial 2	
n	7	7	
Mean	14.22	14.46	Km.h^{-1}
SD	*1.60*	*1.54*	Km.h^{-1}
TE		0.21	Km.h^{-1}
TE (CV%)		1.4	%
95% LOA		0.58	Km.h^{-1}
Lower/Upper	-0.82	0.34	Km.h^{-1}
r		0.98	
p		0.780	

3.6.3.2 Respiratory gas analysis

During Study 2 respiratory gases were analysed using an online gas analysis system, with samples internally mixed (Metalyzer Sport analyser, Cortex, Leipzig, Germany). Following an upgrade of equipment, a breath by breath Metalyzer 3B (Cortex, Leipzig, Germany) was used throughout Studies 3-5. The analyser was pre-warmed for 30 min before a 3-point calibration was completed prior to every trial. Firstly, a correction was made for barometric pressure, determined from a portable barometer (Weather Station, Oregon Scientific, Oregon, USA). The analyser then sampled ambient air and a gas of known concentrations of oxygen and carbon dioxide (15% O_2, 5% CO_2 sourced from Brin Oxygen Company), until the software (Metasoft, Cortex, Germany) identified stabilisation of sensors. Ambient air was sampled from the hot and humid environment where exercise would take place. Finally, the volume transducer was calibrated through simulated inspiration and expiration via manual syringe (3L, Hans Rudolph, Germany) for 5 cycles that elicited an acceptable flow rate between 2-4 L.s^{-1}.

3.6.3.3 Running economy

Ventilatory gases were measured across the exercise protocol breath by breath and converted into 30 second averages. Running economy was estimated from the oxygen consumption from the final minute of the first five stages of GXT 1, providing the oxygen consumption per kilometre, relative to body mass (mL O_2.kg^{-1}.km^{-1}) as per Jones (2006). Data from the sixth, and later stages of GXT 1 were not included to control for the influence of the $\dot{V}O_2$ slow component in the calculation of RE.

Table 10: Reliability of mean running economy across five stages of GXT 1 in hot and humid conditions.

	Trial 1	Trial 2	
n	15	15	
Mean	226.9	225.5	mL O_2.kg^{-1}.km^{-1}
SD	*18.4*	*17.3*	mL O_2.kg^{-1}.km^{-1}
TE		6.8	mL O_2.kg^{-1}.km^{-1}
TE (CV%)		3.0	
95% LOA		18.8	mL O_2.kg^{-1}.km^{-1}
Lower/Upper	-17.4	20.2	mL O_2.kg^{-1}.km^{-1}
r		0.86	
p		0.831	

3.6.3.4 $\dot{V}O_{2max}$

During Study 2 $\dot{V}O_{2max}$ was calculated as the highest 15 s moving average of oxygen consumption recorded during GXT 2. A shorter averaging period was adopted due to the short recovery period between GXT 1 and GXT 2 of 2 min. Recovery was minimal in order to help ensure both physiological and perceptual effects of cooling would still be present whilst testing $\dot{V}O_{2max}$. Therefore, a shorter averaging period was adopted in order to attenuate a potential effect on blunted $\dot{V}O_{2max}$ values. As interventions within Studies 3-5 did not follow the same time course as cooling, a longer rest period was afforded between GXT 1 and GXT 2. Consequently, a more conventional 30 s averaging was used during these chapters as per the recommendations of Saunders and Green (2013) and Jones (1996a).

In accordance with the recommendations of Edvardsen et al. (2014) secondary criteria for $\dot{V}O_{2max}$ were adopted, in keeping with recommended criteria for trained athletes (Jones 2006a, Saunders & Green, 2013). A $\dot{V}O_{2max}$, not $\dot{V}O_{2peak}$, was accepted when a $\dot{V}O_2$ plateau (<2 mL.kg^{-1}.min^{-1} across two successive 30 s fixed-time averages) was observed. In the absence of a plateau, a test was deemed maximal if three out of the following four criteria were met; blood lactate concentration >8 mMol.L^{-1}, HR within 10 beats of age predicted maximum, respiratory exchange ratio >1.1, and RPE at or above 19 RPE.

Table 11: Reliability of $\dot{V}O_{2max}$.

	Trial 1	Trial 2	
n	14	14	
Mean	56.6	57.6	mL.kg^{-1}min^{-1}
SD	*5.9*	*5.2*	mL.kg^{-1}min^{-1}
TE		2.2	mL.kg^{-1}min^{-1}
TE (CV%)		3.9	
95% LOA		6.1	mL.kg^{-1}min^{-1}
Lower/Upper	-7.1	5.2	mL.kg^{-1}min^{-1}
r		0.85	
p		0.670	

3.6.3.5   v$\dot{V}O_{2max}$

Velocity at $\dot{V}O_{2max}$ (v$\dot{V}O_{2max}$) was calculated by multiplying $\dot{V}O_{2max}$ (mL.kg^{-1}.min^{-1}) by 60, divided by the average running economy as per Jones (2006a).

Table 12: Reliability of v$\dot{V}O_{2max}$.

	Trial 1	Trial 2	
n	14	14	
Mean	15.45	15.68	Km.h^{-1}
SD	*2.31*	*2.10*	Km.h^{-1}
TE		0.45	Km.h^{-1}
TE (CV%)		2.9	
95% LOA		1.25	Km.h^{-1}
Lower/Upper	-1.48	1.01	Km.h^{-1}
r		0.96	
p		0.780	

3.6.4   Blood glucose concentration

Blood glucose concentration was assessed from the same fingertip sample as blood lactate concentration, as per the procedure previously detailed and using the same YSI 2300 analyser. The specific reaction that takes place to provide a measure of glucose concentration involves the oxidation of glucose in the presence of glucose oxidase, producing hydrogen peroxide and glucono-lactone.

$$\text{D-Glucose} + H_2O + O_2 \xrightarrow{\text{Glucose Oxidase}} \text{D-Gluconic Acid} + H_2O_2$$

Table 13: Reliability of resting blood glucose concentration.

Rest	Trial 1	Trial 2	
n	30	30	
Mean	5.05	4.95	mMol.L^{-1}
SD	*0.79*	*0.90*	mMol.L^{-1}
TE		0.68	mMol.L^{-1}
TE (CV%)		13.6	%
95% LOA		1.88	mMol.L^{-1}
Lower/Upper	-1.78	1.99	mMol.L^{-1}
r		0.36	
p		0.655	

Table 14: Reliability of exercising blood glucose concentration.

Mean concentration during exercise	Trial 1	Trial 2	
n	7	7	
Mean	4.96	4.94	mMol.L^{-1}
SD	*0.83*	*0.83*	mMol.L^{-1}
TE		0.22	mMol.L^{-1}
TE (CV%)		4.5	%
95% LOA		0.62	mMol.L^{-1}
Lower/Upper	-0.60	0.63	mMol.L^{-1}
r		0.93	
p		0.974	

3.6.5 Core temperature

Core temperature (T_{CORE}) measurement was monitored continuously to ensure participant safety, as well as quantifying the effect of interventions during each experimental chapter. Despite being invasive, and relatively slow to respond to changes in thermal state due to the volume of tissue surrounding the measurement location, the criterion measure during exercise in the heat remains rectal temperature (Ganio et al. 2009). Whilst oesophageal provides a valid measure (mean bias during exercise -0.18°C, Lee et al. 2000) and may be more responsive (Saltin & Hermansen 1966), the associated discomfort necessitates extensive familiarisation (Cranston et al. 1954), and thus is not considered a practical alternative. Telemetric gastro-intestinal pills offer a less intrusive, valid and reliable alternative (mean bias during exercise -0.02°C, Byrne & Lim 2007), but are expensive and susceptible to bias when a cooling impulse is subsequently delivered to the gut as a consequence of inter-individual differences in gut transit time (Lee 2011). Therefore, single-use rectal thermistor probes (Henleys Medical, UK), connected to a metering box (Meter logger Model 401, Yellow Springs Instruments, Missouri, USA) were used to inform T_{CORE} throughout this thesis. Rectal probes were inserted 10cm past the anal sphincter, with the depth and fixation of the probe aided by wrapping a small amount

of zinc oxide tape at the appropriate depth. The manufacturer stated accuracy and reliability are ±0.05°C and ±0.01°C respectively between 0-70°C. Table 15 shows the calculated inter-day reliability of T_{CORE}, based on the data from Studies 1-3 and the control group of Studies 4 and 5. These data are comparable with those in Table 16, which presents acceptable levels of reliability and sensitivity for T_{CORE} and T_{SKIN} as reported in the literature, which serve to inform levels of variation that are currently considered acceptable for measures used in this thesis.

Table 15: Inter-day reliability of T_{CORE}.

	Trial 1	Trial 2	
n	30	30	
Mean	37.07	37.08	°C
SD	*0.29*	*0.27*	°C
TE		0.13	°C
TE (CV%)		0.4	
95% LOA		0.37	°C
Lower/Upper	-0.38	0.37	°C
r		0.77	
p		0.930	

Table 16: Reliability of skin (T_{SKIN}; wired thermistors) and core (T_{CORE}; rectal thermistor) temperature measurement at rest and during exercise.

OUTCOME MEASURE		RELIABILITY MEASURES						Meaningful Δ
		Δ mean (±SI unit)	Pearson (*r*)	ICC	TEM (±SI unit)	TEM (±CV%)	LOA (±)	
T_{SKIN}	REST	0.5°C [1]	>0.9 [1]	>0.90 [1]	<0.3 [1]	<1% [1].	<0.9°C [1]	>0.5°C [1]
	EXERCISE	0.5°C [1]	>0.9 [1]	>0.90 [1]	<0.30 [1]	<1% [1]	<0.9°C [1]	>0.5°C [1]
T_{CORE}	REST	0.10°C [3] 0.12°C [4]	>0.94 [4]	>0.97 [4]	<0.1°C [2]	<1.8% [4]	<0.22°C [2]	>0.1°C [3]
	EXERCISE	0.10°C [3]	>0.94 [4]	>0.97 [4]	<0.1°C [3]	<1.8% [4]	<0.43°C [4]	>0.1°C [3]

Legend to Table 16; 1 Harper Smith et al. 2010, [2] Byrne & Lim 2007, [3] Muir et al. 2001, [4] Ganio et al. 2009.

NB. Values are from both temperate and hot environments. Columns from L-R; respective temperature measurement, a battery of relative and absolute reliability statistics with acceptable levels of error, typical change in the mean to elucidate a statistically significant difference (*p*<0.05) and finally what magnitude of change may be considered meaningful.

3.6.6 Skin temperature

Alongside T_{CORE} measurement, skin temperature (T_{SKIN}) was continuously monitored through all trials. It is through the skin that the body loses or gains heat and as such, T_{SKIN} plays an important role in human thermoregulation. The criterion measure for T_{SKIN} are wired thermistors or thermocouples attached to specified anatomical sites. Following early estimate that advocated up to 12 sites across the body (Hardy & Du Bois 1938) to develop an estimate of mean T_{SKIN}, this has been simplified this to 4 sites; the mid-belly of the pectoralis major, biceps brachii, rectus femoris and gastrocnemius (Ramanathan 1964, see General Methods, Section 3.7.3, Equation 12). Thermistors and thermocouples are non-invasive, but the associated wiring requires familiarisation and a hard-wired connection to a datalogger, making field testing problematic. This in turn, limits the external validity of thermal interventions, which are untested in the field. The recent development of wireless skin thermistors and infrared imaging techniques may provide alternatives to traditional thermistors, particularly as wireless devices appear more accurate than wired thermistors (Harper-Smith et al. 2010) and require no familiarisation. This problem informed the direction of Study 1 (Chapter 4).

Following this initial investigation, the most accurate, reliable and least inhibitive skin temperature measurement system was chosen for use through all subsequent trials. Thereafter, skin temperature (T_{SKIN}) was measured via telemetry thermistors (U-Type connected to Gen II GD38 transmitter, Eltek, UK), attached to the mid-belly of the pectoralis major, biceps brachii, rectus femoris and gastrocnemius. After shaving surface hair and cleaning with a sterilizing wipe, thermistors were attached to each site using a film patch. Breathable film patches (Tegaderm 1632W, 3M, UK) fastened all sensors and ensured thermistors remained in contact and perpendicular to the skin. The same product was used during all trials to maintain consistency of any micro-climate effect. Data were transmitted wirelessly from the transmitter worn on the person, to a datalogger (RX250AL 1000 series Wireless Squirrel Logger, Eltek). The system was set to sample every 10 seconds and log the mean across 30 seconds. Accuracy and reliability data for this system is discussed in detail in Study 1 (Chapter 4). Table 16 presented acceptable levels of reliability and sensitivity for T_{SKIN} as reported in the literature, which serve to inform levels of variation that are currently considered acceptable for the measures that will be used in this thesis. Table 17 presents data from within this thesis that is commensurate with the variability in the literature.

Table 17: Reliability of resting T_{SKIN}.

	Trial 1	Trial 2	
n	30	30	
Mean	34.36	34.29	°C
SD	*0.68*	*0.587*	°C
TE		0.33	°C
TE (CV%)		1.0	
95% LOA		0.93	°C
Lower/Upper	-0.86	0.99	°C
r		0.73	
p		0.687	

3.6.7 Heart rate

During Studies 1, 2, 4 and 5 heart rate (HR) was measured through a moistened chest strap (Polar T30 coded), displayed by short range telemetry on a wrist watch (Polar RS800, Kempele, Finland). For Study 3, a Cortex Metalyzer *SMART Bluetooth HR receiver* logged HR continuously throughout the trial from the same chest strap.

Table 18: Reliability of resting HR.

	Trial 1	Trial 2	
n	30	30	
Mean	56	54	b.min^{-1}
SD	*10*	*9*	b.min^{-1}
TE		3	b.min^{-1}
TE (CV%)		5	
95% LOA		7	b.min^{-1}
Lower/Upper	-5	8	b.min^{-1}
r		0.93	
p		0.569	

3.6.8 Perceived exertion

Rating of perceived exertion (RPE) encompassing effort, exertion, breathlessness and fatigue was quantified during exercise using a Borg RPE scale where 6 equals *very very light* continuing up to 20 representing maximal work; where 19 is *very very hard* (Borg 1998). RPE was recorded at the end of each exercise stage, as a blood sample was being taken, as well as immediately following cessation of exercise during GXT 2.

3.6.9 Thermal sensation

Thermal sensation during rest and exercise was quantified through a thermal sensation scale whereby 0 represents '*unbearably cold*', continuing up to 8 representing '*unbearably hot*' in increments of 0.5 (Gagge et al. 1969). Thermal sensation was recorded at the end of each exercise stage, as a blood sample was being taken, as well as immediately following cessation of exercise during GXT 2.

3.6.10 Sweat rate

Sweat rate ($L.hr^{-1}$) was calculated from the difference in pre and post nude body mass divided by the individual exercise duration. Body mass was calculated using the same scales as detailed earlier in Section 3.6.1, General Methods.

3.7 Calculations

3.7.1 Change in Plasma Volume

In Studies 4 and 5 (Chapters 7 and 8), plasma volume change was estimated from measured haematocrit and haemoglobin concentration.

Equation 10: Change in plasma volume (Dill and Costil 1974). Where 'A' represents the first blood sample and 'B' the second.

$$\Delta PV\% = 100[HbA (1 - HctB \times 10^{-2})] / [HbB (1 - HctA \times 10^{-2})] - 100$$

3.7.2 Physiological strain index

Scale of 1-10, whereby T_{CORE0} and HR_0 denote baseline and T_{CORE1} and HR_1 denotes measurement taken at any time during exercise.

Equation 11: Physiological strain index (Moran et al. 1998)

$$PSI = [5 * (T_{CORE}^1 - T_r^0)* ((39.5 - T_{CORE}^0)] + [5 * (HR^1 - HR^o) * (180-HR^0)]$$

3.7.3 Mean skin temperature

Equation 12: Mean skin temperature (T_{SKIN}) (Ramanathan 1964):

$$Mean\ T_{SKIN} = 0.3(T_{CHEST} + T_{ARM}) + 0.2(T_{THIGH} + T_{CALF})$$

3.8 Statistical analyses

Prior to data collection the number of participants required for each experimental chapter was determined using G*Power v3.1 in accordance with established guidelines (Prajapati et al.

2010; Suresh & Chandrashekara 2012). Where possible effect sizes were utilised from published literature that demonstrated similar experimental design and participant characteristics. Effect sizes were calculated from means and standard deviations where this was not possible. Estimations of sample size were made based upon conventional α (0.05) and β (0.20) levels.

All outcome variables were assessed for normality and sphericity prior to further analysis. Data were analysed in three phases: rest, intervention and exercise. Two way, repeated measures ANOVA (condition*time) were used to test for differences in primary outcome variables such as blood lactate indices, respiratory responses, T_{CORE}, T_{SKIN}, PSI, RPE and TS. One way, repeated measures ANOVA were used to detect differences between singular values such as $\dot{V}O_{2max}$, running time until $\dot{V}O_{2max}$ during GXT 2, $v\dot{V}O_{2max}$ and thermoregulatory variables at rest, where there were more than 2 experimental conditions, with paired samples t-test used when only two conditions. Where appropriate, Bonferroni adjusted pairwise comparisons revealed where differences occurred. Data were analysed using SPSS (Version 20, SPSS Inc, Illinois, USA) with significance set at $p<0.05$ and the data are presented as means ($\pm$SD). Effect sizes for main effects and interactions are presented as partial eta squared (η^2), whilst differences between two related samples were evaluated through Cohen's d_{av} in accordance with (Lakens 2013). Lakens (2013) and other recent articles (Cummings 2013) advise against the use of parameters around Cohen's d as they are considered arbitrary, instead recommending the magnitude of change is compared against other published effects. Additional statistical techniques are reported in the appropriate chapters.

4 Reliability and validity of skin temperature measurement by telemetry thermistors and a thermal camera during exercise in the heat

4.1 Abstract

New technologies have afforded convenient modalities for skin temperature (T_{SKIN}) measurement, notably involving wireless telemetry and non-contact infrared thermometry. The purpose of this study was to investigate the validity and reliability of skin temperature measurements using a telemetry thermistor system (TEL) and thermal camera (TC) during exercise in a hot environment. Each system was compared against a certified thermocouple, measuring the surface temperature of a metal block in a thermostatically controlled waterbath. Fourteen recreational athletes completed two incremental running tests, separated by one week. Skin temperatures were measured simultaneously with TEL and TC compared against a hard-wired thermistor system (HW) throughout rest and exercise. Post hoc calibration based on waterbath results displayed good validity for TEL (mean bias [MB] = -0.18°C, typical error [TE] = 0.18°C) and reliability (MB = -0.05°C, TE = 0.31°C) throughout rest and exercise. Poor validity (MB = -1.4°C, TE = 0.35°C) and reliability (MB = -0.65°C, TE = 0.52°C) was observed for TC, suggesting it may be best suited to controlled, static situations. These findings indicate TEL systems provide a convenient, valid and reliable alternative to HW, useful for measurements in the field where traditional methods may be impractical.

4.2 Introduction

Skin temperature (T_{SKIN}) measurement has application for research (Harper-Smith et al. 2010), occupational health (Kim et al. 2013) and clinical monitoring (Sherman 1996). It is through the skin that the body loses or gains heat and as such, T_{SKIN} plays an important role in human thermoregulation. T_{SKIN} is a consequence of dermis microcirculation, which is mediated through activity of the sympathetic nervous system and regulated by the hypothalamus. Typically T_{SKIN} may initially reduce during exercise as a consequence of sweat on the skin surface and blood shifting towards working skeletal muscles (Torii et al. 1992). However, a steady rise is observed during endurance exercise as core temperature (T_{CORE}) increases, with elevated ambient temperatures increasing the rate of T_{SKIN} increase (Roberts & Wenger 1979). Whilst T_{SKIN} may be interpreted in isolation, it also forms a component of derivative calculations of heat strain, such as body heat content (Jay & Kenny 2007) and mean body temperature (Jay,

Reardon, et al. 2007). Such calculations assist in understanding the mechanisms underpinning practical thermal interventions such as precooling and heat acclimation, by providing an objective measure of whole-body thermal dynamics.

Typically, T_{SKIN} has been measured using thermocouples or wired thermistors with recent literature adopting wired thermistors as the criterion measure when validating new tools (Kelechi et al. 2011, Buono et al. 2007, Burnham et al. 2006). A thermocouple is a temperature-measuring device consisting of two dissimilar conductors that contact each other at one or more joint locations. It produces a measureable electrical potential difference proportional to the temperature difference against another joint, which is set at a reference temperature in another part of the circuit. Thermistors are resistors in which resistance varies with temperature, allowing stored calibration data within the circuit to convert this to a temperature. Such devices have been shown to be robust and accurate to 0.045°C across a range (10-40°C) of waterbath temperatures (Harper-Smith et al. 2010). Thermistors and thermocouples are non-invasive, but the associated wiring requires familiarisation and a hard-wired connection to a datalogger, making field testing problematic. This in turn, limits the external validity of thermal interventions which are untested in the field.

Recent developments in wireless thermometry provides an alternative to hard-wired systems, particularly as some telemetry devices appear more accurate than wired thermistors (Harper-Smith et al. 2010), require little familiarisation and provide freedom of movement for the person being measured. Harper-Smith et al. (2010) examined wireless iButtons (Maxim Integrated Products Inc. California, USA) in a waterbath as well as on human skin during exercise in hot conditions. Typical error was <0.3°C, Pearson and Intra-class correlation (ICC) coefficients >0.9 and coefficient of variation (CV) <1% when compared against wired thermistors which were the criterion measure during exercise. The size and convenience of iButtons undoubtedly affords opportunities for measurements in novel environments, however the lack of real-time data may preclude their use in safety monitoring and research environments. Dermal temperature patches for physiological monitoring systems are wireless and offer live data, but being single-use only, carry significant purchase and consumable costs, which may prohibit use for large sample sizes. Consequently, a newly-developed telemetry system, whereby thermistors are connected to a transmitter worn on the person, may offer the benefits of live data without long, trailing connecting wires or being restricted to the laboratory.

Infrared thermometry is another technique that is used for monitoring T_{SKIN} in research (Costello et al. 2012) and clinical environments (Ring & Ammer 2012). Thermal cameras receive and process infrared radiation emitted from a surface, using this information to display the production and dissipation of heat. The ability of a surface to emit energy by radiation is termed 'emissivity' and allows the temperature of the emitting surface to be calculated. Thermopiles or microbolometers within the cameras absorb this infrared radiation, eliciting a change in electrical resistance that a colour palette can use to display temperatures of an object. Handheld infrared thermometers provide temperature at specific points based on the same principle, and are widely used for measuring core temperature via the tympanic membrane as well as increasingly for T_{SKIN} (Ring & Ammer 2012). Measurements from such devices demonstrate strong association with wired thermistors, providing valid measures of mean T_{SKIN} at rest (r=0.95) and whilst walking in the heat (r=0.98, Buono et al. 2007). This technology appears reliable, with mean inter-examiner intraclass correlation of r = 0.88 (range 0.73–0.99) between T_{SKIN} measurements on consecutive days (Zaproudina et al. 2008). The majority of literature utilising thermal cameras as a measure of T_{SKIN} has involved thermogram images taken at rest being retrospectively analysed using software to identify area average temperatures for specified regions of anatomical interest. Measuring temperature across a region of interest enhances construct validity by helping to avoid inter-individual variation of veins and vascularisation and the consequential non-uniform heat production, a potential confounding error when taking readings from a single spot on an image or from attached thermistors (Chudecka & Lubkowska 2012). Broadly, this technique has been shown to be valid (correlation range r =0.71-0.77, Roy et al. 2006) and reliable (correlation range r =0.82-0.97, Selfe et al. 2006), such that it has been recommended for clinical use (Ng et al. 2004; Ring & Ammer 2012). However, large errors when compared to a thermocouple during rest and exercise have also been reported (-0.75°C, Fernandes et al. 2014), making it unclear within which situations it may have application. Recent developments in thermal camera technology permit high speed imaging, offering a real-time thermal image, such that cameras can produce whole images for post hoc analysis as well as instantaneous spot analysis. These improvements allow simultaneous comparison against other T_{SKIN} measures, facilitating an objective assessment of the potential of thermal cameras as a multi-purpose tool for environmental exercise physiology research.

Wired thermistors, telemetry thermistors and a thermal camera do not appear to have been compared simultaneously for live T_{SKIN} measurement during exercise in hot environments. Therefore, the aim of this study was to compare the reliability and validity of these

measurement tools for live T_{SKIN} measurement in athletes exercising in a hot and humid environment, including subsequent studies within this thesis. It was hypothesised that telemetry thermistors and a thermal camera would provide acceptable levels of error for both reliability and validity when compared against hard-wired thermistors during exercise in the heat.

4.3 Methods

4.3.1 Participants

Fourteen (male) recreational club runners volunteered as participants (mean [±SD]): age 38 (11) years, stature 179 (8) cm, mass 77.3 (7.1) kg, sum of four skinfolds 33.6 (7.7) mm, $\dot{V}O_{2max}$ 57.3 (4) mL.kg^{-1}.min^{-1}. No contraindications for testing were violated prior to commencing any experimental session in accordance with previously detailed procedures (General Methods Section 3.3).

4.3.2 Experimental design

The study was organised into two parts; a waterbath comparison and human skin temperature measurement during exercise. Both parts of the study assessed validity and reliability of tools. During the waterbath analysis, data were collected for 20 minutes across seven stable temperatures within the range 25-40°C. Stability was defined as a deviation of no more than 0.1°C measured by the criterion thermocouple over 5 min consecutively. Retest reliability was examined on the following day. In order to assess the measurement tools in a relevant context for endurance exercise in the heat, an incremental exercise test was completed on each athlete volunteer. Re-tests of T_{SKIN} measurements were separated by one week to prevent an acclimation effect (Barnett & Maughan 1993) and taken at the same time of day (Winget 1985), with the second trial data used for validity analysis.

4.3.3 Measurement tools

During the waterbath tests, measurements from all thermistors and the thermal camera (TC) were referenced against a multi-point calibrated and certified thermocouple (Type K probe attached to Fluke 51 ll instrument, range -200°C - +1000°C, divisions 0.1°C, Washington, US). This thermocouple had been calibrated in a certified laboratory in the last 6 months.

During exercise, the criterion measure comprised of four hard-wired (HW) skin thermistors (Eltek U-Type EUS-U-VS5-0, Eltek Ltd, Cambridge, UK) connected to a datalogger (Grant Squirrel 1000 series, Grant Instruments, Cambridge, UK). The manufacturer stated accuracy was ±0.2°C.

This type of device has been adopted as a criterion during similar validity comparisons (Kelechi et al. 2011; Buono et al. 2007, Burnham et al. 2006). The telemetry system (TEL) comprised four skin thermistors (ELEU-U-VS-02-, Eltek U-Type, Eltek Ltd, Cambridge, UK) connected to a transmitter (Gen II GD38, Eltek, Cambridge, UK, dimensions 6x8x5cm). Data is transmitted wirelessly to a datalogger (Eltek RX250AL 1000 series Wireless Squirrel Logger, Eltek, Cambridge, UK), up to a distance of 2 km. The datalogger was placed outside of the environmental chamber approximately 3 m away. The manufacturer stated accuracy was ±0.1°C. Both dataloggers were synchronised and sampled every 30 s with minute mean logged. Data were downloaded using *Squirrelwire* and *Darca Plus* software for the HW and TEL systems, respectively. The TC was a FLIR e40BX (Flir tools, Oregon, US) with 160x120 focal plane array, uncooled microbolometer with thermal sensitivity of <0.045°C at 30°C, 7.5 to 13µm spectral range, 60 Hz frame rate and a 'live-view' colour palette offering multiple spot analysis and area average functions. The manufacturer stated accuracy was ±2°C or ±2%. Emissivity was set at *e*=0.98 in accordance with the data of Steketee (1973).

4.3.4 Procedures

4.3.4.1 Waterbath

All thermistors and the criterion thermocouple (CT) were affixed within a 3cm^2 area, each was separated by 1cm, in the centre of the top surface of a cast iron block (dimensions: 15 x 12 x 12 cm) placed in a waterbath (Fischer Scientific DMU19). Whilst a waterbath allows comparison of tools throughout a range of temperatures, water is not an appropriate body to measure using infrared thermometry. Thus, the waterbath provided controlled plateau temperatures, with the metal block providing the thermal surface for measurements. The thermal conductivity of iron results in a uniform temperature which helps avoid erroneous readings from convection currents within a large waterbath. The metal block was submerged to within 1mm of the surface, which remained dry at all times. Breathable film patches (Tegaderm 1632W, 3M, UK) fastened all sensors and ensured thermistors remained in contact and perpendicular to the surface. The same patches were used during exercise trials to maintain consistency of any micro-climate effect (Tyler 2011). Black electrical tape with a known emissivity of 0.95 marked the TC measurement site.

The camera was mounted one metre above the block, at an angle of 90° to the plane of the block, with reflected environmental temperature and environmental conditions adjusted accordingly. The incorporated laser pointer ensured readings were consistently taken from the

centre of the designated area. Both dataloggers were set to sample and log temperature values every minute, with TC and CT temperatures recorded each minute.

4.3.4.2 Exercise test

Upon arrival, participants self-inserted a single-use rectal probe and attached skin thermistors from both HW and TEL to the mid-belly of the pectoralis major, biceps brachii, rectus femoris and gastrocnemius on the right of the body, as shown in Figure 26. Thermistors were attached using a film patch as per the waterbath procedures. Participants completed all trials in running trainers, sports shorts and without a t-shirt.

Figure 26: showing location of thermistors on chest, upper arm and thigh. Thermal camera measurements were taken beside the thermistors, ensuring the patches did not interfere with measurement.

Participants then entered the environmental chamber (TISS, Hampshire, UK) with conditions (mean [±SD]) 31.9 (1)°C, 61 (8.9)% relative humidity. The trial began with participants sitting for 40 minutes with measurements taken every five minutes. All thermal camera measurements were taken handheld, at a distance of 1 m, with the camera at 90° to the relevant site as per the manufacturer's instructions. Temperatures were taken from the spot analysis function, which displays the live temperature of an area within the viewfinder.

The measurement site for the thermal camera was 1cm adjacent to the edge of the film patch fixing the thermistors. This equated to a distance of 3cm between the furthest thermistor and TC measurement site at each anatomical location. After 40 minutes, a 5 minute warm-up at 8 km.h^{-1} was completed on a motorized treadmill (Woodway ELG2, Weil am Rhein, Germany) before participants began an incremental exercise test with starting speed between 8 − 10 km.h^{-1}. Each participant completed five stages of three minutes, with speed increasing by 1 km.h^{-1}. At the end of each stage the participant would straddle the treadmill belt and the thermal camera would be used to measure T$_{SKIN}$ at each site; the procedure took approximately 30 s. At the end of the test nude body mass was recorded before participants were actively cooled using a large fan and cold drinks.

4.3.5 Statistical Analyses

4.3.5.1 Waterbath

Linear regression analysis was used to derive correction formulae for each measurement tool, relative to the criterion thermocouple. In order to develop robust formulae, all data from both day one and day two of the waterbath were included, which equated to fourteen temperature measures (seven each day) within the range 25-40°C and two hundred and eighty data points for each equation. However, statistical analyses were conducted on the measures of temperatures taken over a typical range of values that would be expected during exercise. These temperatures were 33°C, 35°C and 38°C. Validity and reliability comparisons were made on the grouped data for each tool, rather than at each temperature (33, 35, 38°C). Data were corrected to one decimal place before analysis as CT and TC are precise to 0.1°C whereas HW and TEL read to 0.01°C. Mean values of the four thermistors in each system are reported, although data from individual thermistors was adjusted based on CT and the corrected values used in subsequent analysis. Differences between tools (validity) for raw and corrected data were investigated using a two way repeated measures ANOVA (correction*tool) with Bonferroni adjustments.

Additionally, the following battery of relative and absolute reliability statistics was calculated for each tool; mean bias, typical error of the measure (TEM), calculated from the standard deviation of the mean difference for each pair of trials using the formula TE = SD$_{(diff)}$/√2 and expressed as a mean coefficient of variation (CV), intra-class correlation (ICC) and limits of agreement (LOA). Reliability comparisons compared waterbath day 1 data against day 2 data. To eliminate variation from the waterbath itself, all of day 2 data were corrected based on a linear regression equation formed from the two criterion datasets. This prevented a

systematic difference between trials, such as the failure to achieve precisely the same plateau temperature on day 2 which would invalidate reliability analyses of individual tools.

Validity comparisons were completed on waterbath day 1 data. Similar statistics were calculated for HW, TEL and TC relative to the criterion to assess validity; mean bias, ICC, typical error of the estimate (TEE), providing standard or typical error of the predicted y-value for each x, and LOA. Differences between trials for each tool were investigated using two way repeated measures ANOVA (tool*trial) with Bonferroni post hoc adjustments. Statistical tests were completed using SPSS 20 (SPSS Inc, Chicago, USA) with significance set at p <0.05 throughout. Data are presented as mean (±SD).

4.3.5.2 Exercise test

Analysis of T_{SKIN} measurements was conducted on the derivative calculation of mean T_{SKIN}, using the formula of Ramanathan (1964) (Equation 12, General Methods, Section 3.6.6). Two way ANOVA (trial*time) with Bonferroni correction were used to identify differences between exercise trials for each tool (reliability). This included a comparison of the entire dataset from trial 1 against trial 2 data (main effect trial) to assess whether a systematic change had occurred between trials which may affect the interpretation of other statistics. A two way ANOVA (tool*time) was used to identify differences between tools (validity). Sweat rate (L.hr^{-1}) was calculated from the difference in pre and post nude body mass divided by the individual exercise duration.

4.3.5.3 Analytical limits

Analytical limits may assist in completing an objective and robust assessment of a measure (Atkinson & Nevill 1998). Such limits can be predefined on what constitutes a meaningful physiological change, limits adopted by similar research and the precision of the criterion thermometer. The *a priori* analytical limits are shown in Table 19.

Table 19: Analytical limits adopted for both part 1 (waterbath) and part 2 (T_{SKIN} measurement) of this study. Sig = relative to criterion, with thermocouple the criterion during the waterbath and hard wired thermistors criterion during exercise.

	Δ mean (°C)	Sig.	TEE/TEM (°C)	TE (CV%)	ICC	LOA (°C)
Waterbath	<0.2	p>0.05	<0.1	<1%	>0.9	<0.3
Exercise	<0.5	p>0.05	<0.3	<1%	>0.9	<0.9

Columns left to right; change in mean (Δ mean), statistical difference (Sig), typical error of the estimate (TEE)/measure (TEM), typical error as a coefficient of variation (TE [CV%]), intraclass correlation coefficient (ICC) and limits of agreement (LOA).

4.4 Results

4.4.1 Waterbath reliability comparison

A difference was observed between all day 1 and all day 2 data (p=0.01), however this did not remain after day 2 data were corrected based on the differences measured by the criterion thermocouple (p=0.658) using the correction formula $\gamma = (1.009\,x) - 0.365$. The raw data also displayed differences between days for every tool. After correction, this difference was eliminated for CT (p=0.124), but remained for all other tools (Table 20).

A summary of reliability comparisons is shown in Table 20. Prior to correction, all tools displayed a mean bias below 0.2°C. Correction reduced the mean bias for both HW and TEL, but a small bias remained for TC. Absolute typical error (TEM), relative typical error (CV) and ICC calculated from the raw data were acceptable against the *a priori* analytical limits for all tools. As a linear correction factor was applied to the data, the variation in differences between trials did not change, so TEM and the associated coefficients of variation remained the same after correction. Acceptable LOA were observed for TEL, with HW marginally exceeding the limits of 0.3°C and TC displaying the largest range.

Table 20: Reliability of wired thermistors, telemetry thermistors and thermal camera after

	Δ mean (°C) (95% CI)	Sig	TEM (°C)	TE (CV%)	ICC	LOA (°C) (95% CI)
Analytical limit:	*<0.2°C*	*p>0.05*	*<0.1°C*	*<1%*	*>0.9*	*<0.3°C*
Wired	-0.10 (-0.15- -0.05)	p=0.005	0.14	0.3	0.996	0.38 (-0.28-0.48)
Telemetry	-0.04 (-0.06- -0.02)	*	0.05	0.2	1.000	0.14 (-0.10-0.18)
Thermal camera	0.14 (0.08-0.20)	*	0.16	0.5	0.996	0.44 (-0.06-0.30)

correction to account for difference in bath temperature between trials 1 and 2.

Columns left to right; change in mean between trials (Δ mean), statistical difference (Sig), typical error of the measure (TEM), typical error as a coefficient of variation (TE [CV%]), intraclass correlation coefficient (ICC) and limits of agreement (LOA). * denotes p<0.001, '95% CI' = 95% confidence interval.

4.4.2 Waterbath validity comparison

Variation in block temperature was low throughout each temperature plateau period with mean standard deviation across all temperatures below 0.07°C. Indicated water temperature was greater than displayed by CT for each temperature (block: water, 32.8:34°C, 34.9:37°C, 38.1:40°C). Individual corrections were made to the data of each thermistor. Mean values from the four thermistors of both HW and TEL systems produced the following equations; HW; $y = (0.978\,x) + 0.484$ and TEL; $y = (1.019\,x) - 0.518$. The formula used to correct all TC data was $y = (1.146\,x) - 3.121$. Formulae were derived from the fourteen plateau temperatures and provided TEE across this range of 0.13°C, 0.13°C and 0.10°C for HW, TEL and TC, respectively. The largest error for an individual thermistor was 0.18°C, with mean TEE of 0.16°C for HW and 0.14°C for TEL.

Prior to correction, the accuracy of each tool was very close to the manufacturer stated accuracy and all improved following correction (Table 21). Only TEL displayed a mean bias below 0.2°C, although HW nearly achieved this, only 0.02°C greater, with uncorrected data plotted in Figure 27. The largest error was observed for TC, with raw mean bias ten times greater than the limit. Correction brought all tools within an acceptable level of bias. Significant differences from CT were observed for all tools in the raw data, but only for HW after correction ($p<0.001$), with the corrected data significantly different to the raw data ($p<0.001$). After correction, all devices displayed acceptable levels of absolute and relative TEE, whilst correlations were just below the *a priori* acceptable limit. Limits of agreement were acceptable for HW and TEL pre and post correction, with a difference of just 0.01°C preventing TC meeting this limit.

Figure 27: Mean uncorrected data from hard-wired, telemetry system and thermal camera during each waterbath plateau temperature across the range 25-40°C. The dark line represents the criterion thermocouple (CT) against which thermistors and camera data were corrected.

Table 21: Validity of wired thermistors, telemetry thermistors and thermal camera relative to the criterion thermocouple. Mean values from 33, 35 and 38°C are presented.

	Manufacturer accuracy (±°C)	Δ mean (°C) (95% CI)	Sig	TEE (°C)	TE (CV%)	ICC	LOA (°C) (95% CI)
Raw data							
Analytical limit:		*<0.2°C*	*p>0.05*	*<0.1°C*	*<1%*	*>0.9*	*<0.3°C*
Wired	0.2	-0.22 (-0.24 - -0.20)	*	0.07	0.2	0.85	0.13 (-0.35- -0.09)
Telemetry	0.1	0.15 (0.13-0.17)	*	0.07	0.2	0.86	0.16 (0.00-0.31)
Thermal camera	2	2.01 (1.91-2.10)	*	0.17	0.5	0.99	0.74 (1.26-2.75)
Corrected							
Wired	0.2	0.08 (0.05-0.11)	*	0.07	0.2	0.85	0.21 (-0.13-0.29)
Telemetry	0.1	0.02 (0.00-0.04)	*p*=0.267	0.08	0.2	0.86	0.15 (-0.13-0.17)
Thermal camera	2	-0.02 (-0.07-0.02)	*p*>0.99	0.16	0.5	0.86	0.31 (-0.34-0.29)

Columns left to right; manufacturer stated accuracy, change in mean (Δ mean), statistical difference (Sig), typical error of the estimate (TEE), typical error as a coefficient of variation (TE [CV%]), intraclass correlation coefficient (ICC) and limits of agreement (LOA). * denotes *p*<0.001, '95% CI' = 95% confidence interval.

4.4.3 Skin temperature reliability at rest and during exercise

Sweat rate did not differ between trials (Trial 1; 1.52 L.hr^{-1}, Trial 2; 1.48 L.hr^{-1}, *p*=0.726). No differences in T_{SKIN} were observed between trials (*p*=0.137) for HW (Figure 28). The HW system displayed a consistently small mean bias with a mean value of 0.01°C across rest and 0.18°C during exercise (Table 22). Across the statistics adopted, the largest differences occurred during the first 20 minutes of rest, with the greatest reliability after 30 minutes of rest. Initially TE was high (0.53°C), but improved throughout the rest period (mean=0.34°C), reaching 0.2°C after 30 minutes and remaining just above the analytical limit throughout exercise (mean=0.31°C). Similarly, LOA was initially high (1.47°C after 10 minutes), but acceptable at the end of rest (35

minutes; 0.56°C, mean=0.95°C). This further improved during exercise, with the mean value 0.86°C. Correlation between the trials across rest (*r*=0.67) and exercise (*r*=0.62) was low, although after 30 minutes of the rest period the correlation increased (*r*=0.84) to a level just below the predefined limit.

Table 22: Reliability of wired thermistors, telemetry thermistors and thermal camera measuring skin temperature at rest and during exercise.

		Δ mean (°C) (95% CI)	TEM (°C)	TE (CV%)	ICC	LOA (°C) (95% CI)
	Analytical limit:	*<0.5°C*	*<0.3°C*	*<1%*	*>0.9*	*<0.9°C*
HW	Rest	0.01 (-0.27-0.29)	0.34	1	0.67	0.95 (-0.95-0.94)
	Exercise	0.18 (-0.46-0.09)	0.31	0.88	0.62	0.86 (-0.62-1.09)
TEL	Rest	0.10 (-0.22-0.42)	0.38	1.11	0.64	1.04 (-1.14-0.94)
	Exercise	-0.19 (-0.42-0.04)	0.24	0.7	0.84	0.67 (-0.48-0.87)
TC	Rest	-0.38 (-0.83-0.07)	0.53	1.58	0.52	1.46 (-1.08-1.85)
	Exercise	-0.92 (-1.35-0.49)	0.5	1.5	0.56	1.40 (-0.60-2.20)

Columns left to right; change in mean (Δ mean), typical error of the measure (TEM), typical error as a coefficient of variation (TE [CV%]), intraclass correlation coefficient (ICC) and limits of agreement (LOA). 'CI' = 95% confidence interval.

No main effect between trials was observed (*p*=0.343) for TEL, however a time*trial interaction was present (*p*=0.040) with differences during the final two exercise stages (Figure 28). A small mean bias was observed throughout the TEL trial 2 (-0.05°C) which was within acceptable limits, as was bias during rest (-0.1°C) and exercise (-0.19°C). Typical error within TEL was greatest at the start of rest (0.48°C), reducing to 0.29°C after 30 minutes, and remained low for the remainder of the trial (mean during exercise 0.24°C, overall mean 0.31°C), either below or very close to the acceptable limit. As with HW, despite overall correlation for the entire protocol being below 0.9 (*r*=0.74), points of strong agreement were observed for TEL, in particular during exercise with correlation coefficients of *r*=0.89, 0.95 and 0.96 for the final three stages.

A main effect was observed between trials (*p*=0.023) for TC reflecting a systematic difference from trial 1 to trial 2. Similarly, a trial*time interaction was observed (*p*=0.001) with differences in TC identified from the second exercise stage and continuing to the end of the

trial (Figure 28). Agreement was poor for all statistics with none consistently meeting the acceptable limits (Table 22). A large negative bias was observed throughout trial 2 (-0.65°C).

Figure 28: Mean (±SD) reliability of wired thermistors, telemetry thermistors and thermal camera measuring skin temperature. "R" = rest, "Ex" = exercise. "*" denotes a difference between trials (*p*<0.05).

4.4.4 Skin temperature validity comparison

A main effect for tool (p=0.002) was observed with differences between HW and TEL (p=0.03) and HW and TC (p=0.007). A tool*time interaction effect (p<0.001) was also observed. A tabular report detailing individual differences is shown in Table 23. The TEL system showed good agreement with HW throughout the protocol. Mean bias was consistently low (mean: -0.18°C), achieving the predefined limit. This is supported by low mean typical error (0.18°C) and strong correlation (r=0.92). Similarly, mean LOA are narrow and within the acceptable limits (0.39°C). Unlike TEL, TC did not show good agreement with HW. A large bias was recorded throughout rest (-0.87°C) and exercise (-1.92°C) phases. Typical error consistently exceeded the limits and correlations were low throughout the trial (mean r=0.45). These errors translated into large limits of agreement, consistently above the predefined limit (1.30°C).

	Rest 1	Rest 2	Rest 3	Rest 4	Rest 5	Exercise 1	Exercise 2	Exercise 3	Exercise 4	Exercise 5

Table 23: Tabular report of validity comparisons between hard wired thermistors, telemetry thermistors and thermal camera at rest and during exercise.

	10 min	*20 min*	*25 min*	*30 min*	*35 min*	*4 min*	*8 min*	*12 min*	*16 min*	*20 min*
HW – mean (*sd)*	33.81 (*0.44)*	33.93 (*0.52)*	34.10 (*0.43)*	34.10 (*0.39)*	34.16 (*0.41)*	34.75 (*0.50)*	35.26 (*0.31)*	35.19 (*0.31)*	35.19 (*0.40)*	35.25 (*0.56)*
TEL – mean (*sd)*	33.72 (*0.47)*	33.77 (*0.46)*	33.96 (*0.38)*	33.94 (*0.39)*	34.05 (*0.40)*	34.51 (*0.54)*	35.02 (*0.32)*	34.96 (*0.45)*	34.95 (*0.56)*	35.02 (*0.69)*
Δ mean (°C) (95% CI)	-0.09 (-0.23-0.05)	-0.16 (-0.34-0.02)	-0.13 (-0.27-0.00)	-0.15 (-0.29--0.02)	-0.12 (-0.26-0.03)	-0.23* (-0.34--0.12)	-0.25* (-0.34--0.16)	-0.22 (-0.35--0.10)	-0.24 (-0.42--0.06)	-0.24 (-0.47-0.00)
TEE (CV%)	0.21 (0.6)	0.25 (0.7)	0.20 (0.6)	0.20 (0.6)	0.20 (0.6)	0.15 (0.4)	0.11 (0.3)	0.10 (0.3)	0.15 (0.4)	0.26 (0.7)
ICC	0.91	0.92	0.91	0.90	0.91	0.97	0.96	0.92	0.92	0.92
LOA (°C) (95% CI)	0.41 (-0.32-0.50)	0.45 (-0.29-0.61)	0.38 (-0.24-0.51)	0.38 (-0.23-0.54)	0.39 (-0.27-0.50)	0.30 (-0.07-0.53)	0.21 (0.04-0.46)	0.35 (-0.13-0.57)	0.46 (-0.22-0.70)	0.59 (-0.36-0.83)
HW – mean (*sd)*	33.81 (*0.44)*	33.93 (*0.52)*	34.10 (*0.43)*	34.10 (*0.39)*	34.16 (*0.41)*	34.75 (*0.50)*	35.26 (*0.31)*	35.19 (*0.31)*	35.19 (*0.40)*	35.25 (*0.56)*
TC – mean (*sd)*	33.06 (*0.65)*	33.04 (*0.81)*	33.06 (*0.83)*	33.27 (*0.67)*	33.31 (*0.80)*	32.63 (*1.13)*	33.45 (*0.54)*	33.26 (*0.85)*	33.33 (*0.94)*	33.32 (*0.99)*
Change in mean (°C)	-0.75 (-1.19--0.31)	-0.89 * (-1.30--0.48)	-1.04 * (-1.46--0.61)	-0.83 * (-1.18--0.48)	-0.85 ** (-1.29--0.41)	-2.12 * (-2.93--1.31)	-1.82 * (-2.29--1.35)	-1.93 * (-2.39--1.46)	-1.86 * (-2.37--1.36)	-1.94 * (-2.50--1.37)
TEE (CV %)	0.43 (1.3)	0.37 (1.1)	0.31 (0.9)	0.32 (0.9)	0.33 (1.0)	0.51 (1.5)	0.33 (1.0)	0.22 (0.6)	0.24 (0.7)	0.45 (1.3)
ICC	0.33	0.75	0.65	0.60	0.58	0.18	0.23	0.55	0.65	0.64
LOA (°C) (95% CI)	1.28 (-0.54-2.03)	1.05 (-0.16-1.94)	1.17 (-0.13-2.20)	1.01 (-0.18-1.84)	1.21 (-0.36-2.06)	2.22 (-0.11-4.34)	1.10 (0.72-2.92)	1.27 (0.66-3.19)	1.28 (0.58-3.15)	1.44 (0.49-3.38)

From top to bottom; descriptive statistics, change in mean (Δ mean), typical error of the estimate (TEE), intraclass correlation coefficient (ICC), limits of agreement (LOA). '*' denotes significant difference whereby $p<0.05$, 'CI' = 95% confidence interval.

4.5 Discussion

Assessing novel tools in a controlled, static environment as well as ecologically valid situations allows objective assessments of measurement accuracy and interpretation of meaningful change. A telemetry thermistor system (TEL) offers a technologically similar, but more convenient option to traditional hard-wired systems. Thermal cameras (TC) measure the emitted infrared radiation from a body, permitting live non-contact infrared measurements, but have not previously been assessed across a range of running speeds in a hot, humid environment. The aim of this study was to compare the validity and reliability of these measurement tools when measuring T_{SKIN} whilst resting and running in a hot and humid environment. As expected, hard-wired thermistors (HW) demonstrated good agreement with the criterion thermocouple (CT) during the initial waterbath comparison and were reliable between trials. The TEL system demonstrated the smallest error and greatest agreement with CT during static waterbath measurement. This system also performed well when measuring T_{SKIN} at rest and during exercise, displaying the least error and largest correlations between trials, as well as the strongest agreement with the criterion suggesting it is appropriate for use within environmental physiology research. The thermal camera also demonstrated a small error during the waterbath measures, although a large systematic bias was detected. Using the current protocol, the camera did not perform as well as the other devices when providing live, handheld measurements of T_{SKIN} during exercise and is not recommended for use in this manner, as it has a tendency to under-read during exercise which presents safety implications.

4.5.1 Waterbath validity comparison

The correction of collected data based on a criterion measure has been advocated by previous research (Harper-Smith et al. 2010). This in-house calibration procedure is time consuming and requires specialist equipment, but is relatively inexpensive and the differences between raw and corrected data in this study suggest it is worthwhile when accurate results are required. Furthermore, completing this process prior to subsequent human testing will assist in partitioning error into biological, environmental and random error, incorporating the equipment error itself. Prior to correction, overall reliability from both HW and TEL was acceptable based on the predefined analytical limits (Table 19). Such limits were adopted based on what constitutes a meaningful physiological change, the limits adopted by similar research and the precision of the criterion thermometer. Of particular note was the mean bias (2°C) and LOA (0.74°C) observed in TC, which despite meeting the manufacturer stated accuracy, far exceeds the acceptable error (0.2°C) for safe and meaningful measurements of T_{SKIN}. Although

TC over-estimated across all waterbath temperatures, a heteroscedastic trend is observed with the greatest errors occurring at the highest temperatures (Figure 27). Within the range assessed, the indicated water temperature was above that measured by CT. Despite being setup in accordance with manufacturer's guidelines, it is possible TC partially detected water temperature rather than solely the metal block. Thermal cameras are usually calibrated by taking readings on an electronically heated black body and corrected as necessary. The purpose of the waterbath was not to calibrate the tools, but to allow a simultaneous comparison of all tools to facilitate a communal correction to help detect differences. This method was adopted given the traditional method for thermistor comparison was inappropriate for including TC and following correspondence with the TC manufacturer. These data may indicate a limitation for TC when measuring against a background which is hotter than the object in question. These errors were minimised after correction, such that TC accuracy was acceptable in terms of mean bias, no significant difference and low CV. Other statistics were extremely close to acceptable limits.

4.5.2 Waterbath reliability comparison

Data collected during trial 2 of the waterbath analysis were corrected based on the differences observed by the criterion between trials. This was necessary due to a difference between plateau temperatures (0.1°C, $p<0.001$) on day two. Correction allowed the remaining differences to be attributed to the tools themselves rather than the waterbath conditions. The correction reduced the mean bias for all tools and eliminated the significant difference between trials for HW, but not for TEL and TC. However, significance testing alone does not preclude a device from being considered reliable or valid, which is why a battery of statistics was completed. When a significant difference is identified, it is typically the magnitude of this difference that is relevant to the end user (Hopkins et al. 2009). Cohen's d effect sizes state these differences to be trivial. Therefore, where a significant difference was observed, in the context of the accepted limits the difference was not critical. TEL was the most reliable tool, achieving all other *a priori* limits, both with the raw and corrected data. Wired thermistors and TC failed to meet the acceptance levels for TE and LOA, however both displayed low mean bias, strong correlations and low CV, so were deemed to have performed reliably.

4.5.3 Skin temperature validity comparison

During rest and exercise HW recorded a consistently higher temperature than TEL and TC. However, there was strong agreement between HW and TEL, with all *a priori* limits achieved, meaning TEL can be considered a valid measurement tool. Conversely, TC showed poor

agreement and could not be considered valid for measurement of T_{SKIN} using this method or similar conditions with an error of 2°C potentially affecting mean body temperature by 0.4°C (Jay & Kenny 2007).

During exercise, both TEL and TC recorded lower temperatures than HW, despite reading higher during the waterbath. The mean difference from HW for TEL was small (-0.18°C) with the largest difference during exercise (-0.25°C) still within predefined acceptable limits (<0.3°C). In the context of previous research on T_{SKIN} measurements, such differences appear very small, with Harper-Smith et al. (2010) reporting differences of 0.26-1.36°C between thermistors and iButtons. Furthermore, when retest variation of both HW and TEL are considered, this difference becomes negligible and may not even exist. Such results may afford opportunities to measure T_{SKIN} in extreme environments using TEL with the receiver unit not needing to be connected to the exercising individual. Until now, the potential for this type of research has been limited by the need for trailing wires when using HW systems.

However, the differences observed in T_{SKIN} from TC were sizeable. Utilising TC in a dynamic, ecologically valid situation is in contrast to the techniques adopted in the majority of previous research with thermal cameras that has shown broadly encouraging results (Merla et al. 2010; Ferreira et al. 2008; Chudecka & Lubkowska 2012), albeit not unanimously (Fernandes et al. 2014). Specifically, tripod mounted cameras have been used to generate thermograms of participants who have spent up to 40 minutes in thermo-neutral conditions. Thermograms are then analysed post hoc using area averages to identify temperatures within specific anatomical regions. For an exercise physiologist, the problem with such an approach is the lack of real-time data upon which decisions can be made. Consequently, the rationale for including TC in this study was to examine whether TC could provide accurate and reliable data through the live-view function during an exercise test. Johnstone et al. (2012) also reported poor validity of an IR sensor within a physiological monitoring system that provided live data during exercise in the heat. Despite an acceptable mean bias (0.49°C), they reported a large random error with the LOA range -1.36-4.14°C. There are a range of difficulties associated with exercise that may explain the erroneous readings in both studies and why these findings differ from previous research that has successfully used this technology. A fundamental difference between thermistor and IR measurement techniques is covering of the measurement site. Thermistors were attached using breathable film patches, which may lessen a micro-climate effect relative to other fixation methods, but may still over-estimate relative to uncovered skin (Tyler 2011). Whilst secure fixation for thermistors is necessary to minimise small angular changes during exercise, the precise angle and distance of IR measurements in both studies was not fixed,

which could lead to increased measurement error (Hershler et al. 1992). An aim of this study was to assess tools taking live measurements in an ecologically valid setting. As such, a widely used incremental, discontinuous protocol was completed which permitted short, but regular measurement opportunities at the end of each stage. Temperatures at four body sites were required in approximately 30 s and although precautions were taken, some variation in angle between measurements is to be expected. The short measurement period prevented ensuring that the skin was completely dry for measurements. Protocol sweat rates were approaching 2 L.hr^{-1}, which compromised drying the skin and completing measurements within the permitted time. Water may alter the emissivity of the skin, so causing the camera to under-read; a trend observed not only here, but by Johnstone et al. (2012). Further, the effect of the waterbath correction procedure on subsequent exercising values should be considered. As previously mentioned, TC may have partially detected the surrounding water temperature, rather than simply the metal block, resulting in over-estimation of values. In this instance the correction formula may be inaccurate, falsely suggesting TC under-estimated during exercise. However, as a linear correction was applied to the data, the high levels of random error TC displayed throughout T_{SKIN} measurements remain an obstacle for dynamic use.

Modifications to the TC protocol are required if accurate results are going to be yielded from such a test. Subsequent pilot testing (unpublished data) suggests improved accuracy is possible with this model of camera when camera positions are fixed and longer periods of time are available for measurements to be taken. When transferring tools to applied situations, a compromise must be made between maximising measurement accuracy and maintaining ecological validity of the protocol. Consequently, whilst TC may still have a role to play in static, controlled situations within environmental physiology, using the current protocol it cannot be considered a valid alternative to either of the thermistor systems for live monitoring of T_{SKIN} during exercise.

4.5.4 Skin temperature reliability comparison

The reliability of both thermistor systems was acceptable throughout the exercise tests, whilst TC again displayed large errors. The lack of difference in sweat rate between trials would indicate that TC did not under-read as a consequence of an adaptation eliciting a greater sweat response from the heat exposure during trial 1. Moreover, allowing 7 days between trials is likely to have prevented this effect (Barnett & Maughan 1993). The graphs for both HW and TEL (Figure 28) display converging means throughout the rest period, with dissociation during the latter part of exercise, which is when skin wettedness is likely to be greater as body

temperature increases. This supports the need for a long stabilisation period and suggests that temperature differences occurred as exercise intensity rises. Reduced reliability is associated with challenges to homeostasis and this occurs throughout incremental tests where thermal equilibrium is never achieved. Other literature has made comparisons during steady-state exercise (Buono et al. 2007; Harper Smith et al. 2010), so the small differences observed may be a characteristic of this test and the reliability of all tools may improve when examined under similar circumstances.

In conclusion, a telemetry thermistor system offers a valid and reliable alternative measure of skin temperature to traditional hard-wired thermistors. Such a system may provide for convenient and wireless data collection, allowing ecologically valid measurements to be taken in the field. Thermal cameras may still be useful tools for measuring skin temperature in static and controlled environments, however their use is not recommended for live monitoring during exercise.

5 Physiological responses to incremental exercise in the heat following internal and external precooling

The initial investigation compared different skin temperature measures and identified telemetry thermistors as the most suitable device for use through the subsequent investigations. With there being limited understanding of the effect heat has on the determinants of endurance performance, this study will investigate the efficacy of two prominent acute, precooling strategies, previously untested during endurance running, for enhancing the determinants of endurance performance in the heat. For this experiment the same participants were used as in Chapter 4, with data collection occurring simultaneously.

5.1 Abstract

Twelve males completed three incremental, discontinuous treadmill tests in the heat (31.9 [1.0]°C, 61.9 [8.9]%) to determine speed at two fixed blood lactate concentrations (2 & 3.5 mMol.L^{-1}), running economy (RE) and maximum oxygen uptake ($\dot{V}O_{2max}$). Trials involved 20 min of either internal cooling (7.5 g.kg^{-1} ice slurry ingestion, ICE) or mixed-methods external cooling (EXT, cold towels, forearm immersion, ice vest and cooling shorts), alongside a no intervention control (CON). Following precooling, participants ran 0.3 km.h^{-1} faster at 2 mMol.L^{-1} and 0.2 km.h^{-1} faster at 3.5 mMol.L^{-1} (p=0.04, partialη^2=0.27). Statistical differences were observed versus CON for ICE (p=0.03, d=0.15), but not EXT (p=0.12, d=0.15). There was no effect of precooling on RE (p=0.81, partialη^2=0.02), nor on $\dot{V}O_{2max}$ (p=0.69, partialη^2=0.04). A main effect for cooling on physiological strain index was observed (p<0.01, partialη^2=0.41), with differences versus CON for EXT (p=0.02, d=0.36), but not ICE (p=0.06, d=0.36). Precooling reduced thermal sensation (p<0.01, partialη_2=0.66) in both cooling groups (p<0.01). Results indicate ICE and EXT provide similar physiological responses for exercise up to 30 min duration in the heat. Differing thermoregulatory responses are suggestive of specific event characteristics determining the choice of cooling. Precooling appears to reduce blood lactate accumulation and reduce thermoregulatory and perceptual strain during incremental exercise.

5.2 Introduction

Endurance exercise is underpinned by the ability to transfer chemical energy into a given exercise velocity (Coyle 1999). The status of this biological process can be assessed using physiological markers such as the lactate thresholds, running economy (RE) and maximum oxygen uptake ($\dot{V}O_{2max}$). Under normothermic conditions, when combined with the velocity at $\dot{V}O_{2max}$ ($v\dot{V}O_{2max}$), these markers have been shown to account for 97.8% of the variation in 16

km run time (McLaughlin et al. 2010). McLaughlin et al. (2010) highlighted that $\dot{V}O_{2max}$ accounted for 90.2% of variation in running time in a group with hetergenous $\dot{V}O_{2max}$ values. Furthermore, Lorenzo et al. (2011) has shown the lactate turnpoint (LTP) to be a strong predictor of time trial performance in both cold ($r =0.89$) and hot ($r =0.87$) environments.

The addition of heat stress during endurance running is characterised by an enhanced metabolic (Parkin et al. 1999), cardiovascular (González-Alonso et al. 2008) and sensory strain (Villanova et al. 1997) as core temperature (T_{CORE}) increases. Furthermore, the consequential heat strain is associated with reductions in the LTP (Lorenzo et al. 2011) and $\dot{V}O_{2max}$ (Nybo et al. 2014). The reduction in LTP in the heat is of particular importance given it remains a valid predictor of endurance performance in hot environments (Lorenzo et al. 2011). This decline may be associated with the shift towards carbohydrate oxidation (Fink et al. 1975; Parkin et al. 1999) and the increased blood lactate concentration observed during heat strain (Hargreaves 2008). At maximal exercise intensities, $\dot{V}O_{2max}$ is attenuated due to increased skin blood flow required for heat dissipation, which leads to a reduction in stroke volume as a consequence of cutaneous pooling, and ultimately limits muscular blood flow and oxygen delivery (Gonzalez-Alonso et al. 2008). Enhanced oxygen consumption has also been reported during heat strain (Consolazio et al. 1973), although not all studies observed this effect (Gonzalez-Alonso et al. 1999). Furthermore, during prolonged or intense endurance exercise, a protective reduction in central nervous system (CNS) motor output may be observed as core temperature approaches 40°C (Cheung 2007). Thus, thermal interventions such as precooling that reduce body temperature thereby increasing heat storage capacity or reducing the rate of heat storage, have been shown to benefit endurance exercise in the heat.

A dichotomous approach towards precooling is apparent, with interventions either cooling externally or internally, eliciting different skin, core and muscle temperatures and therefore potentially different physiological responses. The attenuated LTP in the heat may be in part a consequence of increased muscular glycolysis (Fink et al. 1975; Parkin et al. 1999), an alteration in muscle metabolism that external body cooling has previously been shown to mediate (Kozłowski et al. 1985; Hsu et al. 2005). Therefore, an external precooling technique may help reduce plasma lactate accumulation and could have an effect on LTP. Similarly, by reducing T_{CORE}, internal cooling may reduce the cutaneous circulation that can inhibit cardiac filling, thereby ameliorating cardiovascular strain that ultimately causes a reduction in $\dot{V}O_{2max}$. Therefore, accurately quantifying any differences in the responses to different types of cooling is important to optimise cooling strategies.

The vast number of cooling techniques reflects the challenge of providing a large cooling impulse through a technique that remains practical for use across a number of venues. Considerable growing evidence supports the use of internal cooling through ice slurry ingestion (ICE) as an ergogen for endurance exercise in the heat (Jones et al. 2012; Siegel & Laursen 2012; Wegmann et al. 2012). In addition to increasing heat storage capacity, direct cooling of sensitive thermoreceptors within the splanchnic region may contribute to a reduced perceived thermal strain (Villanova et al. 1997). Further, visceral cooling may preserve splanchnic flow that reduces during heat strain (Rowell et al. 1968) as well as prevent against endotoxin leakage that has been associated with impaired muscle force generation (Supinski et al. 2000) and exertional heat illness (Sawka et al. 2011). ICE typically elicits a substantial reduction in T_{CORE} of 0.3-0.6°C (Siegel et al. 2010; Siegel et al. 2012) and has been shown to aid time trial performance in the heat, improving a 40 km laboratory cycle by 6.5% compared with no cooling (Ihsan et al. 2010). ICE appears to permit similar running time to exhaustion as the gold standard technique of cold water immersion (Siegel et al. 2012), with recent systematic reviews advocating ICE to avoid impracticalities with water immersion (Jones et al. 2012; Siegel & Laursen 2012; Ross et al. 2013) Moreover, ICE is a simple strategy that may complement hydration and nutritional strategies during competition in the heat (Ross et al. 2011).

From an exogenous perspective, mixed method whole body external cooling (EXT) is gaining prominence following the apparent limited effectiveness of individual cooling garments on endurance performance (Ranalli et al. 2010; Jones et al. 2012). Duffield et al. (2009) combined cooling garments to enhance the cooling volume and reported a blunted rise in T_{CORE} during exercise, resulting in increased work during 30 min of intermittent sprinting. External cooling may not always elicit a reduction in T_{CORE} prior to exercise (Minett et al. 2011; Minett, et al. 2012), but appears to permit a reduced rate of heat storage during exercise by enhancing heat dissipation through an increased core-to-skin gradient (Kay et al. 2010). The reduced T_{SKIN} will also lead to reduced vasodilation of peripheral capillary beds, potentially lowering cardiovascular (CV) strain. Cooling of the skin may be an important mediator of thermal sensation (Schlader, Simmons et al. 2011b) and has been associated with a 6% increase in self-selected exercise intensity during a 30 min cycling trial when an initial reduction in T_{CORE} was not observed (Kay et al. 1999). Minett et al. (2011) has subsequently identified a dose-dependent response with skin cooling surface area coverage as the critical factor for exercise capacity, adopting a mixed methods approach to increase total work during intermittent sprinting in the heat, compared with no cooling (Minett et al. 2011, Minett et al. 2012). As with ICE, EXT is a simple and practical technique, however it has yet to be assessed prior to

endurance running or on the physiological markers that are strongly associated with endurance exercise, such as the determinants of endurance performance derived from a graded exercise test.

The aim of this study was to compare the physiological responses to practical and evidenced internal and external precooling techniques through the markers of lactate thresholds, RE and $\dot{V}O_{2max}$. The first hypothesis stated both precooling techniques would increase lactate threshold, improve running economy and increase $\dot{V}O_{2max,}$ relative to no cooling. The second hypothesis stated internal cooling would elicit the greatest improvement within these markers when compared to EXT due to the magnitude of T_{CORE} reduction, and the size of effects previously reported for this technique.

5.3 Method

5.3.1 Participants

Twelve male recreational club runners volunteered as participants (mean [±SD]): age 38 (11) years, stature 177.8 (7) cm, mass 76.1 (5.7) kg, sum of four skinfolds 32.6 (7.1) mm, $\dot{V}O_{2max}$ 57.5 (4) mL.kg^{-1}.min^{-1}. All participants trained at least three times per week and met the training status criteria defined in the General Methods (Section 3.2). No contraindications for testing were violated prior to commencing any experimental session in accordance with previously detailed procedures. (General Methods, Section 3.3).

5.3.2 Experimental design

A randomised controlled design was used with each participant performing experimental trials under three conditions; control (no cooling, CON), internal cooling (ice slurry ingestion, ICE) and external cooling (mixed methods, EXT). Four trials, each comprising of two graded exercise tests during each visit were completed, with the first trial serving as a familiarisation. The subsequent three trials were completed in a randomised order, separated by 7-10 days. An overview of each trial is provided in Figure 29. Briefly, trials comprised four phases; 10 min rest, 20 min precooling, 5 min warm-up and then the graded exercise tests (GXT 1 and GXT 2), with the entire trial within a hot and humid environment (31.9 (1.0)°C, 61 (8.9)% relative humidity). In order to replicate a competition schedule, the exercise test began 15 min after cooling.

Figure 29: Protocol overview. Entire protocol completed in hot environment. 'GXT 1' involved 3 min exercise stages with increments of 1km.h-1. 'GXT 2' denotes gradient based test to exhaustion incorporating 1 min stages with increments of 1%.

5.3.3 Cooling interventions

During ICE, participants ingested 7.5 g.kg^{-1} body mass of ice slurry (-1°C) during the cooling phase. Such a volume has previously been shown to elicit large reductions in T_{CORE} without inducing gastrointestinal distress (Siegel et al. 2012). Of this volume, the drink consisted of two thirds shaved ice using a snow cone maker (JM Posner, Watford, UK) and one third diluted drinking cordial (High Juice, 7.3 g carbohydrate per 100 ml of diluted drink, Tesco Stores Ltd, Cheshunt, UK). This cordial is a non-carbonated syrup made from fruit juice, water and carbohydrate, that was diluted one part cordial to four parts water. Slurry was dispensed in equal amounts every five min over the 20 min precooling period to prevent gastrointestinal discomfort, with total drink volumes typically between 500-600 ml.

During EXT, participants were cooled using the manoeuvre adopted by Minett et al. (2011). This involved wet, iced towels covering the head and neck, forearm and hand immersion in cold water (9°C), an ice vest on the torso (Artic Heat, Queensland, Australia) and ice packs affixed to the quadriceps using cooling shorts as shown in Figure 30. Towels were swapped after 10 min and hand immersion water temperature was actively maintained throughout. During EXT and CON, the same volume of squash was provided to match the sugar intake between all conditions, with the drink delivered to the hot environment from an normothermic laboratory (21°C), following a 15 min stabilisation to room temperature.

Figure 30: Mixed methods external cooling involving cooling shorts, hand immersion, ice vest and cold towel around the head and neck.

5.3.4 Graded exercise tests

Following rest and precooling phases in the environmental chamber, a five min warm-up was completed. The exercise test is described in the General Methods (Section 3.4). Briefly, the test comprised two parts; GXT 1 was a submaximal incremental speed protocol, followed by GXT 2; an incremental gradient protocol to volitional exhaustion. Starting speeds were between 8–10 km.h^{-1} (1% gradient), with each participant completing 6-8 stages, using speed increments of 1 km.h^{-1}. The first capillary sample was taken 18 min after hand immersion ceased, of which 8 min was exercise. Following a 2 min rest, GXT 2 began at a speed 2 km.h^{-1} below the previous final speed with gradient increasing by 1% each min and continuing until volitional exhaustion.

5.3.5 Physiological measures

During the familiarisation trial, anthropometric data were collected for stature, body mass and a four site skin fold calliper assessment, as described in the General Methods. During all trials, following confirmation of euhydration, participants self-inserted a rectal probe and had skin thermistors attached as previously described. Heart rate (HR), T_{CORE}, T_{SKIN}, rating of perceived exertion (RPE,) and thermal sensation were noted every five min during rest and precooling and at the end of each stage during exercise.

The following physiological responses were calculated; lactate thresholds, running economy (RE), maximum oxygen consumption ($\dot{V}O_{2max}$) and velocity at $\dot{V}O_{2max}$ ($v\dot{V}O_{2max}$) as previously described (General Methods, Section 3.6.3). Ventilatory gases were measured using 30 s averaging from the final min of each stage to provide RE, ventilation (V_E) and respiratory

exchange ratio (RER). Mean running economy across the first 5 exercise stages is presented, although the data from each individual stage was used for analysis. During the $\dot{V}O_{2max}$ test, the highest 15 s moving average recorded represented $\dot{V}O_{2max}$. A shortened data averaging approach was adopted due to the short recovery period between the two parts of the test to attenuate a potential effect on blunted $\dot{V}O_{2max}$ values. Recovery between GXTs was minimal in order to help ensure both physiological and perceptual effects of cooling would still be present whilst testing $\dot{V}O_{2max}$.

5.3.6 Statistical Analyses and Derivative Calculations

Derivative calculations of mean T_{SKIN} and PSI, as well as estimations of sweat rate, were calculated as previously described in General Methods, Section 3.7. All outcome variables were assessed for normality and sphericity prior to further analysis. Data were analysed in three phases: rest, cooling and exercise. Two way, repeated measures ANOVA (cooling type*time) were used to test for differences in blood lactate indices, respiratory responses, T_{CORE}, T_{SKIN}, PSI, RPE and TS. One way, repeated measures ANOVA were used to detect differences in $\dot{V}O_{2max}$, running time until $\dot{V}O_{2max}$ during GXT 2, $v\dot{V}O_{2max}$, thermoregulatory variables at rest, sweat rate, absolute change during cooling and change during exercise.

5.4 Results

5.4.1 Physiological responses

A main effect of cooling condition on running speed was observed across both fixed lactate concentrations (F=3.78, p=0.04, partial η^2=0.27). Mean values at 2 mMol.L^{-1} were ICE 12.3(1.1) km.h^{-1}, EXT 12.3(1.1) km.h^{-1}, CON 12.0(1.1) km.h^{-1} and at 3.5 mMol.L^{-1}, ICE 13.8(1.0) km.h^{-1}, EXT 13.8(1.0) km.h^{-1}, CON 13.6(1.0)km.h^{-1}. Bonferroni comparisons identified a difference between ICE and CON (p=0.03, d=0.15), but not between EXT and CON (p=0.12, d=0.15), or between ICE and EXT (p>0.99, d<0.001). The mean blood lactate response is displayed in Figure 31, whilst lactate and oxygen uptake during GXT 1 are plotted in Figure 32. There was no effect of cooling on RE (ICE 231[18] mL.kg^{-1}.km^{-1}, EXT 230[17] mL.kg^{-1}.km^{-1}, CON 228[13] mL.kg^{-1}.km^{-1}, p=0.79, partial η^2=0.02), nor for $\dot{V}O_{2max}$ (ICE 57.5[5.6] mL.kg^{-1}.min^{-1}, EXT 58.4[4.7] mL.kg^{-1}.min^{-1}, CON 57.3[4.9] mL.kg^{-1}.min^{-1}, p=0.69, partial η^2=0.04). No statistical difference in running time until exhaustion during GXT 2 was observed (p=0.707, partial η^2=0.03). The mean running time of both precooling groups was greater than CON (368(79) s, ICE 375(57) s, EXT 381(73) s) which equated to a 5.0% (d=0.11) following ICE and 6.4% (d=0.17) difference following EXT. Times for EXT were 1.6% (d=0.08) greater than ICE. No statistical difference was found in $v\dot{V}O_{2max}$ (p=0.49, partial η^2=0.08), although speed after EXT (15.4[1.3] km.h^{-1}, ICE 15.0[1.4] km.h^{-1}, CON 15.0[1.7]

km.h^{-1}) equated to a 3.2% difference versus CON (d=0.26) and a 3.0 % (d=0.31) difference versus ICE. Speed following ICE was not different to that of CON (-0.2%, d=0.02). No differences in heart rate (p=0.81, partial η^2=0.20) were observed between groups; CON 146(16) b.min^{-1}, ICE 145(15) b.min^{-1}, EXT 143(15) b.min^{-1}.

Figure 31: Mean (±SD) lactate response over six incremental sub-maximal exercise stages, with error bars displaying standard deviation. Each stage constituted 3 min exercise and 1 min blood sampling, with increments of 1 km.h^{-1}. Horizontal dotted line indicates blood lactate concentration of 2 mMol.L^{-1} from which individual running speeds were calculated to represent lactate threshold. All participants completed a minimum of 6 stages, with some participants completing additional stages before displaying blood lactate concentrations exceeding 3.5 mMol.L^{-1}. A main effect for cooling type was observed (p=0.04, partial η^2=0.27), with differences identified between CON and ICE.

Figure 32: Mean (±SD) blood lactate versus oxygen uptake during GXT 1. Horizontal dotted line indicates blood lactate concentration of 2 mMol.L^{-1} from which individual running speeds were calculated to represent lactate threshold. Error bars represent one standard deviation.

No differences were observed throughout the submaximal exercise test in either V_E (mean across exercise; CON 85.6[12.5] L.min^{-1}, ICE 85.0[12.5] L.min^{-1}, EXT 85.0[11.6] L.min^{-1}, p=0.90, partial η^2=0.01) or RER (mean across exercise; CON 0.97[0.03], ICE 0.97[0.03], EXT 0.98[0.06], p=0.44, partial η^2=0.08) were observed across six exercise stages. Similarly, no interaction effects were observed for V_E (p=0.149, partial η^2=0.131) or RER (p=0.11, partial η^2=0.16).

5.4.2 Thermoregulatory responses

Figure 33A illustrates mean T_{CORE} data for each condition. After the 10 min rest period there were no differences between conditions (CON 37.13[0.23]°C, ICE 37.18[0.25]°C, EXT 37.13[0.31]°C, p=0.43, partial η^2=0.08). During the cooling phase, an interaction between cooling type and time was observed (F=25.86, p<0.001, partial η^2=0.70). Ice slurry ingestion resulted in a greater reduction in T_{CORE} (-0.32[0.11]°C) than EXT (-0.05[0.08]°C, p<0.001, d=2.97) and CON (-0.05[0.07]°C, p<0.001, d=3.03). No main effect for cooling type during exercise was found (p=0.13, partial η^2=0.17), although a trend towards a higher T_{CORE} for CON is apparent. The failure to detect an effect for cooling type may be explained by the presence of a cooling type*time interaction (F=4.38, p=0.01, partial η^2=0.29). As shown in Table 24 the overall change in T_{CORE} across the exercise phase was greatest during ICE (1.34[0.27]°C) compared with EXT

(1.01[0.25]°C, *p*=0.001 *d*=1.29). However, there was no statistical difference vs CON (1.11[0.29]°C, *p*=0.10, *d*=0.82) and between CON and EXT (*p*=0.44, *d*=0.40). Finishing T_{CORE} for each group at $\dot{V}O_{2max}$ were CON 39.03(0.45)°C, ICE 38.96(0.55)°C and EXT 38.88(0.38)°C.

Figure 33B illustrates mean T_{SKIN} data for each condition. A difference was observed between conditions at rest (CON 33.70 [0.5]°C, ICE 33.94[0.39]°C, EXT 33.48[0.60]°C, *p*=0.04 partial η^2=0.28), however this effect was not detected by Bonferroni post hoc. ANOVA revealed a main effect for cooling type (F=230.53, *p*<0.001, partial η^2=0.96) and a cooling type*time interaction (F=5.74, *p*<0.001, partial η^2=0.37) during the cooling phase with EXT displaying the lowest mean T_{SKIN} temperatures throughout. EXT also resulted in a greater reduction in T_{SKIN} (-6.64[1.46]°C) than ICE (-0.17[0.52] °C, *p*<0.001, *d*=6.90) and CON (-0.40[0.39]°C, *p*<0.001, *d*=7.62). There was no difference between CON and ICE (*p*=0.51, *d*=0.52). An effect for cooling type was apparent during exercise (F=44.20, *p*<0.001, partial n^2=0.82) with EXT (pre-post; 32.32[0.6]-35.01[0.59]°C) lower than CON (pre-post; 34.56[0.55]-35.27[0.67]°C, *p*<0.001, *d*=1.03) and ICE (pre-post; 34.94[0.39]-35.28[0.68]°C, *p*<0.001, *d*=1.29). A cooling type*time interaction was observed (F=44.14, *p*<0.001, partial n^2=0.72), with a greater rate of increase within EXT resulting in no differences versus CON after stage 4 (*p*=0.058) and versus ICE after stage 5 (*p*=0.07). Consequently, EXT also displayed the largest overall change in T_{SKIN} during exercise (2.69[0.61]°C) with post hoc analysis revealing differences versus CON (0.71[0.42], *p*<0.001, *d*=3.86) or ICE (0.69[0.46], *p*<0.001, *d*=3.73) as shown in Table 24.

Table 24: Mean (±SD) change in core temperature and mean skin temperature across six incremental exercise stages (left) and change per 5 min (right). Differences (*p*<0.05) against control are denoted by '*' and differences between internal and external cooling groups by '†'.

	Δ total			Δ per 5 min		
Cooling type	Control	Ice slurry	External	Control	Ice slurry	External
Δ T_{CORE} (°C)	1.11	1.38†	1.01†	0.23	0.29	0.21
(SD)	(0.29)	(0.26)	(0.25)	(0.06)	(0.05)	(0.05)
Δ Mean T_{SKIN} (°C)	0.71	0.69*†	2.69*†	0.15	0.14*†	0.56*†
(SD)	(0.42)	(0.46)	(0.61)	(0.09)	(0.10)	(0.13)

Figure 33A: Mean (±SD) core temperature response B: Mean (±SD) skin temperature response across protocol. '*' denotes difference between Ice slurry and Control, '#' denotes difference between External and Control, '†' denotes difference between Ice slurry and External ($p<0.05$). Error bars represent one standard deviation.

An effect for cooling on PSI during exercise was observed (F=6.91, p=0.005, partial η^2=0.41), with differences between CON (CON 5.2[1.6]) and EXT (4.6[1.6], p=0.02, d=0.36), and a non-significant trend for ICE (4.58[1.8], p=0.058, d=0.36). A cooling type*time interaction was also observed (F=3.98, p=0.01, partial η^2=0.26) due to different rates of increase between groups, with trends displayed in Figure 34. Sweat rates did not differ between groups (F=2.00, p=0.16,

partial η^2=1.66) with groups means as follows; (mean [±SD], percentage of body mass); CON 1.4(0.7) $L.hr^{-1}$ (1.2%), ICE 1.6(0.6) $L.hr^{-1}$ (1.4%) and EXT 1.6(0.5) $L.hr^{-1}$ (1.4%).

Figure 34: Mean (±SD) physiological strain index across 6 incremental exercise stages. '*' denotes difference (p<0.05) between internal vs control and '†' denotes difference between external vs control. Error bars represent one standard deviation.

5.4.3 Perceptual measures

Thermal sensation did not differ after 10 min rest, between trials (F=0.897, p=0.422, partial η^2=0.075). A main effect for cooling type was observed in thermal sensation (F=59.422, p<0.01, partial η^2=0.844), as well as an interaction effect (F=3.549, p=0.004, partial η^2=0.0244). Both EXT and ICE lowered thermal sensation relative to CON, whilst EXT was also lower than ICE, as shown in Figure 35. Precooling reduced thermal sensation during exercise (F=20.98, p<0.01, partial η_2 = 0.66) with CON (6.2 [0.8]) higher than ICE (5.7 [0.9], p=0.005, d=0.50) and EXT (5.4 [0.8], p<0.001, d=0.98). However, this reduction did not remain throughout exercise, evidenced by a cooling type*time interaction effect (F=4.98, p<0.001, partial η_2 =0.31). No differences in RPE (F=1.96, p=0.54, partial η^2=0.06) were observed between groups.

Figure 35: Mean (±SD) thermal sensation across rest, precooling and exercise phases of each condition. There were no differences at rest. Statistical differences were observed across all subsequent time points for internal vs control, external vs control and internal vs external, aside of the first cooling measure where internal did not differ from control. Statistical symbols removed for clarity. Error bars represent one standard deviation.

5.5 Discussion

The aims of this study were to compare the physiological responses from internal and external precooling methods during graded exercise tests in the heat. In accordance with the first hypothesis, both precooling interventions resulted in modest improvements in running speeds at fixed blood lactate of 2 and 3.5 mMol.L^{-1} compared with no cooling. However, in contrast to the second hypothesis, no differences in running economy or $\dot{V}O_{2max}$ were observed. Furthermore, no difference in the physiological responses between ICE and EXT were found despite a larger pre-exercise reduction in T$_{CORE}$ following ICE. Finally, there were different thermoregulatory responses from the cooling techniques, suggesting specific event characteristics will determine the choice of cooling.

5.5.1 Effects of cooling

In this study, fixed blood lactate concentrations of 2 and 3.5 mMol.L^{-1} were used to represent the lactate threshold and lactate turnpoint, respectively (Saunders & Green 2013). This approach accounted for differences in the number of stages completed, removed subjectivity of experimenter identification and provided precision to less than 1 km.h^{-1}. ICE displayed a statistically significant greater running speed across both markers relative to CON, whilst a trend was observed for EXT. Such differences may be important given LTP remains a valid predictor of endurance performance in the heat (Lorenzo et al. 2011). Both precooling

techniques displayed the same mean difference to CON at 2 (+0.3 km.h^{-1}) and 3.5 mMol.L^{-1} (+0.2 km.h^{-1}), as well as the same overall effect size. As a result, a magnitude based inference statistical approach may conclude that both interventions had the same effect on lactate indices (Hopkins et al. 2009). Such changes in blood lactate response following precooling are small and likely fall at the upper end of what may be considered day to day variation of 0.2 mMol.L^{-1} for this type of test (Saunders & Green 2013), possibly as a consequence of differences in muscle glycogen status (Yoshida 1984). However, the modest differences observed were consistent throughout the 23 min trial and appear proportional to previously reported reductions in blood lactate, relative to the T$_{CORE}$ reduction from hand cooling during exercise. During 1 hour cycling at 60% $\dot{V}O_{2max}$, Hsu et al. (2005) reported a ~-0.5 mMol.L^{-1} reduction in blood lactate concentration, when T$_{CORE}$ was ~-0.6°C lower. Furthermore, this constitutes a 2% improvement in running speed at the lactate threshold, which may be meaningful as it exceeds the 1.5% coefficient of variation for speed at lactate threshold (Hopkins et al. 2001). For this participant cohort, when running at lactate turnpoint pace, such a difference would equate to 31 s over 5 km. Figure 32 illustrates the lactate $\dot{V}O_2$ relationship during GXT 1 and provides some further evidence of a trend towards precooling eliciting a modest effect on the lactate thresholds during this test. The lack of difference in ventilation or RER suggests that under this level of heat strain cooling directly elicits an effect on lactate production or clearance rather than an altered $\dot{V}O_2$ in the muscle. This is supported by the apparent mediated metabolic strain not observed through improved running economy in the heat. Despite competing demands for cardiac output for both exercising skeletal muscle and cutaneous vasodilation, it is apparent that muscular blood flow is maintained at submaximal intensities (Nybo et al. 2014; Gonzalez-Alonso et al. 2008). Therefore, any changes in lactate are unlikely to be explained by cooling eliciting alterations in muscular blood flow. Rather, as thermoregulatory strain leads to a reduction in visceral circulation during exercise (Rowell et al. 1968), it is more likely that by directly cooling T$_{CORE}$, ICE may elicit a heat sink away from the skin and maintain the rate of lactate-pyruvate conversion in the liver. Similarly, by substantially lowering T$_{SKIN}$, EXT may also reduce cutaneous blood flow, thereby preserving splanchnic circulation and enhancing lactate clearance. An increase in splanchnic blood flow during exercise has previously been proposed as a mechanism to explain the enhancement in LTP following heat acclimation (Lorenzo et al. 2010). It should be noted that the alterations in the lactate response occurred at moderately elevated levels of T$_{CORE,}$ with mean T$_{CORE}$ 38.3°C in CON after 23 minutes of graded exercise. Thus these results warrant an investigation into whether a greater effect of cooling on lactate occurs when individuals are hotter, and where metabolic alterations would be expected to be more pronounced.

Running economy incorporates both biomechanical as well as physiological parameters and is thought to account for differences between elite athletes who display similar $\dot{V}O_{2max}$ values (Bassett & Howley 2000). Despite indications that precooling may benefit some determinants of RE such as oxygen uptake (Lee & Haymes 1995), stride length (Folland et al. 2006) and neuromuscular function (Siegel et al. 2011), no differences were found, supporting the results of Winke and Yates (2008) who examined RE following ice slurry precooling. Thus, beneficial effects of precooling do not appear to present through improved RE whilst exercising for relatively short durations in the heat.

Despite evidence that maximum oxygen consumption is attenuated in the heat (Lorenzo et al. 2011), cooling had no effect on subsequent $\dot{V}O_{2max}$. González-Alonso and Calbet (2003) have demonstrated that under heat stress, cardiac output and mean arterial pressure reduce at maximal exercise intensities, leading to a reduction in skeletal muscle blood flow. Thus, a cooler body would be expected to mediate the decline in $\dot{V}O_{2max}$ under heat stress through reducing competition for blood flow and maintaining cardiac output and mean arterial pressure for longer. Such a mechanism is supported by the maintenance of skeletal muscle blood flow and $\dot{V}O_{2max}$ in thermoneutral, relative to hot, conditions (Périard et al. 2011). However, it would appear that thermometric effects of both cooling techniques were no longer present at the point of $\dot{V}O_{2max}$, as evidenced by the similar finishing T_{CORE} between cooling techniques. The time-course of the current protocol shares similarities with many athletic events whereby a precooling intervention must occur prior to a warmup, with individuals completing 24-32 min of exercise, which may culminate in an all-out end-spurt. It remains plausible however, that cooling may still elicit an effect on $\dot{V}O_{2max}$ if a greater cooling impulse is provided or $\dot{V}O_{2max}$ is measured during a shorter duration protocol.

Interestingly, despite reductions in body temperature and thermal sensation following both cooling methods, relative to control, RPE remained unchanged. This may indicate that enhanced performance in the heat and associated pacing adjustments following precooling are closer linked to thermal sensation rather than overall perceived exertion. Such a relationship could have implications for future cooling techniques more aggressively targeting locations that determine thermal sensation.

5.5.2 Responses to internal and external precooling

The physiological responses from each cooling technique were similar, with neither ICE nor EXT having an effect on $\dot{V}O_{2max}$ or RE, and the magnitude of the effect on lactate similar between techniques. Although a small mean difference suggests an enhanced $v\dot{V}O_{2max}$

following EXT, no statistical difference between treatments was observed. Further, whilst a change of 3.2% (0.4 $km.h^{-1}$) in EXT compared with ICE and CON could be interpreted as meaningful, this change is below what has been suggested to constitute a meaningful difference of 0.5 $km.h^{-1}$ (Billat & Koralsztein 1996). Therefore, it would appear that the estimate of the maximum speed that can be maintained by oxidative phosphorylation is similar, irrespective of cooling method.

Although both precooling manoeuvres produced a similar, lowered PSI throughout the trial, there were markedly different thermoregulatory responses which may determine application. Whilst ICE resulted in a 0.3°C reduction in T_{CORE}, in keeping with other literature (Ihsan et al. 2010; Ross et al. 2011; Siegel et al. 2012), EXT did not elicit a reduction in T_{CORE}. This is not uncommon following external cooling techniques, as an 'after-drop' may be observed whereby T_{CORE} remains unchanged during cooling, before falling at the start of exercise as vasoconstriction dissipates and warm blood from the core is subsequently cooled in the periphery. Whilst an after-drop was not observed through a reduction in T_{CORE}, the rate of increase in T_{CORE} during exercise following EXT was smaller than both ICE and CON. The lack of an after-drop may be attributable to differences in the time-course and intensity of exercise following cooling compared with other research (Kay et al. 1999). Similarly, Duffield (2009) did not report a reduction in T_{CORE} following EXT and subsequently observed a reduced T_{CORE} throughout exercise. Both Uckert & Joch (2007) and Duffield et al. (2010) have reported performance benefits following external cooling techniques that did not elicit an initial reduction in T_{CORE}, as greater pace may be achieved through a reduced rate of heat storage or T_{SKIN}. Such a lower T_{SKIN} may be associated with reduced thermal discomfort (Gagge & Gonzalez 1974) and appears to be a key mediator of behavioural thermoregulation, contributing towards a greater selected intensity during self-paced prolonged exercise (Schlader, Simmons, et al. 2011a). Indeed, the size of reduction in thermal sensation relative to CON was greatest following EXT as shown in Figure 35, in accordance with a reduced T_{SKIN}. Previous research suggests ICE affects performance through an enhanced absolute heat storage capacity that prevents or delays CNS motor drive reduction, again permitting greater exercise intensity, relative to not being cooled (Siegel et al. 2011). In addition to an enhanced capacity for storing heat, functional magnetic resonance imaging has indicated that thermoreceptors within the splanchnic region may activate pleasure centres of the brain, possibly leading to a deceptive effect concerning overall thermal strain (Guest et al. 2007). The current results may suggest splanchnic thermoreceptors are less sensitive than those on the skin in order to alter thermal sensation. However, the lack of a self-paced performance test within the current study limits

the extrapolation of findings to endurance performance, therefore future research should investigate how a reduced thermal sensation following EXT transfers into running speed during self-paced protocols relative to ICE.

Similar trends in the T_{CORE} response were observed by Siegel et al. (2012) during fixed intensity exercise, whereby ICE produced a faster rise in T_{CORE} relative to cold water immersion, the rise from which was in turn slower than a no cooling control condition. Sawka et al. (2012) suggested that cooling the core without a concurrent reduction in T_{SKIN} decreases the core-to-skin gradient, impairing the ability to dissipate heat to environment. Such an interaction between the rates of rise in T_{CORE} indicates ICE would be better suited to shorter duration endurance events, with the rate of heat storage during EXT making it also appropriate for longer duration exercise. Future research should consider combining ICE and EXT to increase heat storage capacity and promote a reduced rate of heat storage during exercise. Techniques may be utilised sequentially in order not to impair heat dissipation through a reduced core-to-skin gradient, however time constraints within competition may necessitate concurrent use if such an additive effect exists.

In conclusion, internal and external precooling induced similar improvements in the lactate thresholds, whilst neither altered RE or $\dot{V}O_{2max}$ during incremental treadmill exercise up to 30 minutes. However, different thermoregulatory responses from the cooling techniques are suggestive of specific event characteristics determining the choice of cooling. Internal cooling may be limited to shorter duration events whilst external cooling may remain appropriate for exercise over 30 min due to the reduced rate of heat storage. Finally, precooling appears to reduce blood lactate accumulation in the heat and reduce thermoregulatory and perceptual strain during incremental exercise.

6 Ischaemic preconditioning does not alter the determinants of endurance running performance in the heat.

Study 2 compared two prominent acute, precooling interventions and did not observe large effects across the determinants of endurance performance. This study will investigate the effect of another acute, but non-thermal, intervention on the determinants. Ischaemic preconditioning has been shown to enhance endurance performance, but has not yet been adopted in the heat.

6.1 Abstract

Ischaemic preconditioning (IP) has been shown to be ergogenic for endurance performance in normothermic conditions, potentially through haemodynamic and/or metabolic mechanisms. Exertional hyperthermia is characterised by competition for blood flow between the muscles and skin, an enhanced metabolic strain and impaired endurance performance. This study investigated the effect of IP on the determinants of endurance performance, through an incremental exercise test in the heat. Eleven males completed two experimental trials in the heat (32°C, 62% RH), preceded by 4x5 min bouts of occlusion, at either 220 (IP) or 50 mmHg (CON). IP did not improve running speeds at fixed blood lactate concentrations of 2 and 4 mMol.L^{-1} ($p=0.828$), or affect blood glucose concentration throughout the trial (mean [±SD]; CON–5.03 [0.94] mMol.L^{-1}, IP–5.47[1.38] mMol.L^{-1}, $p=0.260$). There was no difference in $\dot{V}O_{2max}$ (CON-55.5[3.7] mL.kg^{-1}min^{-1}, IP 56.0[2.6] mL.kg^{-1}min^{-1}, $p=0.436$), running economy (CON–222.3 [18.0] mL.kg^{-1}.km^{-1}, IP–218.9 [16.5] mL.kg^{-1}.km^{-1}, $p=0.125$), or total running time during graded exercise (CON–347[42] s, IP–379[68] s, $p=0.166$). The IP procedure did not change muscle temperature (CON $\Delta= 0.55[0.57]$°C, IP $\Delta=0.78[0.85]$°C, $p=0.568$), but did reduce T_{CORE} during exercise (~-0.1°C, $p=0.001$). IP does not appear to offer any benefit across the determinants of endurance performance in the heat.

6.2 Introduction

Repeated bouts of occlusion and reperfusion, termed ischaemic preconditioning (IP), are established within clinical practice to prepare cardiac muscle for subsequent stresses arising from surgically induced hypoxia, infarction and reperfusion (Hausenloy & Yellon 2008). Skeletal muscle may also be exposed to modest exercise-induced arterial hypoxemia and ischaemic threat (Noakes 2000). Therefore, this simple, acute intervention has been utilised prior to a range of exercise protocols and shown to offer meaningful improvements in both

high intensity and endurance events (Salvador et al. 2015), as well as different environmental conditions (Foster et al. 2014).

Of note, Crisafulli et al. (2011) reported a 4% increase in maximal power output and a ~40 s increase in total exercise time during an incremental cycling test to exhaustion immediately following IP, whilst de Groot et al (2010) observed a 3% increase in $\dot{V}O_{2max}$ alongside a 1.6% increase in maximal power output during incremental cycling. In swimming, it has been suggested IP may provide a particular benefit as a consequence of the relative exercise-induced hypoxia that occurs as a result of specified breathing patterns (Noakes 2000), leading to a decreased arterial partial pressure of oxygen and decreased blood pH (Craig 1986; Sharp et al. 1991). Accordingly, Jean-St-Michel et al. (2011) reported a meaningful and statistically significant 0.7 s improvement in 100m swim time (-1.1%, p=0.02) following IP, improving personal best times within a highly trained cohort. Few studies have considered endurance running, however Bailey et al. (2012a) reported lower blood lactate concentration (mean difference -1.07 mMol.L^{-1}) throughout an incremental submaximal running test, although no subsequent effect on $\dot{V}O_{2max}$ was observed. This was followed by improved 5 km running time with a mean improvement of-34 s (2.5%, p=0.03), highlighting the potential for use prior to endurance running. Exercising in extreme environments may also elicit a heightened physiological strain and as such, IP has been shown to be beneficial under hypoxia (Foster et al. 2011; Foster et al. 2014), but has yet to be adopted under heat stress.

Early investigations concerning the protective effects of IP before surgery suggested the artificially induced metabolic stress provided cellular protection through a heat shock protein response (Marber 1994). However, Bushell and Klenerman (2002) observed no change in heat-shock protein content following IP and highlighted the need for a relatively acute and transient stress to elicit protection, which is not in keeping with stimulating the expression of a transcribed protection factor. Bushell and Klenerman (2002) have mimicked the protective effects of IP through adenosine infusion. Adenosine may proffer beneficial clinical and exertional effects by acting as a vasodilator (Gustafsson et al. 1993), and/or opening potassium (K_{ATP}) channels, which in turn preserves energy (Pang et al. 1997; Cohen et al. 2000), possibly through reduced mitochondrial oxygen consumption (Cooper & Brown 2008). There is also likely an effect of IP in promoting endothelial nitric oxide biosynthesis (Kimura et al. 2007), which appears to maintain vascular function that may be impaired following high intensity exercise (Bailey et al. 2012b). In turn, this maintains the supply of oxygen and energy substrates, whilst removing metabolites during exercise, affording the ability to exercise at a greater %$\dot{V}O_{2max}$. The opening of K_{ATP} channels may present a more efficient muscular action,

facilitating greater work to be completed for the same energy expenditure (Crisafulli et al. 2011; Pang et al. 1995), although De Groot et al. (2010) did not observe differences in power output and oxygen consumption at submaximal intensities. Clevidence et al. (2012) speculated that IP appears to be effective when an ischaemic event is most likely and may require a minimum metabolic stress to be achieved during the exercise bout to be effective during exercise. Thus, extreme environmental conditions such as heat stress and hypoxia (Foster et al. 2014), higher intensity exercise (Crisafulli et al. 2011) and/or activities such as running (Bailey et al. 2012a) and swimming (Jean-St-Michel et al. 2011), that elicit significant physiological strain, warrant investigation. In addition to haemodynamic and metabolic mechanisms, IP may influence afferent feedback, facilitating a greater self-selected exercise intensity due to a desensitising of group III and group IV afferents (Sharma et al. 2015).

Accordingly, environmental heat stress provides an additional stressor to exercise performance (Galloway & Maughan 1997). Indeed, endurance running in the heat is characterised by a myriad of physiological perturbations (Nybo et al. 2014), including competition for blood flow between the muscles and skin as individuals become hyperthermic (Gonzalez-Alonso et al. 2008), as well as metabolic alterations including increased blood lactate production, carbohydrate oxidation and glycogen depletion (Hargreaves 2008). Collectively, such alterations contribute towards the widely reduced exercise duration and intensity during exercise under heat stress (Nybo et al. 2014), whilst an increase in glycogenolysis may have implications for prolonged exercise or regular training in the heat (Hargreaves 2008). Mechanisms underpinning the shift towards carbohydrate metabolism may relate to compromised blood flow due to enhanced skin blood flow for thermoregulation, a Q10 effect upon glycolysis enzymes and/or elevated plasma adrenaline levels (Febbraio 2001). Therefore, preserving muscle blood flow during exercise in the heat may ameliorate some of these metabolic changes. Alongside these changes, the lactate turnpoint (Lorenzo et al. 2011) and $\dot{V}O_{2max}$ (Nybo et al. 2014) are impaired in the heat, markers which help determine endurance performance, when combined with running economy. These changes are significant given that the lactate turnpoint (LTP) remains a strong predictor of time trial performance in hot environments (Lorenzo et al. 2011) and $\dot{V}O_{2max}$ may be the best individual predictor of performance, explaining 90.2% of the total variance in a 16-km run (McLaughlin et al. 2010). Through effecting a change in muscle blood flow and/or mitochondrial function, it is possible that IP could help to mediate some of these metabolic changes in the heat, and maintain markers such as LTP and $\dot{V}O_{2max}$, thereby reducing the fractional utilisation of $\dot{V}O_{2max}$ during endurance performance in the heat. Theoretically therefore, IP may be an appropriate acute

intervention for athletes to consider, in addition to traditional techniques such as precooling (James et al. 2015; Randall et al. 2015) and heat acclimation (Gibson et al. 2015; Mee et al. 2015; Willmott et al. 2016), that seek to alleviate the significant impairment to endurance performance afforded by heat stress (Guy et al. 2015).

This study investigated the effect of IP across a range of submaximal and maximal physiological markers through the determinants of endurance performance model (Bassett & Howley 2000) during incremental exercise in the heat. It was hypothesised that IP would ameliorate the decline in the lactate turn-point and $\dot{V}O_{2max}$ during endurance running in the heat, providing potential as a simple, acute ergogenic technique for use prior to competition.

6.3 Methods

6.3.1 Participants

Eleven male, recreational club runners volunteered as participants (mean [±SD]): age 37 (12) years, stature 178.3 (5.9) cm, mass 74.4 (6.1) kg, sum of four skinfolds 25.1 (3.3) mm, $\dot{V}O_{2max}$ 57.6 (3.2) mL.kg^{-1}.min^{-1}. All participants trained at least three times per week and had run a sub 22 min 5 km in the previous month (mean [±SD] 20:06 [1.0] min), as defined in the General Methods (Section 3.2). No contraindications for testing were violated prior to commencing any experimental session in accordance with previously detailed procedures (General methods, Section 3.3). Hydration status was assessed upon arrival via urine osmolality and urine specific gravity, in accordance with the General Methods (Section 3.6.2)

6.3.2 Experimental Design

A repeated measures, crossover design was used to investigate the effect of a brief period of remote IP on the determinants of endurance performance in the heat, with two conditions; IP and control (CON). All participants undertook a prior, cooler familiarisation trial (24°C, 50% relative humidity [RH]), containing no treatment, before two experimental trials in the heat (32°C, 60% RH).

6.3.3 Ischaemic Preconditioning

In both IP and CON trials, whilst in the supine position, participants were fitted with automated tourniquet thigh cuffs (10 x 30 cm), positioned around the upper thigh of both legs. Each leg was exposed to 5 min of occlusion, 220 mmHg (IP) or 50 mmHg (CON), followed by 5 min of reperfusion and rest for 4 consecutive cycles, as has previously been shown to be effective (Jean-St-Michel et al. 2011; Bailey et al. 2012a). An overview of the preconditioning protocol

and subsequent graded exercise test (GXT) is shown in Figure 36. The occlusion pressure of 220 mmHg was chosen following pilot testing to identify the limb occlusion pressure when blood flow ceased within the popliteal artery, utilising Doppler ultrasound (Shenzhen Delicate Electronics Co. Ltd., Shenzhen, China) and is supported by similar IP literature (Bailey et al. 2012a; de Groot et al. 2010). Occlusion pressure for the group was between 160-170 mmHg, therefore 220 mmHg ensured the pressure was at least 50 mmHg above systolic blood pressure.

Figure 36: Protocol overview. The entire protocol was completed in a hot environment. 'R' and 'L' represent occlusion of right and left legs, respectively. 'GXT 1' denotes 3 min exercise stages with increments of $1km.h^{-1}$. 'GXT 2' denotes gradient based test to exhaustion, incorporating 1 min stages with increments of 1%.

In order to preserve participant naivety and control for a *Hawthorne* effect during the second part of the exercise test (GXT 2), participants were informed the study was investigating the optimal pressure of an intervention that had the potential to be ergogenic in both conditions. At the end of each trial standardised questions were used to ascertain how convincing the placebo had been. Blood flow data of the popliteal artery collected during pilot testing, and published literature (Gibson et al. 2013), identified 50 mmHg as an appropriate control pressure, as it provides a sensation of pressure, without impairing arterial flow. Participants completed an extended rest period during the familiarisation trial, prior to exercise.

6.3.4 Graded exercise tests

A graded exercise test, split into two parts; GXT 1 and GXT 2, was adopted as previously described (General Methods, Section 3.4). Briefly, following rest and preconditioning phases, a low intensity five min warm-up was completed. GXT 1 was a submaximal incremental speed protocol followed by GXT 2, an incremental gradient protocol to volitional exhaustion. Starting speeds were between 8–11 km.h^{-1} (1% gradient, Jones & Doust [1996]) depending upon recent running performance, with each participant completing a minimum of six stages, using speed increments of 1 km.h^{-1}. Following a 10 min rest, GXT 2 began at a speed 2 km.h^{-1} below the previous GXT 1 final speed, with gradient increasing by 1% each min and continuing until volitional exhaustion (Jones, 2006a).

6.3.5 Physiological measures

During the familiarisation trial, anthropometric data were collected for stature, body mass and a four site skin fold calliper assessment (Harpenden, Burgess Hill, UK) across iliac crest, subscapular, triceps and biceps (Durnin & Womersley 1974). Measures of T_{CORE}, T_{SKIN} and HR were taken throughout the trial as detailed in the General Methods (Section 3.6). Heart rate, T_{CORE}, T_{SKIN}, RPE and TS were noted every 5 min during rest and the IP protocol, and at the end of each stage during exercise. The following physiological responses were calculated; lactate thresholds, running economy (RE), $\dot{V}O_{2max}$ and velocity at $\dot{V}O_{2max}$ (v$\dot{V}O_{2max}$), as described in the General Methods (Section 3.6.3).

Pre and post the IP protocol, muscle temperature was measured through a needle thermistor probe (Type MLA connected to DM 852 Medical Precision Thermometer, Ellab A/S, Hilleroad, Denmark) inserted to a depth of two centimetres into the right thigh at the mid-point between the greater trochanter and tibial tuberosity, in the vastus lateralis.

6.3.6 Statistical Analyses and Derivative Calculations

All outcome variables were assessed for normality and sphericity prior to further analysis. Where assumptions of normality were not met, data was log transformed as appropriate. Two way, repeated measures ANOVA (treatment*time) were used to test for differences in blood lactate indices, expired metabolic gases, T_{CORE}, T_{SKIN}, PSI, RPE and TS. Where appropriate, Bonferroni adjusted pairwise comparisons revealed where differences occurred. Paired samples t-tests were used to detect differences between total running time during GXT 2, v$\dot{V}O_{2max}$ sweat rate, environmental conditions and muscle temperature. Effect sizes for main effects and interaction effects are presented as partial eta squared ($\eta2$), whilst differences

between two related samples were evaluated through Cohen's d_{av} (d) in accordance with Lakens (2013).

6.4   Results

Environmental conditions did not differ between trials; CON 32.6 (0.3)°C, 61.8 (3.1)% RH, IP 32.4 (0.3)°C, 60.8 (3.6)% RH (Wet bulb globe temperature CON 28.2 [0.5]°C, IP 27.8 [0.6]°C, t=1.531, p=0.157). Similarly, comparisons of physiological variables between CON and IP, at rest, prior to cuff inflation did not reveal differences between conditions (Table 25).

Table 25: Comparisons of physiological variables at rest during CON and IP, prior to cuff inflation (mean ±SD).

	CON	IP	t	p	d
Hydration (mOsmol.kg^{-1} H$_2$O)	247 (191)	322 (208)	0.842	0.419	0.37
Blood lactate (mMol.L^{-1})	0.85 (0.38)	0.80 (0.29)	0.533	0.605	0.11
Blood glucose (mMol.L^{-1})	5.17 (0.94)	5.08 (1.26)	0.432	0.675	0.11
Heart rate (b.min^{-1})	55 (6)	52 (8)	2.138	0.058	0.52
T_{CORE}(°C)	37.00 (0.28)	37.04 (0.36)	0.463	0.654	0.11
T_{SKIN} (°C)	34.15 (0.74)	34.13 (0.36)	0.107	0.918	0.03
T_{THIGH} (°C)	33.90 (0.69)	33.84 (0.57)	0.274	0.792	0.10
T_{CALF} (°C)	33.69 (0.74)	33.54 (0.58)	0.605	0.564	0.23
T_{MUSC} (°C)	34.82 (0.69)	34.50 (0.96)	0.732	0.483	0.39

T_{CORE} = core temperature, T_{SKIN} = skin temperature, T_{THIGH} = thigh temperature, T_{CALF} = calf temperature, T_{MUSC} = muscle temperature, t = t-test value, p = significance value, d = Cohen's d effect size.

During GXT 1 IP did not improve running speeds at fixed blood lactate concentrations of 2 and 4 mMol.L^{-1} as shown in Figure 37 and Figure 38. At 2 mMol.L^{-1} the running speed in CON was 11.57 (1.17) km.h^{-1} and 11.65 (1.36) km.h^{-1} in IP. At 4 mMol.L^{-1} running speed in CON was 13.55 (1.03) km.h^{-1}, and 13.53 (1.24) km.h^{-1} in IP. There were no main effects between conditions (F=0.050, p=0.828, partial η2= 0.005), or interaction effects across the levels of blood lactate concentration (F=1.319, p=0.277, partial η2= 0.117). Similarly, during exercise, no main effect of treatment on blood glucose concentration was observed (CON – 5.03 [0.94] mMol.L^{-1}, IP – 5.47 [1.38] mMol.L^{-1}, F=1.423, p=0.260, partial η2= 0.125), nor an interaction effect (F=2.056, p=0.087, partial η2= 0.171).

Figure 37: Running speed at 2 & 4 mMol.L^{-1} respectively. Grey columns represent group means (±SD), with individual data shown by grey lines.

Figure 38: Mean (±SD) blood lactate concentration during GXT 1 for IP and CON. 'Famil' represents cooler familiarisation, and is presented in grey, without error bars for clarity.

There was no main effect for treatment in RE (CON – 222.3 [18.0] mL.kg^{-1}.km^{-1}, IP – 218.9 [16.5] mL.kg^{-1}.km^{-1}, F=2.806, p=0.125, partial η2= 0.219), nor an interaction effect (F=0.446, p=0.774, partial η2= 0.043). No main effect was observed on V$_E$ for treatment (F=0.013, p=0.910, partial η2= 0.001), nor an interaction effect (F=0.843, p=0.477, partial η2= 0.078). Similarly, there was no main effect for RER (F=1.593, p=0.236, partial η2= 0.137), or an interaction effect (F=1.318, p=0.272, partial η2= 0.116).

$\dot{V}O_{2max}$, as defined by specified criteria, was achieved in 16 trials, with the remaining 6 trials demonstrating $\dot{V}O_{2peak}$. There were 7 $\dot{V}O_{2max}$ and 4 $\dot{V}O_{2peak}$ in CON, and 9 $\dot{V}O_{2max}$ and 2 $\dot{V}O_{2peak}$ following IP. There was no difference in $\dot{V}O_{2max}$ (CON - 55.5 (3.7) mL.kg^{-1}.min^{-1}, IP 56.0 (2.6) mL.kg^{-1}.min^{-1}, $t=-0.812$, $p=0.436$, $d=0.17$), as shown in Figure 39, nor in total running time during GXT 2 (CON – 347 [42] s, IP – 379 [68] s, $t=1.496$, $p=0.166$, $d=0.58$) (Figure 40). However, differences in $v\dot{V}O_{2max}$ were observed (Figure 41), with IP demonstrating a greater predicted running speed (CON 15.0 [1.2] km.h^{-1}, 15.4 [1.2] km.h^{-1}, $t=2.727$, $p=0.021$, $d=0.33$).

Figure 39: $\dot{V}O_{2max}$ during familiarisation, Control and IP. Grey columns represent group means (±SD), with individual data shown by grey lines. 'Famil' represents normothermic familiarisation.

Figure 40: Total running time during GXT 2. Grey columns represent group means (±SD), with individual data shown by grey lines.

Figure 41: v$\dot{V}O_{2max}$ during GXT 2 between conditions. Grey columns represent group means (±SD), with individual data shown by grey lines. * represents a statistical difference ($p<0.05$).

Resting heart rate was not different between groups (Table 25), however heart rate, as noted at the end of each 5 min occlusion period, was consistently lower whilst undergoing IP, compared with CON (CON 60 [10] b.min^{-1}, IP - 56 [10] b.min^{-1}, $F=9.977$, $p=0.010$, partial $\eta2=$ 0.499). The lower pre-exercise heart rate did not continue during exercise, with HR similar throughout GXT 1 ($F=0.132$, $p=0.724$ partial $\eta2=$ 0.13), and at maximum during GXT 2 (CON 186 [10] b.min^{-1}, 186 [11] b.min^{-1}, $t=0.341$, $p=0.740$, $d=0.03$). A main effect for PSI was observed, with PSI lower following IP ($F=5.548$, $p=0.043$, partial $\eta2=$ 0.381), with no interaction effect ($F=1.380$, $p=0.250$, partial $\eta2=$ 0.133). No differences were observed between IP and CON for RPE ($F=0.028$, $p=0.870$, partial $\eta2=$ 0.003), or thermal sensation ($F=0.054$, $p=0.821$, partial $\eta2=$ 0.005).

There were no differences in thermoregulatory variables at rest as shown in Table 25. There was also no main effect for treatment on T_{CORE} whilst IP occured ($F=0.666$, $p=0.433$, partial $\eta2=$ 0.062). However, there was a progressive decrease in T_{CORE} during IP, which was not matched by the control group, providing an interaction effect ($F=6.098$, $p=0.009$, partial $\eta2=$ 0.379) as shown in Figure 42. However, Bonferroni post hoc did not detect differences at any time points. The reduced T_{CORE} following IP continued during GXT 1, with IP lower throughout ($F=27.886$, $p=0.001$, partial $\eta2=$ 0.756), however the rate of increase was similar between the groups, so no interaction effect was seen ($F=3.308$, $p=0.05$, partial $\eta2=$ 0.269). IP did not elicit a change in muscle temperature (CON Δ = 0.55[0.57]°C, IP Δ= 0.78[0.85]°C, $t=-0.593$, $p=0.568$,

d=0.33). There was no main effect for treatment on mean T_{SKIN} throughout IP (*F*=2.177, *p*=0.184, partial η2= 0.237), nor during exercise (*F*=0.001, *p*=0.976, partial η2= 0.000). Furthermore, no differences in localised skin temperature between treatments were observed during the preconditioning intervention, for the calf (*F*= 0.001, *P*= 0.971, partial η2= 0.000) or thigh (*F*=4.522, *p*=0.071, partial η2= 0.392). Similarly, calf (*F*=0.804, *p*=0.400, partial η2= 0.103) and thigh (*F*=0.475, *p*=0.513, partial η2= 0.064) temperatures did not differ between conditions during exercise. Finally, sweat loss did not differ between conditions (CON – 2.76 [0.54], IP 2.87 [0.83] L.hr^{-1}, *t*=-0.471, *p*=0.648, *d*=0.15).

Figure 42: Mean (±SD) Core temperature throughout the protcocol. Error bars represent one standard deviation. IP-R represents ichaemic preconditioning of the right leg, IP-L the left leg.

6.5 Discussion

This study investigated the effect of IP on the primary physiological determinants of endurance running, notably the lactate turn-point, $\dot{V}O_{2max}$ and running economy under heat stress. Whilst the enhanced cardiovascular and metabolic strain are not limiting factors leading to the cessation of exercise during sub-maximal exercise in the heat (González-Alonso et al. 2008), such a competitive demand for blood flow may impair performance in competition or training, as well as invalidate the use of blood indices to monitor training intensity in the heat accurately, relative to normothermic conditions. It was hypothesised that the novel application of IP in the heat would alleviate some of the deleterious effects of heat strain, possibly by enhancing skeletal muscle vasodilation and/or influencing muscle metabolism, leading to a mediated metabolic strain, as has been demonstrated under normothermic conditions.

However, these data indicate IP does not proffer any benefit across the determinants of endurance performance in the heat.

The lack of difference in blood lactate accumulation during GXT 1 is distinctly different from the results of Bailey et al. (2012a) who reported a significantly lower blood lactate concentration throughout an incremental, submaximal run (mean difference -1.07 mMol.L^{-1}). They attributed the subsequently improved 5 km performance to improvements in vascular function, facilitating enhanced removal and transport of lactate for uptake and use. Bailey et al. (2012a) speculated that such changes may increase lactate oxidation in the mitochondria of working muscles, and/or reflect energy sparing, through augmented mitochondrial flux or increased excitation-contraction coupling efficiency, as has previously been observed in preconditioned pig muscle (Pang et al. 1995). Recently observed improvements in isometric endurance exercise of the forearm suggests such alterations in muscle metabolism may occur without concomitant haemodynamic changes (Barbosa et al. 2015). Previously, the improvements to vascular function following IP have been attributed to biochemical changes that follow ATP degradation during periods of occlusion. For example, the accumulation of intracellular adenosine interacts with adenosine receptors A_1 and A_2 (Marongiu & Crisafulli 2014), and ultimately mediates vasodilation (Riksen et al. 2008). Adenosine accumulation has also been suggested to maintain endothelium function, when it is normally impaired following strenuous exercise (Bailey et al. 2012b). Alongside adenosine, ischaemia stimulates increased endothelial nitric oxide (NO) release that attenuates sympathetic vasoconstriction, inducing smooth muscle relaxation as part of the secondary plateau phase of the bimodal cutaneous response to local hyperthermia (Kellogg et al. 1998). It is possible that under heat stress, any reduction in skeletal muscle energy consumption were too small for these measures to detect or, from a haemodynamic perspective, skeletal muscle may not be sensitive to further increases in NO following IP, due to the apparently greater role of prostaglandins, relative to NO (Kellogg et al. 2005). Moreover, it is possible that blood vessels are already maximally dilated from heat stress (Lorenzo & Minson 2010).

Among other studies to have found no significant effect from IP during submaximal exercise, Clevidence et al (2012) suggested their incremental cycling exercise protocol may not have provided sufficient ischaemic challenge to realise the benefits of IP, although the authors also acknowledge they may not have achieved full occlusion during the IP procedure. Notwithstanding, the elevated physiological and metabolic strain observed during submaximal running in the heat, compared with familiarisation data collected under cooler conditions (Figure 38), is supportive of an ischaemic challenge, especially given the distinct improvements

following IP observed by Bailey et al. (2012a) during a similar normothermic incremental running protocol. However, Febbraio (2001) has highlighted that oxygen availability is not the main factor mediating the augmented glycogenolysis and elevated blood lactate concentration during moderate exercise under heat stress, although they did not rule out a role for decreased muscular blood flow influencing metabolic processes in an oxygen independent manner. Indeed, Febbraio et al. (1994) has previously highlighted a strong relationship between reduced body temperature and reduced plasma adrenaline levels in explaining metabolic changes following heat acclimation, notably a reduced RER, indicating greater fatty acid or ketone oxidation and less glycogenolysis. These findings were in the apparent absence of changes in fibre type recruitment and with changes far exceeding that expected from a Q10 effect on glycogenolytic and glycolytic processes. It is through the lowering of body temperature that other interventions such as heat acclimation and precooling may prevent such metabolic alterations, with a cooler body preserving splanchnic circulation and thus maintaining the conversion of lactate to pyruvate in the liver (Lorenzo et al. 2010). However, given the sensitivity of splanchnic blood flow to changes in body temperature, where splanchnic blood flow may reduce by 40% during hyperthermia (Rowell 1973), this same mechanism following IP would appear unlikely. Therefore, despite potentially greater blood flow to the muscle following IP, metabolic alterations and metabolite production by the muscle were maintained. Consequently, the unchanged circulating blood lactate and glucose concentrations are in keeping with elevated muscle temperature and adrenaline being the prominent drivers of increased intramuscular carbohydrate utilisation in the heat, rather than localised ischemia (Febbraio 2001), although it is acknowledged these were not directly measured in the current study.

$\dot{V}O_{2max}$ is attenuated in the heat (Nybo et al. 2014), which is likely a direct consequence of cardiovascular strain and insufficient muscular blood flow at maximal intensities (Gonzalez-Alonso et al. 2008). Given that mean finishing T_{CORE} in both conditions was elevated (39.0°C CON, 38.9°C IP), and $\dot{V}O_{2max}$ was lower than the normothermic familiarisation (~-2 mL.kg^{-1}min^{-1}, Figure 39), it is probable $\dot{V}O_{2max}$ was indeed impaired in this cohort. Notwithstanding that IP was applied under different environmental conditions, effects of IP on $\dot{V}O_{2max}$ appear equivocal (Marocolo et al. 2015). A potential explanation for such variability in $\dot{V}O_{2max}$ following IP are methodological differences between exercise protocols, time of exercise following IP, training status of participants, as well as data analysis techniques, such as $\dot{V}O_2$ averaging time. Furthermore, it is unclear if other studies adopted strict $\dot{V}O_{2max}$ criteria as in the present study, or may in fact be reporting $\dot{V}O_{2peak}$. Although the adoption of $\dot{V}O_{2max}$ criteria is not without

criticism (Poole et al. 2008), the use of a verification phase is supported to reaffirm $\dot{V}O_{2max}$ attainment (Midgley & Carroll 2009), but no studies pertaining to IP have adopted this procedure. In the current study, a verification phase would be confounded by the environmental conditions, given $\dot{V}O_{2max}$ impairments are determined by the progressive influence of heat strain (Gonzalez-Alonso et al. 2008). Interestingly, there was a statistical difference in $v\dot{V}O_{2max}$, a composite measure of running economy and $\dot{V}O_{2max}$, which could indicate small improvements in both of these variables, which were not substantial enough to be seen individually, but is apparent from the combined calculation. However, the mean difference of 0.38 $km.h^{-1}$, does not exceed the calculated typical error of 0.51 km^{-1} established during pilot testing, indicating that changes may not be meaningful.

Crisafulli et al. (2011) has highlighted a role for altered fatigue perception to explain enhanced performance following IP, after observing no differences in physiological parameters including $\dot{V}O_{2max}$, maximal stroke volume and maximal cardiac output. These data cannot support, or refute this, as testing occurred under different environmental conditions. However, no differences were found in RPE, and whilst a mean improvement in total running time of 32 s during GXT 2 could be considered meaningful, this was not statistically significant, nor did it far exceed the laboratory typical measurement error of 27 s. Figure 40 displays the individual responses of total running time to IP, with two individuals demonstrating large (>150 s) improvements. The physiological data of these individuals does not reveal clear evidence for the large increases in performance. Similar cardiovascular, respiratory and thermoregulatory responses were observed between tests. However, lower RPE values were reported during GXT 1, indicating these participants may have experienced a reduced perceived exertional strain following IP, enabling them to continue running for longer during GXT 2. Recently, Tocco et al. (2014) did not observe an effect from IP with highly trained athletes during free paced 5 km time trials on a track. They also suggested altered fatigue sensations could account for some of the benefits observed by IP, and that training status modifies the sensitivity to this alteration, or indeed changes in blood flow. Such a suggestion would help explain the difference between effects in sub-elite (Bailey et al. 2012a) and elite runners (Tocco et al. 2014), but does not appear to be universal, with Jean-St-Michel et al. (2011) having reported improvements in elite swimmers. Indeed, the two individuals who may have experienced a reduced perceived strain in this trial, facilitating longer running times during GXT 2, were close to the group mean for recent 5 km performance and $\dot{V}O_{2max}$. Alongside changes in fatigue perception, the possibility of a 'placebo effect' should be considered, as IP is difficult to blind. In the current study, procedures were implemented to try and maintain participant naivety to the true purpose of

the study, and this was reinforced by questioning after the study about their beliefs and perceptions of the trials. No participant reported a prior belief that the IP condition may have provided a benefit to their performance. However, blinding measures and such actions to mitigate against a *Hawthorne* effect are not widely reported elsewhere within the IP literature, so it is difficult to ascertain whether IP performance may have been influenced by a placebo effect.

Furthermore, such variability in submaximal and maximal exercise responses to IP could relate to the technique itself. Clevidence et al (2012) speculated that individual variation in limb occlusion pressure may have resulted in a different level of ischaemia to Jean-St-Michel et al. (2011). Arterial flow is unlikely to be completely blocked in the legs at 250 mmHg (Iida et al. 2007), and a greater stimulus may be achieved when occlusion occurs, rather than ischaemia. Therefore, it is possible that preconditioning of the arms, as in the study of Jean-St-Michel et al. (2011), resulted in a greater response, due to the same pressure being used on a smaller muscle mass. Gibson et al. (2013) have also suggested a pressure of 220 mmHg to be too low for leg occlusion, as arterial pulses may still be detected at 250 mmHg (Suga et al. 2010). However, the current study adopted a pressure previously shown to produce beneficial effects (de Groot et al. 2010; Bailey et al. 2012a). Prior to experimental trials, Doppler ultrasound was used to establish individual limb occlusion pressure to ensure a safety margin of at least 50 mmHg across all participants. Collectively, this suggests this cohort experienced an appropriate level of occlusion.

Muscle temperature was taken pre and post IP to quantify the thermal effects of such large, cyclical alterations in major limb blood flow. This measurement would appear to be novel within IP studies and the lack of change in muscle temperature suggests passive heating maintenance or rewarming to be unnecessary when IP is adopted, in order that muscle temperature is maintained for the start of an anaerobic exercise bout. While muscle temperature did not change following IP, interestingly IP appeared to elicit a small change in resting T_{CORE} (~0.1°C). This likely reflects a consistent redistribution of blood away from the core to the periphery as part of recovery for the previously occluded limb. During exercise this difference was maintained, presenting a small, but consistent change in T_{CORE}, which in turn influenced PSI. However, the small magnitude of difference in T_{CORE} between conditions (~0.1°C) explains the lack of an independent effect of body temperature on performance measures such as $\dot{V}O_{2max}$ and blood lactate indices. Whilst this change is small in comparison to the change in T_{CORE} following precooling techniques, future research may consider how this could complement a cooling strategy.

In conclusion, this study investigated the novel application of IP during exercise in the heat. These data indicate IP does not proffer any benefit across the determinants of endurance performance in the heat and metabolic alterations under heat stress appear insensitive to IP signalling. Furthermore, four cycles of IP did not influence deep muscle temperature, but elicited a modest influence on T_{CORE} during subsequent exercise. Consequently, in comparison to alternative acute interventions such as precooling, IP is not recommended for use when completing endurance exercise in the heat.

7 Short term heat acclimation improves the determinants of endurance performance and running time trial performance in the heat.

Previous investigations have demonstrated modest effects from acute interventions on the determinants of endurance performance. This investigation will now consider the chronic approach of heat acclimation, and investigate a practical 5-day strategy. The effect of heat acclimation on the determinants of endurance performance model has yet to be investigated. Furthermore, the decrement to the model afforded by heat stress is unclear. A 5 km time trial protocol will be completed to complement findings from the incremental exercise protocol.

7.1 Abstract

Despite widespread use of heat acclimation training, no studies have quantified the effect on the determinants of endurance performance and time trial performance in runners. Therefore, the aim of this study was to investigate the effect of 5 days controlled hyperthermia heat acclimation (STHA), with permissive dehydration, on $\dot{V}O_{2max}$, LT, LTP, RE, $v\dot{V}O_{2max}$ and 5 km running time trial performance in the heat, as well as quantify the impairment to the determinants in hot versus cool conditions. A control group (CON) matched for training volume and intensity was adopted to differentiate the effect of heat stress and exercise components of the acclimation programme on the subsequent exercise performance. Seventeen participants (10 STHA, 7 CON) completed graded exercise tests (GXT) in cool (13°C, 50% RH, pre training) and hot (32°C, 60% RH) conditions (pre and post training), as well as 5 km treadmill time trials in the heat (32°C) pre and post training. Interaction effects were observed for total running time during the GXT (STHA;78 (43) s, CON; 18 [44] s, p=0.006, partial η2=0.457) and TT performance (STHA 6.2 [5.5]%, CON; 0.6 [1.7]%, p=0.029, partial η2= 0.296), with greater improvements in STHA. $\dot{V}O_{2max}$ was improved across both groups (p=0.004, partial η2=0.517), with no interaction (p=0.228). However, large differences in the mean improvements were observed; STHA +4.0 (2.2) mL.kg^{-1}.min^{-1} (7.3 [4.0]%, d_{av}=0.47), CON; +1.9 (3.7) mL.kg^{-1}.min^{-1} (3.8 [7.2] %, d_{av}=0.30). Alongside improved TT performance, following training, the finishing T_{SKIN} was lower (p=0.041, partial η2= 0.283) in STHA (Pre; 35.7 [0.35]°C, Post; 34.6 [0.31] °C), but not in CON (Pre; 35.7 [0.43]°C, Post; 35.5 [0.38] °C). There were improvements in both groups for the lactate threshold (p=0.021, partial η2=0.306), lactate turnpoint (p=0.005, partial η2=0.413) and $v\dot{V}O_{2max}$ (p=0.031, partial η2=0.332), but no interactions. Running economy was impaired following both training programmes (p=0.008, partial η2=0.459), with no interaction (p=0.341).

The independent effect of training in the heat appears to account for improvements in running time to exhaustion, TT performance and $\dot{V}O_{2max}$, however similar improvements in LT and LTP were seen following both training programmes. Training in the heat provides specific thermal adaptations that improve endurance performance further than high intensity normothermic training, despite normothermic training affording similar systematic improvement to the traditional model of endurance physiology.

7.2 Introduction

A deleterious effect of heat stress on endurance performance is well established (Galloway & Maughan 1997). Moreover, this impairment extends to the primary determinants of endurance performance; $\dot{V}O_{2max}$, (Sawka et al. 1985), blood lactate indices (Lorenzo et al. 2011) whilst the influence on running economy (RE) is unclear (Saunders et al. 2004a). Consequently, considerable evidence documents the effectiveness of transient thermal adaptations, proffered through heat acclimation training (HA), in alleviating physiological and thermoregulatory strain during exercise in both temperate and hot conditions (Corbett et al. 2014; Neal et al., 2015), as well as the ergogenic potential for endurance performance in the heat (Lorenzo et al. 2010; Garrett et al. 2012; Racinais et al. 2015b; Keiser et al. 2015). The HA phenotype arises from physiological adaptation across multiple systems notably pertaining to; sudomotor function (Lorenzo & Minson 2010), cardiovascular stability (Rowell 1967), skeletal muscle metabolism (Febbraio et al. 1994), cutaneous blood flow (Lorenzo and Minson, 2010), central thermoregulatory control (Buono et al. 1998) and cellular function (McClung et al. 2008). In turn, observable and prominent HA adaptations include decreased resting and exercising, core (T_{CORE}) and skin (T_{SKIN}) temperatures, alongside a reduction in exercising heart rate (HR) in the heat, which likely arises through a combination of increases in plasma volume (PV), cardiac contractility and sudomotor function (Sawka et al. 2011). Such adaptations collectively ameliorate the deleterious effect of peripheral vasodilation in the hyperthermic individual, which is a predominant limitation during maximal aerobic exercise in the heat (González-Alonso et al. 2003; Périard et al. 2011). Concomitantly, improved perception of both thermal strain and perceived exertion during heat stress may be observed following HA, alluding to a role for behavioural alterations to improve free-paced, sub-maximal exercise in the heat (Flouris & Schlader 2015). Although greater physiological adaptations are typically observed following MTHA and LTHA (Pandolf 1998; Shapiro et al. 1998; Garrett et al. 2009), the rapid induction of cardiovascular, thermoregulatory and perceptual adaptations after ~4–6 days (Armstrong & Maresh 1991, Tyler et al. 2016) helps explain the relative prominence of short-term acclimation

(STHA; ≤7 days) as a time-efficient alternative for competitive preparation (Garrett et al. 2011; Chalmers et al. 2014).

In addition to improving TT performance in the heat, HA appears to enhance the determinants of endurance performance directly, although the holistic effect on the traditional model (Bassett & Howley 2000) during running is not well documented. Recently, Racinais et al. (2015b) demonstrated a partially and then almost fully ameliorated 43 km cycling TT performance in the field (~37°C) following 1 and 2 weeks of heat adaptation respectively, compared with cool conditions (~8°C). Racinais et al. (2015b) attributed the initial pre-acclimation reduction and subsequent improvement in TT performance under heat stress, to increased power output, albeit with relative intensity (%$\dot{V}O_{2max}$) maintained. This pattern is commensurate with a reduced, and then enhanced, $\dot{V}O_{2max}$ as individuals arrive and then adapt to a hot environment respectively (Nybo et al. 2014). It is likely HA does not entirely abate the decline in $\dot{V}O_{2max}$ in the heat, with Sawka et al. (1985) reporting a 3.5% increase in $\dot{V}O_{2max}$ in the heat, compared with the initial ~8% reduction. Similarly, Lorenzo et al. (2010) reported an 8% improvement in $\dot{V}O_{2max}$, relative to an initial ~20% reduction under heat stress. An enhanced lactate threshold (LT), or lactate turnpoint (LTP) following HA is also widely reported (Lorenzo et al. 2010; Chalmers et al. 2014; Neal et al. 2015), likely facilitated by the maintenance of splanchnic blood flow and conversion to glucose in the liver (Rowell 1973), as well as metabolic adaptations in the muscle, ameliorating the shift to glycogenolysis under heat stress (Febbraio et al. 1994). Theoretically, HA may enhance running economy (RE), through hypervolaemia and a reduced cardiovascular strain (Morgan et al. 1989; Saunders et al. 2004a), however whether RE is impaired by heat stress remains a topic of contention (Bailey & Pate 1991), with both enhanced (MacDougal et al. 1974) and reduced (Rowell et al. 1967) submaximal $\dot{V}O_2$ reported in the heat. Notwithstanding this ambiguity, the traditional model of endurance performance (Coyle 1995; Basset & Howley 2000; Jones & Carter 2000; McLaughlin et al. 2010) appears to remain sensitive for characterising decrements in performance under heat stress, as well as subsequent improvements following HA.

Despite the efficacy of HA in improving exercise performance under heat stress, there is a dearth of research concerning running time trials. A greater metabolic heat production, reduced convective cooling and potentially enhanced individual variability of economy of movement within runners (Millet et al. 2009), may all elicit a non-uniform response when compared with cycling research. Indeed, given the propensity for a heightened thermal strain in running (Chan et al. 2008), larger effects following HA may be apparent. Therefore, an investigation into the effect of HA on the determinants of endurance performance and TT

performance in runners, would appear to be warranted. Moreover, a lack of synergy within HA regimes exists, with different durations (short, medium, long term), environmental conditions and models (fixed intensity, self-regulated, controlled hyperthermia) adopted, making it difficult to extrapolate results between studies. Given the maximal adaptation that may be observed, medium and long term HA strategies are more widely researched than STHA, despite the apparent preference for STHA by athletes (Garrett et al. 2011; Chalmers et al. 2014). Furthermore, fixed intensity models that prescribe exercise at %$\dot{V}O_{2max}$ may provide a sub-optimal adaptation stimulus as adaptation occurs (Taylor 2014). This reduces the relative strain, as well as presenting systematically altered changes in T_{CORE} and sweat rate due to differences in MHP and the evaporative requirement (E_{req}) across individuals of varying $\dot{V}O_{2max}$ or body mass (Jay et al. 2011, Taylor 2014). Conversely, controlled hyperthermia HA maintains the relative strain by achieving the same internal temperature each day (Gibson et al. 2015c; Mee et al. 2015b). Furthermore, higher intensity exercise complements controlled hyperthermia to rapidly, initially increase T_{CORE}, which is pertinent as this may result in training at sport-specific intensities (Houmard et al. 1990; Sunderland et al. 2008), allowing HA to complement rather than compromise, a competition taper (Spilsbury et al. 2015). However, high intensity training may elicit a training effect independently of thermal strain, but no study has yet to closely match a high intensity HA programme in a control group, making it hard to differentiate the precise roles of thermal versus exertional strain in promoting physiological adaptation in this type of training, which could further optimise HA practices.

Therefore, the aim of this study was to investigate the effect of controlled hyperthermia STHA on the determinants of endurance performance and TT performance in endurance runners, as well as quantify the decrement in the determinants in hot versus cool conditions. A matched-intensity control group was included to differentiate the effect of heat stress and exercise components of the acclimation programme on the subsequent exercise performance. It was hypothesised that STHA would elicit larger improvements in both the physiological determinants and TT performance than normothermic training.

7.3   Methods

7.3.1   Participants

Seventeen local runners volunteered as participants for this experiment. Participant details and between groups comparisons are provided in Table 26 below. Ten participants (9 male, 1 female) completed short term heat acclimation training (STHA), whilst seven (male) participants completed control training (CON). All participants were recreational club runners

who met the inclusion criteria (General Methods, Section 3.2) of training at least 3 times per week and running a sub-22 min 5 km or sub 44 min 10 km race in the previous 2 months. Mean (±SD) recent 5 km performances in the STHA group were 20:51 (1:41) and 19:48 (1:39) in the CON group. As well as previously described preparation criteria (General methods, Section 3.3), participants were asked to prepare for each trial as a competition. Furthermore, given the number of repeat trials, participants completed a 24-hour food diary prior to each test and indicated sleeping hours, motivation, muscle soreness and stress on 5-point Likert scales upon arrival.

7.3.2 Experimental design

A mixed model independent groups design was adopted, with participants assigned to either STHA or CON training. Where possible, the CON group were individually matched against the STHA group for anthropometry, $\dot{V}O_{2max}$ and recent running performance, in order to elicit a similar relative and absolute training intensity. Following instrumented familiarisations, participants initially completed a normothermic graded exercise test (GXT) (13°C, 50% RH), before a GXT and 5 km time trial (TT) in the heat (32°C, 60% RH), pre and post five consecutive days of training. The GXTs were ordered pre and post training to prevent additional heat exposures from the TT influencing HA criteria. All pre and post trials were completed within 1 week of training. An overview of the design is provided in Figure 43 below.

Figure 43: Overview of experimental design.

7.3.3 Graded exercise test

Markers of heat acclimation were assessed for the STHA group from resting and exercise responses during GXTs in the heat, pre and post training. These markers are in accordance with recent literature (Sawka et al. 2011; Périard et al. 2015) and are displayed in Table 27. During the pre and post GXTs in the heat, STHA participants entered the environmental chamber and lay in the supine position for 30 min to assess resting responses.

For all trials, following rest, a five min warm-up was completed, before beginning the exercise test as described in the General Methods (Section 3.4). Briefly, the test comprised two parts; GXT 1 was a submaximal incremental speed protocol, followed by GXT 2; an incremental gradient protocol to volitional exhaustion. During GXT 1 each participant completed a minimum of six stages, using speed increments of 1 $km.h^{-1}$. Following a 10 min rest, GXT 2 began at a speed 2 $km.h^{-1}$ below the previous final speed with gradient increasing by 1% each min and continuing until volitional exhaustion (Jones 2006a).

For all experimental trials and training sessions, pre exercise hydration was assessed and pre and post (towel-dried) nude body mass were recorded to estimate sweat loss (General methods, Section 3.6.2). Measures of HR, T_{CORE}, T_{SKIN}, respiratory gases, sweat loss, RPE and TS were also reported Plasma volume expansion was estimated using the equation of Dill and Costill (1974) (Equation 10, General methods, Section 3.7.1). Measures of both haemoglobin (Hb) and haematocrit (Hct) were taken in triplicate from resting fingertip capillary samples using a portable haemoglobin photometer (EFK Diagnostics Hemo Control Analyser, Magdeburg, Germany), providing an estimate of Hb to 1 $g.L^{-1}$. To measure Hct, blood was collected into 75µl heparinised capillary tubes and spun in a centrifuge at 14,000 rpm for 3 minutes (Haematospin 1300, Hawksley and Sons Ltd, Lancing, England). Following centrifugation, Hct was read using capillary tube slide measure (Hawksley and Sons Ltd, Lancing, England). A mean of the three samples was taken for each measure. In addition to markers of heat acclimation, the determinants of endurance performance were calculated; lactate thresholds, running economy (RE), maximum oxygen consumption ($\dot{V}O_{2max}$) and velocity at $\dot{V}O_{2max}$ ($v\dot{V}O_{2max}$), as previously described (General Methods, Section 3.6.3).

7.3.4 Time trial

Participants completed a minimum of one treadmill TT familiarisation in the heat, in accordance with the recommendations of Laursen et al (2007) for trained runners. Following a 5 min, self-selected warm-up (replicated across all trials), participants completed a 5 km TT on a motorised treadmill. During the TT participants adjusted treadmill speed *ab libitum* (increment

0.2 km.h^{-1}), with the distance completed continuously displayed. Participants were blinded to all other feedback including speed, time, HR and T_{CORE}. Standardised instructions were given at the start of the trial to; 'give your all', 'pace yourself throughout the trial' and 'adjust speed as you see fit' as per similar studies (Stannard et al. 2011). Initially participants straddled the treadmill belt, which was increased to that individual's mean pace from the familiarisation, in order to prevent a small difference in the blinded starting speed influencing the overall performance. The trial began as the participant began running, and they were free to adjust speed immediately. During the run, elapsed time was recorded every km alongside HR, T_{CORE}, T_{SKIN}, RPE and TS.

7.3.5 Training

The STHA group completed five, 90 min daily training sessions in the heat (40°C, 60%) using controlled hyperthermia. This involved participants exercising until T_{CORE} exceeded 38.5°C, with exercise then prescribed intermittently to maintain T_{CORE} above 38.5°C for 60 min, in accordance with the work of Fox et al. (1963). Training was completed on cycle ergometers (Monark, e724, Vansbro, Sweden), which afforded a high level of precision for controlling mechanical work, and therefore metabolic heat production, whilst allowing multiple individuals to train at once. Exercise intensity was initially prescribed relative to body mass, at 2.7 W.kg^{-1}, based on the strength of the relationship between MHP and power output (Gibson et al. 2015d), and subsequently adjusted to maintain the participant's maximum tolerable power in order to minimise exercise volume and ensure participants reached the target T_{CORE} within 30 min. The novel prescription of exercise based on power output, relative to body mass, as opposed to %$\dot{V}O_{2max}$ (Nielsen et al. 1993; Lorenzo et al. 2010; Castle et al. 2011), removes the necessity for an initial cycling $\dot{V}O_{2max}$ test and maintains the relative exercise intensity across training days, independent of adaptation. Measures of T_{CORE}, HR, RPE and TS were taken every 5 min, alongside any adjustments to exercise intensity as appropriate. For each individual, training occurred at the same time of day, with the majority in the morning (07:00-10:00 h) and one participant in the evening (18:00-20:00 h). In accordance with recent evidence supporting the efficacy of permissive dehydration as an additional stressor for PV expansion (Garrett et al. 2011, 2014), no fluid intake was permitted throughout the training sessions.

The purpose of the CON group was to understand the contributing roles of exertional and thermal strain as independent stimuli for subsequent aerobic performance in the heat, rather than assess HA state pre and post, given the volume of evidence supporting the efficacy of this type of protocol for establishing HA. The CON group reflects the typically higher intensity in a

controlled hyperthermia programme, compared to that of fixed intensity (%$\dot{V}O_{2max}$) protocols (Gibson et al. 2015a), which may elicit a training effect that is independent of thermal strain. Therefore, with matched total exercise time and cycling intensity, the CON group completed five consecutive days of training in temperate laboratory conditions (20°C, 40%). Sessions typically lasted 40-45 min, using the same equipment and procedures as in STHA, although participants were permitted cooling fans.

7.3.6 Statistical analyses

All outcome variables were assessed for normality and sphericity prior to further analysis. Heat acclimation criteria for STHA were analysed using one-way repeated-measures ANOVA with Bonferroni post hoc test. Physiological and performance data for CON and STHA from both the GXTs and TTs were analysed using mixed model, 2-way ANOVA (Group*Time) with post hoc Bonferroni adjusted pairwise comparisons used where significant main or interaction effects occurred. Data from STHA and CON were pooled for comparisons between GXTs in cool and hot environments, to quantify the effect of heat stress. Data were analysed using SPSS (Version 21, SPSS Inc, Illinois, USA) with statistical significance set at $p<0.05$ and data presented as means and standard deviation (±SD). Effect sizes for main effects and interaction effects are presented as partial eta squared (partial η2), differences between related and independent samples were evaluated through Cohen's d_{av} (d_{av}) and Cohen's d (d) respectively, in accordance with Lakens (2013).

7.4 Results

7.4.1 Participants

Participants in experimental groups were closely matched for body mass, $\dot{V}O_{2max}$, body composition and recent running performance as shown in Table 26. Self-reported motivation, muscle soreness and stress responses did not differ between groups or trials ($p>0.05$). One participant from STHA was unable to complete the final TT due to injury, so for TT comparisons n=9. Furthermore, equipment failures during two trials reduced the STHA group to n=9 for RE and n=8 for $\dot{V}O_{2max}$ and v$\dot{V}O_{2max}$.

Table 26: Comparison of experimental groups. Data are mean (±SD).

	Heat acclimation	Control	*p*
	n = 10	*n* = 7	
Age	34 (16)	27 (3)	0.279
Body mass (kg)	73.2 (9.2)	70.2 (8.9)	0.512
$\dot{V}O_{2max}$ (ml.kg^{-1}.min^{-1})	58.9 (6.7)	62.4 (5.9)	0.280
Sum of skinfolds (mm)	25.8 (3.9)	23.5 (5.3)	0.326

Body surface area (m^2)	1.89 (0.14)	1.88 (0.13)	0.878
Recent 5 km (s)	1253 (103)	1188 (100)	0.214

7.4.2 Heat acclimation adaptation

The attainment of the majority of heat acclimation markers was observed following the 5 day, controlled hyperthermia programme, as shown below in Table 27. Notably, an enhanced heat dissipation capacity was observed, with resting (-0.15°C, p=0.01), exercising (-0.21°C, p=0.04) and change in T$_{CORE}$ during GXT 1 (-0.25°C, p=0.01) all lower following STHA. Furthermore, a reduced mean exercising HR (-3 b.min^{-1}, p=0.02) and RER (-0.08, p=0.03) were observed alongside expansion of the blood plasma volume (5.7%, p=0.03). Finally, although neither the mean TS (p=0.26) or RPE (p=0.34) reduced following HA, the change in TS was lower following STHA (p=0.04), indicating a mediated perceived stress during the submaximal run. A selection of HA adaptations are shown in Figure 44.

Table 27: Effect of 5 days STHA on recognised HA criteria. Data are mean (±SD).

Criteria	Pre	Post	Δ Pre - post	p	d_{av}
Resting HR (b.min^{-1})	52 (5)	49 (8)	-2 (4)	0.115	0.36
Mean exercising HR (b.min^{-1})	163 (14)	159 (13)	-3 (4)	0.023*	0.26
Resting T$_{CORE}$ (°C)	36.97 (0.33)	36.83 (0.32)	-0.15 (0.12)	0.014*	0.45
Mean exercising T$_{CORE}$ (°C)	38.12 (0.36)	37.91 (0.43)	-0.21 (0.25)	0.039*	0.54
Exercising T$_{CORE}$ Δ (°C)	1.26 (0.27)	1.00 (0.28)	-0.25 (0.20)	0.006*	0.91
Resting T$_{SKIN}$ (°C)	34.6 (0.3)	33.8 (1.1)	-0.77 (0.95)	0.046*	1.13
Mean exercising T$_{SKIN}$ (°C)	35.2 (0.8)	34.9 (0.7)	-0.32 (0.52)	0.205	0.44
Exercising T$_{SKIN}$ Δ (°C)	0.1 (1.0)	0.0 (1.1)	-0.14 (0.99)	0.670	0.13
Mean exercising blood glucose (mMol.L^{-1})	4.63 (0.62)	4.80 (0.48)	0.17 (0.40)	0.240	0.31
Exercising blood glucose Δ (mMol.L^{-1})	1.47 (0.77)	1.02 (0.80)	-0.44 (0.54)	0.040*	0.56
Mean exercising RER	1.02 (0.15)	0.94 (0.07)	-0.08 (0.12)	0.032*	0.70
Exercising RER Δ	0.22 (0.06)	0.18 (0.05)	-0.03 (0.05)	0.025*	0.59
Sweat loss (L)	1.35 (0.3)	1.39 (0.39)	0.05 (0.20)	0.503	0.13
Plasma volume	-	-	5.7 (7.1) %	0.031*	1.06
Mean exercising TS	6.0 (0.8)	5.8 (0.6)	-0.2 (0.6)	0.262	0.29
Exercising TS Δ	2.0 (0.5)	1.6 (0.4)	-0.4 (0.6)	0.042*	0.86
Mean exercising RPE	14.1 (0.9)	13.8 (1.1)	0.0 (2.0)	0.342	0.31

NB. Δ= change in variable from stage 1 to stage 6. * denotes p<0.05.

Figure 44: Clockwise from top left: Pre and post STHA HR response (A), exercising T_{CORE} (B), thermal sensation (C) and RER (D) during GXT 1. Data are mean (±SD).

7.4.3 Training

Mean daily session duration was greater for STHA, compared with CON (STHA; 90 [0] min, CON; 41 [2] min). However, training load was closely matched between groups in terms of total work completed (STHA; 2443 [657] kJ, CON; 2530 [336] kJ, $p=0.502$, $d_{av}=0.36$), total exercising time (STHA; 39 [6] min, CON; 41 [2] min, $p=0.289$, $d_{av}=0.57$), relative exercise intensity (STHA; 2.72 [0.28] W.kg^{-1}, CON; 2.9 [0.22] W.kg^{-1}, $p=0.181$, $d_{av}=0.73$) and absolute exercise intensity (STHA; 201 [33] W, CON; 203 [20] W, $p=0.895$, $d_{av}=0.07$). The difference in training environmental conditions (STHA 36.6 [0.8]°C, 59 [9]% RH, CON 20.0 [0.9]°C, 43.3 [6.8]% RH), elicited a markedly greater physiological strain in STHA in terms of; peak session HR (STHA; 176 [9] b.min^{-1}, CON; 157 [19] b.min^{-1}, $p=0.016$, $d_{av}=1.42$), mean session T_{CORE} (see Figure 45 below, STHA; 38.5 [0.2]°C, CON; 37.8 [0.2]°C, $p<0.001$, $d_{av}=4.08$), time above 38.5°C (STHA; 63 [5] min, CON; 2 [2] min, $p<0.001$, $d_{av}=15.8$), mean peak session T_{CORE} (STHA; 39.1 [0.2]°C, CON; 38.2 [0.2]°C, $p<0.001$, $d_{av}=4.91$), sweat loss volume (STHA; -2.3 [0.7] L, CON; -0.5 [0.2] L, $p<0.001$, $d_{av}=3.43$) and sweat loss relative to body mass (STHA; -3.2 [1.1]%, CON; -0.6 [0.2]%, $p<0.001$, $d_{av}=3.24$).

Figure 45: Mean T_{CORE} response to STHA and CON training across 5 days. A T_{CORE} of 38.5°C was consistently achieved within 30 min, and maintained for 60 min during STHA training. Error bars represent ±SD.

7.4.4 Effect of heat stress

The combined data of both training groups demonstrates a holistically elevated physiological strain at the end of GXT 1 during exercising under heat stress (32.1 [1.2]°C, 56.8 [5.8]% RH), compared with cool conditions (12.8 [0.9]°C, 51.7 [6.3]% RH), as shown in Table 28 below.

Table 28: Effect of heat stress on physiological variables. Exercising measures are taken after ~24 min of running during the final stage of (incremental) GXT 1 test.

	Cool (13°C)	Hot (32°C)
Exercising HR (b.min^{-1})	177 (12)	184 (12)
Exercising T$_{CORE}$ (°C)	38.46 (0.41)	38.70 (0.38)
Exercising T$_{SKIN}$ (°C)	28.34 (1.37)	35.34 (1.08)
Exercising blood glucose (mMol.L^{-1})	5.39 (0.80)	5.95 (1.20)
Exercising RER	1.04 (0.07)	1.12 (0.13)
Mean ventilation (L.min^{-1})	93.1 (14.6)	94.2 (13.4)
Sweat loss (L)	0.64 (0.24)	1.31 (0.27)
Exercising TS	5.4 (1.0)	6.9 (0.8)
Exercising RPE	15.9 (1.3)	17.7 (1.4)

Marked reductions were also observed in the determinants of endurance performance, as shown in Figure 46. Blood lactate concentration was elevated during submaximal running in the heat compared with the cool, as shown in Figure 47. Other notable impairments included; -7.7 (5.9)% in $\dot{V}O_{2max}$ (-4.6 [3.3] mL.kg^{-1}.min^{-1}, $p<0.001$, d_{av}=0.70), -4.4 (6.2)% in LT (-0.6 [0.8] km.h^{-1}, p=0.036, d_{av}=0.31), -4.8 (4.5)% in LTP (-0.7 [0.7] km.h^{-1}, p=0.002, d_{av}=0.34) and -4.0 (4.4)% in v$\dot{V}O_{2max}$ (-0.6 [0.7] km.h^{-1}, p=0.030, d_{av}=0.23), whilst a **-19 (10)%** reduction in running time to exhaustion during GXT 2 was also observed (-98 [54] s, $p<0.001$, d_{av}=1.96). Based on the predefined criteria, no difference was observed in the prevalence of $\dot{V}O_{2max}$ and $\dot{V}O_{2peak}$ tests between cool ($\dot{V}O_{2max}$ = 15, $\dot{V}O_{2peak}$ = 2) and hot ($\dot{V}O_{2max}$ = 12, $\dot{V}O_{2peak}$ = 5) conditions (p=0.08). Despite running for a shorter time, there was a modest increase in maximum HR in the heat (189 [9] b.min^{-1}), compared with cool conditions (186 [12] b.min^{-1}, p=0.04, d_{av}=0.27). Interestingly, RE was improved in the heat, with a reduction of -5.3 (4.3)% in oxygen consumption per kilometre (-12.3 [10.1] mL.kg^{-1}.km^{-1}, p=0.004, d_{av}=0.75). No difference was observed in ventilation (p=0.243, partial η2=0.090) between hot and cold conditions.

Figure 46: Mean (±SD) change in the determinants of endurance performance from graded exercise tests under heat stress, relative to cool conditions.

Figure 47: Mean (±SD) blood lactate response during graded exercise under heat stress, compared to cool conditions. Error bars represent one standard deviation.

7.4.5   Determinants of endurance performance after training

Environmental conditions were not different for submaximal tests both between groups (Pre; p=0.372, Post; p=0.894) and within participants (STHA; p=0.505, CON; p>0.99). The training responses for both groups in terms of the determinants are shown in Figure 48. A main effect for time in $\dot{V}O_{2max}$ was observed (p=0.004, partial η2=0.517), indicating both STHA and

CON may have enhanced $\dot{V}O_{2max}$, however no Group*Time interaction effect was observed (p=0.228). The mean increase in $\dot{V}O_{2max}$ pre to post HA in STHA was 4.0 (2.2) mL.kg^{-1}.min^{-1} (7.3 [4.0]%, d_{av}=0.47) and for CON 1.9 (3.7) mL.kg^{-1}.min^{-1} (3.8 [7.2] %, d_{av}=0.30). Maximum HR did not differ following training (p=0.147) and there was no interaction between groups (p=0.671). Similarly, a main effect for time (pre:post training) was observed for LT (p=0.021, partial η2=0.306), whereby change in STHA was 0.4 (0.6) k.hr^{-1} (4.0 [6.0]%, d_{av}=0.24) and in CON 0.4 (0.6) k.hr^{-1} (3.4 [5.3]%, d_{av}=0.24), with no interaction (p=0.923). For LTP there was a main effect for time (p=0.005, partial η2=0.413), whereby change in STHA was 0.3 (0.4) k.hr^{-1} (2.5 [2.9]%, d_{av}=0.20) and in CON 0.2 (0.3) k.hr^{-1} (1.8 [2.2]%, d_{av}=0.16), but no interaction effect (p=0.699). A main effect was also observed for $v\dot{V}O_{2max}$ (p=0.031, partial η2=0.332), where change in STHA was 0.5 (0.8) k.hr^{-1} (3.5 [5.3]%, d_{av}=0.24) and in CON 0.3 (0.8) k.hr^{-1} (2.6 [5.4]%, d_{av}=0.13), but no interaction (p=0.553). However, for running time during GXT 2 both a main effect for time (p=0.002, partial η2=0.532) and interaction effect were observed (p=0.006, partial η2=0.457). Following STHA, the mean running time during GXT 2 increased by 78 (43) s (p<0.001, d_{av}=2.09) which equated to 20.8 (12.7)%, compared with 18 [44] s (9.8 [1.2] %, p>0.99) in CON. Finally, a main effect for RE pre and post training was observed (p=0.008, partial η2=0.459), with a greater amount of oxygen consumed per kilometre following training, but no interaction (p=0.341). For STHA, the mean difference pre to post was 7.3 (7.3) mL.kg^{-1}.km^{-1} (3.5 [3.5]%, d_{av}=0.59), whilst for CON it was -2.4 (5.0) mL.kg^{-1}.km^{-1} (1.1 [2.3]%, d_{av}= 0.12).

Figure 48: Mean (±SD) percentage change in the determinants of endurance performance following STHA and CON. Error bars represent one standard deviation.

7.4.6 Time trial performance

Environmental conditions were not different for TTs both between (Pre; p=0.07, Post; p=0.429) and within (STHA; p=0.787, CON; p=0.436) participants. Time trial performance before training was not different between groups (STHA; Pre 1476 [173] s, CON; 1405 [178] s, p=0.436, d_{av}=0.40). However following training, total time in STHA was 1378 [116] s and CON 1396 [177] s, as shown by an interaction effect (p=0.029, partial $\eta2$= 0.296), highlighting a greater improvement following STHA (+6.2 [5.5]%), than CON (+0.6 [1.7]%). Overall pacing in each condition is shown in Figure 49. Mean HR during the TT was not different following training for either group (p=0.617), and no interaction effect was observed (p=0.336). Alongside improved TT performance, finishing T_{SKIN} was lower in STHA following training (Pre; 35.7 [0.35]°C, Post; 34.6 [0.31] °C, p=0.001, d_{av}=1.07), however the same was not observed in CON (Pre; 35.7 [0.43]°C, Post; 35.5 [0.38] °C, p=0.564), evidenced by an interaction effect (p=0.041, partial $\eta2$= 0.283). Finishing BL was not different between STHA and CON pre (p=0.323) or post (p=0.138), but was higher in STHA after training (Pre; 4.2 [0.5] mMol.L^{-1}, Post; 6.8 [0.6] mMol.L^{-1}, p<0.001), but not CON (Pre; 5.0 [0.5] mMol.L^{-1}, Post; 5.4 [0.6] mMol.L^{-1}, p=0.541). However, no main effects pre-post (Time), or interaction effects (Time*Group) were observed for RPE (Time; p=0.821, Time*Group; p=0.821), TS (Time; p=0.820, Time*Group; p=0.085), finishing T_{CORE} (Time; p=0.142, Time*Group; p=0.142), PSI (Time; p=0.05, Time*Group; p=0.992). Individual and group $\dot{V}O_{2max}$ and TT performances are shown below in Figure 50 .

Figure 49: Mean (±SD) kilometre split times during the 5 km time trial. Error bars represent one standard deviation. Error bars for control pre and post trials are omitted for clarity, but homogeneity of variance was present

Figure 50: Mean (grey columns) and individual data of percentage change in $\dot{V}O_{2max}$ and 5 km time trial performance following heat acclimation (STHA) and normothermic training (CON).

7.5   Discussion

Compared with cool conditions, heat stress impaired $\dot{V}O_{2max}$, LT, LTP, $v\dot{V}O_{2max}$ and running time during GXT 2, but improved RE. Both high intensity normothermic and heat acclimation training lessened the decline in all of these variables. The improvements in $\dot{V}O_{2max}$ and running time during GXT 2 following STHA were greater than in CON. Moreover, heat acclimation demonstrated meaningful improvements in thermoregulatory function, ultimately improving TT performance by a far greater magnitude than CON. These data support the effectiveness of a controlled hyperthermia, STHA programme with permissive dehydration, for improving aerobic running performance in hot and humid conditions.

7.5.1   Heat acclimation

Despite the greater prevalence of LTHA strategies within research, STHA is more widely adopted by athletes and these data demonstrate multi-system adaptations commensurate with the HA phenotype. Notable STHA induced improvements in exercising HR, RER and T_{CORE} were observed, alongside an enhanced PV and mediated elevation of thermal sensation. Research detailing the progressive induction of adaptations would suggest 70-75% of the maximum potential adaptation to be conferred in this cohort (Garrett et al. 2011; Mee et al. 2015b;

Gibson et al. 2015a). The mediated increase in exercising T_{CORE}, relative to pre-acclimation (Figure 44B), represents reduced heat storage during the submaximal run, theoretically affording the acclimated individual a greater heat storage capacity. The unchanged maximal sweat rate following STHA is consistent with previous research (Mee et al. 2015b; Garrett et al. 2009; Garrett et al. 2014), however that this occurred alongside a smaller change in T_{CORE} alludes to increased sweat onset sensitivity. Accordingly, a reduction in exercising T_{SKIN} was observed during GXT 1, which is likely related to the altered thermal sensation (Figure 44C), suggesting both an ameliorated absolute and perceived thermal strain (Schlader, Simmons et al. 2011b; Flouris & Schlader 2015). The modest, but consistent, reduction in exercising HR is indicative of enhanced cardiac output through increased stroke volume, to which thermal adaptations such as reduced T_{CORE} and T_{SKIN} may contribute, by reducing the demand for cutaneous vasodilation, as well as localised factors such as increased cardiac contractility and/or venous tone (Périard et al. 2015). However, the largest contributing factor is likely hypervolaemia, with the PV expansion of 5.3% commensurate with other literature following 5 days (Patterson et al. 2004a) and 8 days (Garrett et al. 2009; Garrett et al. 2012) of training respectively. Hypervolaemia may be in part be attributable to the high intensity training, given that haemodynamic adaptations are inducted first following this type of training (Montero et al. 2015), whilst the level of dehydration achieved during HA training is also thought to stimulate adaptation (Garrett et al. 2011). In light of this, fluid intake was restricted throughout training, resulting in a mean dehydration of ~3.2% of body mass per session, which is greater than Garrett et al. (2012; 2014) of ~-1.8-2.1%. The reduction in RER during exercise (Figure 44D) may represent a lower relative intensity during the GXT following STHA (Jones & Carter 2000), but may also be explained by a mediation of the shift towards glycogenolysis under heat stress, resulting in a relative maintenance of fat oxidation. Similar effects have previously been reported following heat acclimation and appear to arise from a reduced exercising body temperature, that therefore reduced plasma adrenaline levels (Febbraio et al. 1994).

These data also demonstrate the efficacy of a novel exercise prescription method, based on power output relative to body mass (2.7 W.kg^{-1}) (Gibson et al. 2015d). This eliminated the need for a prior cycling maximal test and better controls MHP than %$\dot{V}O_{2max}$, therefore maintaining relative thermal strain independently of any progressive increase in aerobic fitness across a HA programme. This approach also effectively controls thermal strain between individuals, which may not occur when using %$\dot{V}O_{2max}$, given the disparate absolute exercise intensities between those of high and low $\dot{V}O_{2max}$ (Jay et al. 2011; Cramer et al. 2014). Accordingly, the target T_{CORE} of 38.5°C was consistently reached within 30 min by the majority of participants (27 ± 4 min),

across all days, reinforcing the strength of the relationship between exercise intensity and the associated MHP for predicting changes in T_{CORE} during uncompensable heat stress. Allied with a primary aim of this HA programme, to minimise exercise volume and maximise intensity to complement a competition taper, power output was increased when participants felt they could maintain a higher intensity in order to reach the target T_{CORE} in a shorter duration. Similarly, where participants could not maintain 2.7 W.kg^{-1} across consecutive days, such as those who did not habitually cycle, power was dropped and thus may be better characterised as 'maximum tolerable', although the mean intensity remained at 2.7 (0.3) W.kg^{-1}. Therefore, the current methods achieved HA in an efficient manner, whilst the prescription of exercise intensity initially based upon body mass and absolute power may be of interest to practitioners given the simple practical application.

7.5.2 Effect of heat stress during GXT 1

As shown in Table 28, exercising under heat stress broadly enhanced physiological strain, characterised by increased HR (+7 b.min^{-1}), RER (+0.08), T_{CORE} (+0.24°C), T_{SKIN} (+7.0°C), TS (+1.5) and RPE (+1.8). Whilst the determinants of endurance performance effectively predict performance in normothermic conditions, (Joyner 1991; McLaughlin et al. 2010), the reduction that can be expected within trained runners exercising under moderate heat stress, as can be expected at the 2016 Rio de Janeiro Olympics, is not well defined. Such information could benefit coaches and sport scientists preparing athletes for hot competitions, as well as those who complete field testing in warm environments.

Pooled data highlighted the largest decrement in the heat to be in $\dot{V}O_{2max}$ (~8%), with smaller reductions across LT, LTP and v$\dot{V}O_{2max}$ (all <5%), whilst RE was improved (~5%). Furthermore, the total running time during the $\dot{V}O_{2max}$ test (GXT 2) reduced by 19%. Although T_{CORE} at $\dot{V}O_{2max}$ was elevated in the hot condition (38.9°C), compared with cool (38.5°C), the largest difference between conditions was in T_{SKIN}, with mean T_{SKIN} 35.3 °C in the heat, compared with 28.3°C (+7%) in the cool when measured at the end of GXT 1. This supports previous assertions that when T_{CORE} is elevated, a reduction in $\dot{V}O_{2max}$ is more aligned to the T_{SKIN} elevation (Arngrimsson et al. 2004; Sawka et al. 2011). The primary limitation to $\dot{V}O_{2max}$ is thought to be an inability to achieve maximal cardiac output (Rowell 1966), as demand for both cutaneous and muscular circulations compounds venous return (Gonzalez-Alonso & Calbet 2003). This is supported by the apparent lack of change in myocardial systolic or diastolic function during maximal exercise in the heat, compared with normothermic (Smith et al. 2015), despite alterations to the Frank–Starling relationship and cardiac contractility having been

reported during passive heat stress (Wilson et al. 2009). Although the observed ~8% reduction in $\dot{V}O_{2max}$ may be of interest to those completing similar exercise, it should be noted, that the decrement to $\dot{V}O_{2max}$ is progressive, therefore the impairment to $\dot{V}O_{2max}$ may vary under different environmental conditions, or individuals exercise harder or longer than ~24 min of incremental running.

Heat stress induced reductions in the exercise intensity and fractional utilisation at LT and LTP are well established (Tyka et al., 2009; De Barros et al. 2011; Lorenzo et al. 2010), however different methodologies precludes synthesis of typical delta change. The observed decrements in LT and LTP during GXT 1 are similar to that of Lorenzo et al. (2011) (-5%) in hot (40°C) versus cool (13°C) conditions, but smaller than the 17% reduction in maximum lactate steady state observed by De Barros et al. (2011) at 40°C compared with 22°C, which may reflect the prolonged nature of an MLSS protocol that likely resulted in greater relative heat strain. Despite the relative unanimity surrounding the effects of heat stress on $\dot{V}O_{2max}$ and blood lactate thresholds, the effect on RE is less well defined. Enhanced (MacDougal et al. 1974), reduced (Rowell et al. 1967) and unchanged (Rowell et al. 1969; Maron et al. 1976) submaximal $\dot{V}O_2$ have all been reported during submaximal exercise in the heat. Traditionally, the elevation of T_{CORE} has been associated with small increases in metabolic rate and therefore $\dot{V}O_2$ for a given running velocity during prolonged submaximal exercise (Sawka, Pandolf, et al. 1983; Shvartz et al. 1977; MacDougall et al. 1974), however oxygen kinetics remain unchanged under heat stress (Koga et al. 1997; Nybo et al. 2001; Burnley et al. 2002), which is pertinent given the incremental protocol in the current study. An increased energy demand may reflect increased peripheral circulation, sweat gland activity, hyperthermic hyperventilation and an increased mitochondrial metabolic rate, as well as a reduction in efficiency within the mitochondria (Brooks et al. 1971, MacDougall et al., 1974). Of note, hyperventilation under heat stress has been shown to increase $\dot{V}O_2$ by 31–50 ml (0.4-0.6 mL.kg^{-1}. min^{-1}) in the range of 117-147 L.min^{-1} (Aaron, Johnson, et al. 1992; Aaron, Seow, et al. 1992). In spite of these alterations, a warmer muscle is considered more efficient due to a range of mechanisms (Racinais & Oksa 2010), but notably including enhanced neural drive (Racinais et al. 2004) and reduced viscosity of the muscles and joints (Hill 1927). Consequently, in hot conditions, there is a leftward shift of the force-velocity relationship, affording an increased maximum velocity of shortening, with improvements greatest in Type 1 compared with Type 2 muscle fibres, which could accentuate differences in aerobic metabolism (de Ruiter & De Haan 2000). Therefore, an increased biomechanical efficiency under environmental heat stress (Rowell et al. 1969; Saunders et al. 2004a) may explain the reduced $\dot{V}O_2$ during GXT 1 in the heat, with this benefit potentially

dissipating and/or outweighed by energy demanding thermoregulatory processes at higher body temperatures. Accordingly, an increased ventilation rate in the heat was not observed. However it is acknowledged such interpretation is made in the absence of direct measurements of muscle temperature in both hot and cool conditions. Indeed, Bailey and Pate (1991) suggest the diverse $\dot{V}O_2$ responses under heat stress across previous literature may reflect different stages in the progression of heat strain with submaximal $\dot{V}O_2$ reduced at moderate temperatures, but likely to rise as thermoregulatory responses are activated to a greater extent. The practical implications of this observation are unclear, given that the relative contribution of RE for performance increases with event distance (Saunders et al. 2004a) but so too does the prevalence and influence of heat strain on endurance performance (Taylor & Cotter 2006). Notwithstanding, these data evidence a lower oxygen consumption during submaximal running in the heat, that should be controlled for in experimental work.

7.5.3 Effect of training on GXT and time trial performance

Both training methods demonstrated improvements across the determinants of performance, aside of RE. However, an additional benefit of heat training was only observed in the running time until exhaustion during GXT 2, the TT and $\dot{V}O_{2max}$. The observed improvements in the control group for $\dot{V}O_{2max}$, LT, LTP and v$\dot{V}O_{2max}$ would appear novel, but unsurprising, given the high intensity exercise completed (~81% maximum heart rate), whereas previous HA research has compared against resting or low intensity control training. These physiological benefits arise independently of thermal strain from the exercise component of controlled hyperthermia HA and following cycling training, so are not attributable to increased familiarity with the running protocol. However, ultimately they do not improve endurance performance in the heat. This alludes to a greater prominence of specific thermal adaptations to enhance performance in the heat, rather than traditional physiological determinants. Previous research highlights the importance of maintaining the core:skin gradient, which otherwise narrows as T_{CORE} and T_{SKIN} increase, increasing skin blood flow demands for heat dissipation (Cuddy et al. 2015; Cheuvront et al. 2010). As the core:skin gradient narrows, increased blood flow to the skin results in a reduction in stroke volume, which in turn impairs $\dot{V}O_{2max}$ (Périard et al. 2011; Lee et al. 2015). Furthermore, an elevated T_{SKIN} will also influence perceptual responses such as thermal sensation and RPE, which are associated with the voluntary reduction of exercise intensity in the heat (Schlader, Simmons et al. 2011b, Schlader, Stannard, et al. 2011b). Indeed, the interrelation of $T_{SKIN,}$ cardiovascular strain and RPE has previously been highlighted by Schlader, Simmons et al. (2011b), reinforcing the potential for HA to influence performance through a variety of mechanisms (Nybo et al. 2014). This is supported in the current data by the

maintenance of mean HR, RPE and TS across trials, despite running 6.2% faster following STHA. Notwithstanding, $\dot{V}O_{2max}$ likely explains some performance improvement, as there appears to be a greater increase in $\dot{V}O_{2max}$ from STHA than CON. Despite the absence of an interaction effect for $\dot{V}O_{2max}$, the delta change and magnitude of effect for STHA (7.3%, d=0.47), compared with CON (3.8%, d=0.30), are suggestive of meaningful changes between groups. Furthermore, the absolute difference in STHA was 2.1 mL.kg^{-1}.min^{-1} greater than in CON, which exceeds the proposed magnitude of a meaningful change (2 mL.kg^{-1}.min^{-1}, Tanner & Gore 2013). It is also interesting to observe the variability in $\dot{V}O_{2max}$ response in CON as shown in Figure 50, demonstrated by the high standard deviation, suggesting this type of training will benefit some individuals, but not all, in keeping with previous research indicating genetics may help determine the trainability of $\dot{V}O_{2max}$ (Bouchard et al. 1999; Bacon et al. 2013). The individual in CON who experienced the largest increase in $\dot{V}O_{2max}$, had one of the lowest $\dot{V}O_{2max}$ of all participants (58 mL.kg^{-1}.min^{-1}), suggesting the high intensity training provided a larger stimulus than for other individuals with greater aerobic capacity (CON group mean 63 mL.kg^{-1}.min^{-1}). It is apparent STHA remains a potent training intervention for acutely enhancing $\dot{V}O_{2max}$, with an enlarged PV is the most likely mediator given that augmented cardiac compliance and decreased myocardial $\dot{V}O_2$, have been observed in LTHA, rather than STHA (Horowitz et al. 1986).

The degree to which the improved running performance in the heat can be attributed to an improved $\dot{V}O_{2max}$ is unclear. Both Périard et al. (2011; 2013; 2015) and Schlader, Stannard et al. (2011b) have identified $\dot{V}O_{2max}$ as a primary determinant of self-paced endurance performance in the heat, based on individual's propensity to maintain exercise intensity relative to $\dot{V}O_{2max}$. This supports traditional models of endurance performance, where $\dot{V}O_{2max}$ is considered the strongest single predictor of performance (McLaughlin et al. 2010; Bassett & Howley 2000), also indicated by results from Lorenzo et al. (2010) where an observed an 8% improvement in 1 hour cycling TT performance (38°C) paralleled an 8% increase in $\dot{V}O_{2max}$ in the heat following HA. Furthermore, the respective improvements in performance in hot and cool environments were proportional to the increase in $\dot{V}O_{2max}$ in each condition. However, Lorenzo et al (2010) also reported increases in LTP, PV expansion, lower skin temperatures and a larger core-to-skin gradient, each of which have the potential to contribute to performance in the heat (Nybo et al. 2014). Similarly, in the current study $\dot{V}O_{2max}$ alone cannot wholly explain improved TT performance given the $\dot{V}O_{2max}$ improvement observed in CON (+1.9%), that did not transfer to TT performance (+0.6%). Moreover, as shown in Figure 50, the variability in the STHA group for TT performance is far greater than $\dot{V}O_{2max}$ improvement. Accordingly, this indicates other

specific thermal adaptations contribute in addition to improving $\dot{V}O_{2max}$, but may also represent a naivety for pacing following STHA. Indeed, the kilometre splits of the two individuals who demonstrated only a ~1% improvement in TT performance following STHA indicate these individuals may have begun the too fast following STHA, possibly because of reduced thermal discomfort or a pre-determined strategy, as these individuals then slowed considerably during the second half of the TT. Therefore, it may be necessary to re-familiarise individuals when they are heat acclimated, as the afferent feedback they have become accustomed to, has significantly changed. That STHA did not demonstrate greater improvements in blood lactate thresholds is surprising, given the changes reported elsewhere in the literature (Lorenzo et al. 2010; Chalmers et al. 2014; Neal et al. 2015), potentially arising through reduced splanchnic vasoconstriction due to a reduced body heat storage, and reduced glycogenolysis (Febbraio 2001). It is possible the ~2-3% improvement in both LT and LTP from CON has arisen due to the intensity of exercise, with a mean intensity during normothermic cycling training of ~81% of HR maximum, maintained for ~40 min daily across 5 days, which is likely to be at or above LTP for most participants, an intensity that has been shown to elicit a training effect when replicated across a four weeks 2-4 times weekly (Keith et al. 1992). The impaired RE demonstrates a greater oxygen consumption during GXT 1 following both training programmes. Such an observation is not unusual when an enhanced $\dot{V}O_{2max}$ and/or lactate thresholds, due to the interrelation of the determinants of endurance performance (Midgley, McNaughton & Jones 2007), demonstrating a greater absolute energy provision from aerobic metabolism at the same submaximal intensities. Indeed, a trend for an exaggerated effect for greater oxygen consumption in the STHA group (3.5%, d_{av}=0.59) compared with CON (1.1%, d_{av}= 0.12), reflects the changes observed in $\dot{V}O_{2max.}$ Following training, oxygen consumption and therefore RE remained lower than in cool conditions, which likely reflects the differences in ambient, and therefore muscle temperature.

It is acknowledged that the trial order may have contributed to some performance improvement, as heat acclimated trials were consistently ordered as the final trial. However, confidence that a real change has occurred in the STHA group may be taken from the minimal change in CON performance, who would also be expected to improve with further familiarisation, but also that greater familiarisation does not appear necessary for time trials in the heat, compared with a cool environment (Schmit et al. 2016). Whilst familiarisation with the competition conditions is considered important to an athlete, due to the influence of prior familiarisation of a given task on RPE (Lamb et al. 1999), one familiarisation is considered

appropriate when trained runners are used, who typically display less variability than a lesser-trained population (Laursen et al. 2007).

In conclusion, the current study supports the use of 5 days of controlled hyperthermia, with permissive dehydration, for improving endurance running performance in the heat. The independent effect of thermal strain above that of high intensity exercise training accounted for improvements in TT performance and $\dot{V}O_{2max}$, whilst improvements in LT and LTP were seen in both conditions. Finally, specific thermal adaptations appear to characterise improvements in endurance performance in the heat better than the traditional model of endurance physiology.

8 Effect of heat acclimation and precooling on 5 km running time trial performance in the heat

Previous investigations have demonstrated little effect of acute interventions for enhancing the determinants of performance. However, external cooling elicited large influences on skin temperature and perceptual measures, which may promote a greater self-selected running speed during free paced running. A 5-day heat acclimation protocol has been shown to promote physiological adaptations and improve time trial performance in the heat, but it is unclear whether this can be further enhanced with the addition of precooling. Furthermore, a direct comparison between precooling and heat acclimation on running time trial performance has yet to be completed, which could inform training prioritisation for those preparing for hot competitions. For this experiment the same participants were used as in Chapter 7, with data collection occurring simultaneously.

8.1 Abstract

Endurance running performance remains impaired in hot conditions following heat acclimation. Previous investigations that have combined acclimation with precooling demonstrate no additive benefit, albeit utilising intermittent, or cycling protocols, whereby heat strain may be less severe than endurance running. The aim of this study was to investigate the effect of STHA, alongside mixed-methods precooling on endurance running performance, as well as providing a direct comparison between precooling and heat acclimation. Nine participants completed four 5 km treadmill time trials in the heat (32°C, 60% RH); no intervention (CON), precooling (PC), heat acclimation (HA) and heat acclimation with precooling (HA+PC). Group mean (±SD) performance times were; CON 1476 (173) s, PC 1421 (146) s, HA 1378 (116) s and HA+PC 1373 (121) s. Friedman's ANOVA revealed a statistical difference only between HA and CON (p=0.004, d=0.68). However large effect sizes were observed for; HA+PC vs CON (d=0.70), PC vs CON (d=0.34), HA vs PC (d=0.33) and HA+PC vs PC (d=0.36). Reaffirming previous observations, precooling offered no further benefit to performance in the acclimated individual, despite modest alleviation of perceptual and physiological strain. The maintenance of running speed, despite a reduced physiological strain, may indicate an inappropriate pacing strategy. Finally, these data indicate heat acclimation yields a larger ergogenic effect than precooling on endurance running performance in the heat, although precooling would still appear beneficial when acclimation is not possible.

8.2 Introduction

Considerable, recent, evidence documents the ergogenic benefits of both chronic strategies such as heat acclimation (Garrett et al. 2011; Chalmers et al. 2014) and acute strategies, such as precooling (Tyler et al. 2015; Jones et al. 2012; Ross et al. 2013; Bongers et al. 2015), for endurance performance in the heat. Competition strategies that seek to alleviate the deleterious effects of hyperthermia on endurance performance, habitually adopt a uni-dimensional approach with athletes advised to either precool, or undertake (Racinais et al. 2015a). This dichotomous practice pervades despite a relative dearth of direct comparisons between acute and chronic strategies to assess the more effective approach. As detailed in Study 2 (Chapter 5), precooling techniques may be classified as internal or external depending upon how the cooling impulse is delivered. External techniques demonstrate a dose-dependent response, with a greater cooling surface area apparently providing greater benefits (Minett et al. 2011). Consequently, mixed-method, whole body cooling techniques are well supported in comparison with singular cooling garments, with recent meta-analyses reporting large effects on subsequent endurance performance (+7.3%, d =0.72 Bongers et al. 2015, d = 1.91, Tyler et al. 2015). Indeed, Study 2 (Chapter 5) evidences how a recent, practical, technique from Duffield et al. (2009) ameliorated physiological and thermoregulatory strain during fixed intensity endurance exercise in the heat, but this has yet to be evaluated during a free-paced trial where the influence of alterations in T_{SKIN} and thermal perception may be more pronounced (Flouris & Schlader 2015).

From a chronic perspective, as demonstrated in Study 4 (Chapter 7), traditional heat acclimation methods may be optimised through utilising a controlled hyperthermia model, alongside permissive dehydration, in order to maintain the thermal strain throughout acclimation training. Concomitantly, higher intensity exercise reduces training volume, increasing synergy with pre-competition taper strategies. The typical ergogenic effect of short-term HA (<7 days) on endurance performance appears to be 2.4 (3.5)% (Guy et al. 2015). However, in spite of notable thermal adaptations such as an increased plasma volume in the acclimated individual, a notable accentuated cardiovascular challenge persists when exercising at maximal aerobic intensities under heat stress (Gonzalez-Alonso et al. 2008), as a consequence of cutaneous vasodilation for thermoregulation impeding venous return and cardiac filling. Consequently, evidence demonstrates both endurance performance (Lorenzo et al. 2010) and $\dot{V}O_{2max}$ (Sawka et al. 1985) remain impaired following acclimation, relative to temperate conditions, highlighting not only the persistence of heat strain, but the potential to further improve endurance performance in the acclimated individual.

Individual strategies used to prepare for performance in the heat fail to maintain endurance performance relative to normothermic conditions, however combining interventions has yet to demonstrate any clear benefits. An initial study by Castle et al. (2011) reported no additional benefit from ice pack cooling of the quadriceps, during 40 min of intermittent sprinting, following 10 days of heat acclimation (HA). A reduced resting T_{CORE} and increased basal plasma HSP70 concentration evidenced multi-system thermal adaptations commensurate with the HA phenotype, but precooling yielded no additive benefit above the 2% improvement following HA. However prior to acclimation, precooling maintained, rather than improved, performance possibly due to the lower group body mass, compared with previous populations (Castle et al. 2006). Given that body mass, rather than body composition, has an independent effect on thermoregulation when metabolic heat production is matched (Dervis et al. 2014), a lighter population may have experienced an insufficient level of heat strain to impair intermittent sprint cycling performance, due to a smaller metabolically active muscle mass. Notwithstanding anthropometric considerations, it is conceivable the improvement following HA arose independently of an alleviated thermal strain, reinforced by the maintenance of performance in hot versus cool conditions, and facilitated by enhanced cardiovascular stability and reduced glycogenolysis aiding recovery between sprints. Therefore, additive effects may be more apparent under conditions where heat strain remains high.

Compared with long term HA (LTHA) that affords the maximum adaptive potential, short term HA (<7 days, STHA) is more widely adopted by athletes and confers partial, albeit meaningful adaptations (Garrett et al. 2011). Consequently, Brade et al. (2012) investigated precooling following STHA, where heat strain would be expected to be greater than following LTHA, but observed no additive effect during intermittent sprinting, with acclimation again apparently mediating heat strain sufficiently, such that precooling was unwarranted and thus ineffective. A consistent and significant thermal strain is apparent during continuous endurance exercise in the heat (Galloway & Maughan 1997), reinforced by the larger effects of precooling observed in endurance performance, compared with intermittent sprinting (Tyler et al. 2015). Therefore, Schmit et al. (2015) recently tested the effect of an ice vest worn at rest and during the warm-up prior to a 20 km cycling trial, following a 10-day, hot weather acclimatisation training camp. Although, the combination of HA and precooling did not improve performance above HA alone, transient, beneficial pacing alterations following precooling were observed during the first half of the trial, alongside improved perceptual thermal strain. Therefore, more aggressive precooling would appear to be more effective, given the small effects on endurance performance reported from cooling vests alone (Jones et al. 2012).

Thus, it seems plausible that an additive effect may be observed when the event duration is shorter than the ~32 min trial of Schmit et al (2015), and where heat strain remains high, such that HA and precooling alone do not maintain performance relative to cool conditions. This may be achieved by adopting STHA, rather than LTHA, as well as an exercise mode that yields a significant metabolic heat production (MHP). For example, no studies pertain to endurance running, despite running eliciting a greater MHP than cycling and providing reduced convective cooling (Nielsen 1966, Millet et al. 2009), which collectively expedite heat strain (Chan et al. 2008). The aim of this study was therefore, to investigate the potential for an additive effect of STHA, alongside an evidenced whole-body mixed methods precooling technique on endurance running performance, as well as providing a direct comparison between precooling and heat acclimation. It was hypothesised that a combined STHA and precooling strategy would further enhance time trial performance relative to STHA alone, whilst STHA would be more beneficial than precooling.

8.3 Methods

8.3.1 Participants

Nine participants (8 male, 1 female) volunteered as participants (mean [±SD]: age 32 [16] years, stature 175.2 [7] cm, mass 71.9 [8.8] kg, sum of four skinfolds 25.4 [3.8] mm, $\dot{V}O_{2max}$ 59.1 [6.9] mL.kg^{-1}.min^{-1}, recent 5 km time: 20:44 [1:44] min). Given the number of repeat trials, participants completed a 24-hour food diary prior to each test and indicated sleeping hours, motivation, muscle soreness and stress on 5-point Likert scales upon arrival. No contraindications for testing were violated prior to commencing any experimental session in accordance with previously detailed procedures (General methods, Section 3.3).

8.3.2 Experimental design

A within groups, repeated measures design was adopted, with individuals completing two 5 km treadmill time trials and a graded exercise test (GXT) before and after 5 days of HA training. Each GXT was ordered immediately pre and post HA training, whilst time trials (TT) with precooling (PC) and a no intervention control trial (CON) were completed in a counterbalanced order prior to HA. Following acclimation, a TT was completed without any intervention (HA) and another with the addition of precooling (HA+PC), again with the order counterbalanced across the group. An overview is provided in Figure 51. All experimental trials followed instrumented familiarisations of both the graded exercise test and time trial in the heat.

Figure 51: Overview of experimental design. All trials completed in hot and humid conditions.

8.3.3 Precooling

A mixed-methods, whole-body external precooling technique was adopted, as described in Study 2 (Chapter 5). Briefly, this involved wet, iced towels covering the head and neck, forearm and hand immersion in cold water (9°C), an ice vest on the torso and ice packs affixed to the quadriceps using cooling shorts, across a 20 min seated period. Towels were swapped after 10 min and hand immersion water temperature was actively maintained throughout.

External precooling was chosen as the acute strategy to accompany heat acclimation following a comparison of the effects observed from acute interventions across Studies 2 and 3 (Chapters 5 and 6). Ischaemic preconditioning did not elicit meaningful effects on physiological parameters in the heat, whilst Study 2 demonstrated external cooling to have similar effects on the determinants of endurance performance as internal precooling through ice slurry ingestion. However, external cooling demonstrated larger effects on T_{SKIN}, PSI and thermal sensation, each of which may independently influence behavioural thermoregulation and self-selected pace (Nybo et al. 2014; Flouris & Schlader 2015).

8.3.4 Heat acclimation

Heat acclimation involved five, 90 min daily training sessions in the heat (40°C, 60%) using controlled hyperthermia and permissive dehydration, as previously described (Chapter 7). This involved participants exercising until T_{CORE} exceeded 38.5°C, with exercise then completed intermittently, in order to maintain T_{CORE} above 38.5°C for 60 min. Training was completed on cycle ergometers, with the intensity initially prescribed relative to body mass, at 2.7 W.kg^{-1} (Gibson et al. 2015d), and subsequently adjusted to maintain the participant's maximum

tolerable power in order to achieve the target T_{CORE} within 30 min. Training occurred at the same time of day, with the majority in the morning (07:00-10:00 h) and one participant in the evening (18:00-20:00 h).

8.3.5 Exercise trials

Before and after the heat acclimation training, participants completed graded exercise tests and treadmill time trials, as previously described in the General Methods (Section 3.4) and Chapter 7, respectively. For all experimental trials and training sessions, pre exercise hydration was assessed and pre and post nude body mass were recorded to estimate sweat loss (General methods, Section 3.6.2). Prior to every trial, participants rested in the hot environment (32°C, 60% RH) for 10 mins, before a 20 min period when cooling, or additional rest, occurred as appropriate. Following cooling and/or rest participants completed a self-selected 5 min warm-up that was replicated across all trials, before a 5 km TT on a motorised treadmill. Participants adjusted treadmill speed *ab libitum* (increment 0.2 km.h^{-1}), with the distance completed continuously displayed. Participants were blinded to all other feedback, with standardised instructions given at the beginning of the trial and nothing thereafter. During the run, elapsed time was recorded every km alongside HR, T_{CORE}, T_{SKIN}, RPE and TS.

8.3.6 Statistical analyses

Exercise data were analysed using repeated measures Two-way ANOVA (Trial*Time) with post hoc Bonferroni adjusted pairwise comparisons used where significant main or interaction effects occurred as detailed in the General Methods (Section 3.8). Singular data pertaining to mean, delta change or finishing values between trials were analysed with One-way ANOVA. Where assumptions of ANOVA were not met, data were either log transformed or non-parametric statistics adopted. Effect sizes for main effects and interaction effects are presented as partial eta squared (partial η2), differences between related samples were evaluated through Cohen's d_{av} (d_{av}), in accordance with Lakens (2013). Data presented as mean (±SD).

8.4 Results

8.4.1 Graded exercise tests

The heat acclimation strategy resulted in the attainment of the majority of heat acclimation markers following the 5 day, controlled hyperthermia programme, as previously detailed in Chapter 7. Briefly, these adaptations included reductions in resting T_{CORE} (-0.15°C, p=0.01, d_{av}=0.45), exercising T_{CORE} (-0.21°C, p=0.04, d_{av}=0.54), exercising HR (-3 b.min^{-1}, p=0.02, d_{av}=0.26) and RER (-0.08, p=0.03, d_{av}=0.59). Furthermore, blood plasma volume expanded by

5.7% (p=0.03, d_{av}=1.06) and there was a reduction in the change in thermal sensation (p=0.04, d_{av}=0.86). Sweat loss was unchanged following STHA (p= 0.503, d_{av}=0.13).

8.4.2 Time trial performances

Environmental conditions (WBGT) did not differ between trials; CON = 27.4 (0.7)°C, PC = 26.9 (0.5)°C, HA = 27.5 (0.9)°C, HA+PC= 27.0 (0.8)°C (p=0.246, partial η2=0.156). Time trial performance following heat acclimation with precooling (HA+PC) was not normally distributed. Consequently, Friedman's ANOVA revealed a statistical difference in TT performance between conditions (p=0.001). Each intervention appeared to enhance TT performance in the heat, relative to no intervention, as shown in Table 29, however a Wilcoxon tests with Bonferroni correction (whereby significance = p<0.008), only indicated a significant difference between the control trial and HA+PC. Group mean (±SD) performance times were; CON 1476 (173) s, PC 1421 (146) s, HA 1378 (116) s and HA+PC 1373 (121) s. Relative to control, large differences were observed versus HA and HA+PC, with a modest difference with PC. There was no observable difference in running performance between HA and HA+PC trial, whilst the observed effect sizes and mean difference indicate modest improvements in HA and HA+PC compared with PC (Table 29). Individual data is shown below in Figure 52.

Table 29: Relative difference in 5 km time trial performance between trials. *Corrected statistical significance level for Wilcoxon signed-rank test post hoc p<0.008. Previously established typical error following 5 days high intensity normothermic training = 16 s, 1.2%. Data are; mean change (s), percentage change (%), statistical significance (p) and effect size (d).

	Time trial time	Control	Precooling	Heat acclimation	Heat acclimation + precooling
Control	1476 (173) s	-	55 s (3.7%) (p=0.039, d=0.34)	98 s (6.6%) (p=0.004*, d=0.68)	103 s (7.0%) (p=0.012, d=0.70)
Precooling	1421 (146) s	55 s (3.7%) (p=0.039, d=0.34)	-	43 s (3.0%) (p=0.098, d=0.33)	48 s (3.4%) (p=0.023, d=0.36)
Heat acclimation	1378 (116) s	98 s (6.6%) (p=0.004*, d=0.68)	43 s (3.0%) (p=0.098, d=0.33)	-	5 s (0.4%) (p=0.590, d=0.04)
Heat acclimation + precooling	1373 (121) s	103 s (7.0%) (p=0.012, d=0.70)	48 s (3.4%) (p=0.023, d=0.36)	5 s (0.4%) (p=0.590, d=0.04)	-

Figure 52: Individual comparisons between conditions. Group means are shown by bold lines.

Differences in pacing strategy between conditions were not apparent, with no Trial*Kilometre interaction effect observed (p=0.319, partial $\eta2$=0.133). Figure 53 displays the kilometre splits for each trial.

Figure 53: Mean (±SD) kilometre split times during the 5 km time trial. Error bars represent one standard deviation. Error bars for control (CON) and heat acclimation + precooling (HA+PC) trials are omitted for clarity, but homogeneity of variance was present.

Plots of T_{CORE}, T_{SKIN}, core:skin gradient and thermal sensation throughout all trials are shown in Figure 54. Precooling did not elicit a change in T_{CORE} during the 20 min cooling period (p=0.219, partial η2=0.165). Change in T_{CORE} during PC was 0.03 (0.04)°C and 0.04 (0.5) °C during HA+PC, compared with 0.01 (0.03)°C and 0.01 (0.03)°C during CON and HA, respectively. Therefore, starting T_{CORE} did not differ between trials (p=0.697, partial η2=0.075); CON 37.12 (0.22)°C, PC 37.07 (0.30)°C, HA 37.07 (0.23)°C and 37.2 (0.22)°C during HA+PC. However, a large reduction in T_{SKIN} (p<0.001, partial η2=0.906) was observed across the cooling period in the trials containing precooling (-6.9 [2.7]°C PC; -6.8 [1.5]°C HA+PC), whilst non precooling trials were unchanged (CON +0.87 [0.50]°C; HA +0.58 [0.58]°C). This resulted in a reduction in starting T_{SKIN} (p<0.001, partial η2=0.900) in the trials following precooling (26.9 [2.8]°C PC, 26.4 [1.9]°C HA+PC), compared with non-precooled (34.3 [0.7]°C CON, 34.0 [0.4]°C HA). This coincided with a reduced starting thermal sensation (p=0.002, partial η2=0.907) in PC (2.2 [0.8]) and HA+PC (2.4 [0.8]), compared with CON (4.4 [0.6] and HA (3.7 [1.5]). Finally, as a consequence of the reduction in T_{SKIN} following precooling, a greater core:skin gradient (p<0.001, partial η2=0.896) was observed in PC (9.6 [2.6]°C) and HA+PC (10.5 [1.7]°C), compared with CON (2.7 [0.6]°C) and HA (3.2 [0.5]°C).

During the time trials, no differences (p=0.117, partial η2=0.273) in mean T_{CORE} were apparent between trials; CON 38.53 (0.23)°C, PC 38.40 (0.42)°C, HA 38.37 (0.29)°C, HA+PC 38.25 (0.40)°C. However, the change in T_{CORE} was different between trials (p=0.044, partial η2=0.776). The increase during CON (2.22 [0.30]°C) was greatest, followed by HA (2.05 [0.40]°C, PC (1.89 [0.92]°C), with the smallest change during HA+PC (1.71 [0.31]°C). The change during HA+PC was statistically smaller than in both CON (p=0.013, d=1.67) and HA (p=0.033, d=0.96). This resulted in different finishing T_{CORE} across trials (p=0.025, partial η2=0.396), with the highest in CON (39.34 [0.30]°C), followed by PC (39.24 [0.51]°C), HA (39.16 [0.44]°C) and the lowest finishing T_{CORE} in HA+PC (38.96 [0.43]°C).

The differences in starting T_{SKIN} were also observed during the trials (p=0.010, partial η2=0.369). Mean T_{SKIN} was highest during CON (35.3 [1.2]°C), followed by HA (34.6 [0.7]°C), PC (34.6 [1.2]°C) and the lowest was in HA+PC (34.1 [0.9]°C). However, a statistical difference was only observed between CON and PC (p=0.029, d=0.58). Marked differences were observed in change of T_{SKIN} across trials (p<0.001, partial η2=0.877). The largest changes were observed following precooling (PC = 8.5 [2.7]°C, HA+PC 8.5 [2.3]°C), compared with non-precooled trials (CON = 1.4 [1.4]°C, HA 0.6 [0.9]°C). Statistical differences were observed across all trials, except between the two precooled trials (PC & HA+PC, p>0.99) and non-precooled trials (CON & HA, p=0.300) respectively. Differences in finishing T_{SKIN} were also apparent (p=0.037, partial

η2=0.293), although only between CON and HA (p=0.026, d=0.48). Finishing T$_{SKIN}$ for each trial was; CON 35.1 (1.2)°C, PC 35.7 (1.2)°C, HA 35.6 (1.0)°C and HA+PC 34.9 (1.0)°C.

The observed changes in T$_{SKIN}$ resulted in differences in the mean core:skin gradient between trials (p=0.005, partial η2=0.504). The largest gradient was observed in HA+PC (4.2 [1.2]°C), followed by HA (3.7 [0.8]°C), PC (3.6 [1.0]°C) and CON (2.9 [0.9]°C, with statistical differences between CON and HA+PC (p=0.034, d=1.24). Similarly, there were marked differences in the change in the core:skin gradient between trials (p<0.001, partial η2=0.967). The largest change occurred in precooled trials (PC -5.3 [3.0]°C, HA+PC-6.3 [1.8]°C), with modest changes following HA (1.5 [1.0]°C) and CON (0.9 [1.4]°C). Statistical differences were observed across all trials, except between HA and CON (p>0.99). This resulted in different finishing core:skin gradients (p=0.028, partial η2=0.388). The largest was observed in HA (4.3 [1.1]°C) and HA+PC (4.2 [1.2]°C), followed by PC (3.7 [1.0]°C), with the smallest gradient observed in CON (3.4 [1.0]°C).

Figure 54: Clockwise from top left: Mean (±SD) core temperature (A), skin temperature (B), thermal sensation (C) and core:skin gradient (D) during rest, cooling and exercise phases of the time trial protocol. Error bars represent one standard deviation Core temperature error bars omitted for clarity.

Despite differences in T_{SKIN}, no differences were observed in mean TS between trials (p=0.066, partial $\eta2$=0.255). However, marked differences were observed in change in TS (p=0.001, partial $\eta2$=0.470), with the greatest changes following both precooling trials. Mean RPE did not differ between trials (p=0.213, partial $\eta2$=0.168), although there was a difference in the change in RPE (p=0.03, partial $\eta2$=0.306). However, pairwise comparisons did not reveal differences between individual trials. Neither mean heart rate (p=0.252, partial $\eta2$=0.154), change in heart rate versus rest (p=0.458, partial $\eta2$=0.101) or finishing HR (p=0.734, partial $\eta2$=0.051), differed between trials. Similarly, the mean heart rate as a percentage of maximum heart rate (%HRmax) was not different between trials (p=0.089, partial $\eta2$=0.234), as shown in Figure 55. The mean %HRmax for each trial was; CON 93.4 (3.8)%, PC 94.6 (4.9)%, HA 93.3 (3.8)% and HA+PC 91.6 (3.1)%. A difference in the mean Physiological Strain Index (PSI) was also observed between CON and HA+PC (p=0.005, d=0.95), but all other trials did not differ. Sweat loss was different between trials (p=0.008, partial $\eta2$=0.386), with the largest loss in HA (2.5 (0.5) L.hr^{-1}), compared with CON (2.2 (0.8) L.hr^{-1}), PC (1.7 (0.5) L.hr^{-1}) and HA+PC (2.3 (0.6) L.hr^{-1}) respectively. Pairwise comparisons revealed a statistical difference between PC and HA (p=0.006, d=1.50), but not other conditions. The change in blood lactate concentration was not the same across all conditions (p<0.001, partial $\eta2$=0.648), with both HA (p=0.025, d=1.72) and HA+PC (p=0.007, d=1.72) statistically greater than CON. Furthermore, the change in HA+PC was also greater than that in PC (p=0.016, d=0.79). There was also a difference in the change in blood glucose concentration (p=0.004, partial $\eta2$=0.459), with both PC (p=0.040, d=0.79) and HA (p=0.037, d=0.75) greater than CON.

Figure 55: Mean (±SD) percentage of maximum heart rate maintained throughout each trial. Error bars represent one standard deviation. Error bars for control and heat acclimation trials are omitted for clarity, but homogeneity of variance was present.

8.5   Discussion

The foremost aim of this investigation was to assess the efficacy of combining precooling (acute) and heat acclimation (chronic) interventions for improving endurance running in the heat. Previous investigations into an additive effect have not demonstrated significant improvements, albeit utilising intermittent sprint cycling and/or endurance cycling protocols, whereby heat strain may be less severe than endurance running. The current study is the first to investigate a combined strategy prior to running, but also adopted a precooling technique that afforded greater cooling volume than previous studies. Notwithstanding, these data reaffirm previous observations, with precooling offering no further benefit to performance in the acclimated individual following precooling, despite modest alleviation of physiological strain. The second aim was to provide a direct comparison of the individual ergogenic potential of precooling and heat acclimation interventions. Although a statistical difference was not observed between conditions, these data indicate heat acclimation improves endurance running performance in the heat, further than precooling.

8.5.1   Combined heat acclimation and precooling

In contrast with previous research studies that have combined individual cooling garments with acclimation (Castle et al. 2011, Schmit et al. 2015), a whole body mixed methods precooling technique was utilised, which is considered more potent for endurance performance (Bongers et al. 2015, Tyler et al. 2015) and demonstrated large reductions in thermal sensation in Study 2 (Chapter 5). During intermittent sprinting exercise, heat acclimation appears to mediate heat strain sufficiently, such that additional PC provides no further benefit (Castle et al. 2011; Brade et al. 2012). However, as shown in Study 4 (Chapter 7), the magnitude of physiological impairment during endurance running in the heat following HA, relative to cool conditions, remains significant. Study 4 revealed notable impairments to $\dot{V}O_{2max}$ and running time during GXT 2 of 2.4% and 4.4%, respectively following HA. In spite of the theoretical potential to improve running performance further, no performance improvement above the HA was observed. In the only other study investigating HA+PC on endurance exercise, Schmit et al. (2015) highlighted a potentially meaningful greater self-selected exercise intensity during the first half of the trial, alongside a reduced thermal sensation. However, the greater pace was not sustained, reducing alongside the dissipation of PC effects, with a comparable trend in the precooled trials before HA. In the current study, despite a more aggressive cooling strategy in HA+PC that resulted in small, but consistent differences in T_{CORE}, T_{SKIN}, core:skin gradient and thermal sensation during the first half of the trial (Figure 54), a greater initial pace did not occur. The reasons for this are unclear, but speculatively, may represent a different, and

ultimately sub-optimal, pacing strategy being adopted in HA+PC. Recent evidence indicates athletes target a flatter pacing profile with familiarisation in the heat (Schmit, Duffield, et al. 2015) and as shown in Figure 53, HA+PC displays the smallest decrement in performance and therefore the most even pacing profile. A more cautious pacing strategy is supported by a slightly lower %HRmax during HA+PC until 4 km into the trial (Figure 55). Therefore, both the mediated physiological and thermoregulatory strain afforded by HA+PC during the first half of the trial, may not have been exploited, as individuals targeted an even pace. Previous studies have suggested the induced cardiovascular and thermoregulatory adaptations from HA may reduce the ergogenic effects of precooling by influencing the same mechanisms, such as a larger core:skin gradient and reduced cardiovascular strain, creating an insensitivity or 'ceiling effect' (Castle et al. 2011; Schmit et al. 2015). However, when an aggressive precooling technique is adopted and heat strain remains severe, these data would contend otherwise, evidencing small changes, notably in %HRmax and T_{SKIN}, that indicate future investigations may want to familiarise individuals with HA+PC to ensure pacing is optimised.

8.5.2 Comparison of heat acclimation and precooling

A secondary aim was to directly compare the effect of acute and chronic interventions on endurance running performance, which has received little attention within the literature. Participants ran 43 s (3%) faster following HA than in PC, which exceeds the typical error for this trial 16 s (1.2%). In-turn PC afforded a 55 s (3.7%) improvement over control, whilst the improvement was 98 s (6.6%) following HA, which was the only statistically significant comparison in the analyses. That no other comparisons were statistically different likely reflects a disparity in running performance within this cohort as shown in Figure 52, as well as the adoption of a more conservative non-parametric statistical test, with both the large mean differences and effect sizes indicative of meaningful changes between conditions. Elapsed time was similar between HA and PC at 2 km (PC; 547 [46] s, HA; 538 [45] s), before PC demonstrated a greater reduction between 2- 4 km (elapsed time at 4 km PC; 1135 [111] s, HA; 1108 [106] s). As shown in Figure 54, this reduction in running speed during PC coincides with the dissipation of alleviated thermoregulatory strain, where both T_{SKIN} and the core:skin gradient are no longer improved relative to HA. The flatter pacing profile in HA may reflect the trial order, as PC was not randomised with HA and repeated trials (Racinais et al. 2015b) and familiarisation to the heat (Schmit, Duffield, et al. 2015) may result in a flatter pacing profile. However, it is more likely that the greater reduction in running speed in PC reflects the associated physiological strain, given the aforementioned dissipation of both a reduced T_{SKIN} and core:skin ratio. Concomitantly, this may result in a greater progressive reduction in $\dot{V}O_{2max}$,

necessitating a reduced running speed to maintain relative intensity during PC. The reduction in maximum aerobic capacity has been suggested to be the most plausible explanation for the decline in endurance performance under heat stress (Ely et al. 2009), whilst relative intensity has been shown to be maintained across both hot, cold and hypoxic conditions (Racinais et al. 2015b; Périard & Racinais 2015). Given the transient nature, precooling does not provide prolonged alleviation of cardiovascular and thermoregulatory stress, as shown by the ineffectiveness of precooling on $\dot{V}O_{2max}$ after approximately 30 min of exercise in Study 2 (Chapter 5). Conversely, Study 4 (Chapter 7) evidences meaningful improvements in $\dot{V}O_{2max}$ following acclimation, which would facilitate a greater maintained running speed, despite the inevitable progressive decline in $\dot{V}O_{2max}$. The enhanced $\dot{V}O_{2max}$ following acclimation arises through enhanced plasma volume (Lorenzo et al. 2010) and possibly peripheral adaptation from the high intensity training (Helgerud et al. 2007), whilst the rate of decline in $\dot{V}O_{2max}$ during exercise may be mediated by increased heat dissipation. Peripheral cutaneous and sudomotor adaptations better maintain the core:skin gradient, which otherwise narrows as T_{CORE} and T_{SKIN} increase, thereby reducing cutaneous blood flow demands (Cheuvront et al. 2010). In turn, a greater core:skin gradient will maintain stroke volume and $\dot{V}O_{2max}$ (Périard et al. 2011), delaying exercise termination under heat stress (Cuddy et al. 2014). Alongside a mediated cardiovascular strain, heat acclimation will afford an improved perceived thermal strain, both independently (Chapter 7), and concomitantly with reduced T_{SKIN} (Schlader, Simmons, et al. 2011a). Elevated T_{SKIN} and perceived thermal strain are associated with the voluntary reduction of exercise intensity in the heat (Schlader, Stannard et al. 2011a; Flouris & Schlader 2015), although Ely et al. (2009), suggest this may to be to a lesser extent than the decrement in $\dot{V}O_{2max}$, given the magnitude of $\dot{V}O_{2max}$ impairment. However, relative intensity and perceived thermal strain alone cannot fully explain the differences between HA and PC, given the different starting speeds. This could reflect a lower training status of the current cohort of runners, who began trials with a predetermined even-paced strategy, in comparison to the highly experienced cyclists in the study of Racinais et al. (2015b) who maintained a fixed relative intensity (%$\dot{V}O_{2max}$) from the start of the trial. Alternatively, a naivety of the optimum pacing strategy following precooling would also seem plausible, given the marked reduction in T_{SKIN} that persists through the first half of the trial, which will differ from the afferent feedback participants are accustomed to, and accordingly interpret, to determine self-selected running speed in the heat (Flouris & Schlader 2015). Indeed, anecdotally, participants highlighted ambiguity about how to maximise performance in PC, reinforcing the notion that pacing must be practiced in advance of adopting PC in competition. Therefore, in conclusion, these data

would appear to be the first to demonstrate a marked advantage from STHA over acute precooling prior to endurance running.

Despite the reduced performance compared with HA, these data reaffirm the potential for mixed methods, pre cooling to benefit endurance performance in the heat when heat acclimation is not possible. Although the use of mixed methods cooling is well supported (Bongers et al. 2015; Tyler et al. 2015), this appears to be the first time this particular aggressive and practical technique has been assessed during free-paced endurance exercise in the heat. Despite suggestions that a lighter body mass may limit the effectiveness of precooling, due to the amount of recruited muscle mass (Castle et al. 2011), these data demonstrate benefits from precooling in individuals of similar body mass to those of Castle et al. (2011) (~70 kg), potentially due to a greater thermal strain in running versus intermittent cycling. As per previous research (Duffield et al. 2009) and in Study 2 (Chapter 5), PC did not elicit a reduction in T_{CORE} during the cooling phase. Similarly, an 'after-drop' was not observed, whereby vasoconstriction dissipates and warm blood is subsequently cooled in the periphery (Webb 1986), which is likely a result of the high metabolic heat production from the exercise intensity during treadmill running. A reduced rate of T_{CORE} increase may be inferred, given similar response to CON, but at higher running speeds.

It should be acknowledged that the lack of suitable air-flow, as might be experienced outdoors, may over-estimate the magnitude of the reported PC effect (Morrison et al. 2014), although the influence will be less severe than in cycling due to the reduced air velocity during running. Another potential limitation of this design is the failure to counterbalance the order of the pre and post training time trials, therefore the magnitude of improvement may be exaggerated. However, when compared against the typical error of 16 s (1.2%) following 5 days high intensity normothermic training as in Study 4 (Chapter 7), the reported improvements all appear to represent true differences.

In conclusion, these results suggest athletes and coaches should prioritise a HA strategy where possible prior to endurance exercise in the heat. When this is not possible, a precooling strategy that provides a large cooling impulse would appear to remain beneficial, although time should be taken to familiarise with pacing strategies. Combined HA and PC appears to elicit a better maintained core:skin gradient, which did not transfer into improved time trial performance. Future investigations should consider familiarising individuals with HA+PC to ensure pacing strategies maximise the alleviation of physiological strain.

9 Efficacy of the determinants of endurance performance for predicting time trial performance in the heat

9.1 Introduction

In addition to investigating a range of interventions that seek to improve endurance performance in the heat, a secondary aim of the thesis was to characterise the physiology underpinning endurance running performance in the heat better. This not only facilitates a more informed application of interventions, but also aids the prediction of performance and informs training prioritisation. This Chapter will present a retrospective analysis investigating the relationship between the determinants of endurance performance and 5 km treadmill time trial performance, when the determinants are measured in both hot (32°C, 60%) and cool (13°C, 50%) conditions. Therein, the findings of this analysis will help to characterise endurance performance in the heat better with regard to the role of the physiological determinants in producing a free-paced performance under heat stress, and may reinforce, or question, the appropriateness of utilising the traditional determinants of endurance performance model for research on endurance running in the heat.

The following questions were identified:

- What percentage of the variation of 5 km running performance in the heat amongst heterogeneous runners can be explained by the determinants of endurance performance when measured in the heat?
- What percentage of the variation of 5 km running performance in the heat amongst heterogeneous runners can be explained by the determinants of endurance performance when measured in the cool?
- What is the strongest single predictor of endurance performance in the heat?

9.2 Methods

Data were taken from the hot and cool GXTs in Study 4 (Chapter 7), following familiarisation, but prior to training. Data were pooled from both the heat acclimation and control groups (n=17). Linear regression was adopted in order to determine the best predictor of time trial performance from the LT, LTP, RE, $\dot{V}O_{2max}$ and $v\dot{V}O_{2max}$. Tests for normality were conducted and indicated the 5 km time trial and all respective predictor variables from both

hot and cool conditions to be normally distributed. All analysis was conducted using SPSS (Version 20, SPSS Inc, Illinois, USA). As stated in Study 4 (Chapter 7), technical faults resulted in no data for one measure of both $\dot{V}O_{2max}$ and RE (different individuals). Consequently, correlations are derived from n=17 for LT & LTP, n=16 for $\dot{V}O_{2max}$ & RE and n=15 for $v\dot{V}O_{2max}$.

9.3 Results

Significant linear regression relationships were observed for LT, LTP, $\dot{V}O_{2max}$ and $v\dot{V}O_{2max}$ versus time trial performance when measured in the heat, but not RE, as shown in Table 31 below. Therefore, all variables aside of RE, were entered into a full model (standard) multiple regression, revealing a significant relationship with time trial performance (F=6.508, p=0.008, R^2=0.72, standard error of the estimate [SEE]=105.6 s). The model revealed the following formula for predicting 5 km performance based on measures derived from a GXT in the heat.

5 km time (s) = 2352.608 + (-2.377*$\dot{V}O_{2max}$) + (-48.629*$v\dot{V}O_{2max}$) + (-69.266*LT) + (57.706*LTP).

Although the full model multiple regression approach does not quantify the precise variance explained by each independent variable as a stepwise approach would, the full model revealed the strongest relationship with time trial performance. Indeed, a stepwise approach, which accepts or excludes additional variables into the model that improve the prediction (probably of F to enter ≤0.050 and to remove ≥0.100), revealed a model whereby only $v\dot{V}O_{2max}$ was accepted into the model (F=29.27, p<0.001, R^2=0.69, SEE=97.5 s).

When measured in the cool, significant relationships with time trial performance were observed for all variables; LT, LTP, RE, $\dot{V}O_{2max}$ and $v\dot{V}O_{2max}$, as shown in Table 30 below. Entering the same variables as per the hot condition (excluding RE) into a full model multiple regression revealed a significant relationship with time trial performance (F=11.396, p=0.001, R^2=0.82, SEE=85 s). However, adding RE to these variables further improved the model (F=11.465, p=0.001, R^2=0.86, SEE=77.8 s). The model revealed the following formula for predicting 5 km performance based on measures derived from the cool GXT.

5 km time (s) = -220.569 + (-31.288*$\dot{V}O_{2max}$) + (40.994*$v\dot{V}O_{2max}$) + (-43.484*LT) + (61.911*LTP) + (11.117*RE).

As with predictions from the hot conditions, this model explained time trial performance better than a stepwise model, whereby again only $v\dot{V}O_{2max}$ was accepted (F=58.10, p<0.001, R^2=0.82, SEE=75.1 s) into the prediction equation.

Table 30: Correlations (*r*) between the determinants of endurance performance and time trial performance in existing literature and from this thesis (shown in bold). Where fixed blood lactate concentrations other than 2 & 4 mMol.L^{-1} have been used, the respective value is stated below. 'INFL' represents determination of the LT or LTP based on an inflection point, where blood lactate concentration exceeds steady state (LT) or demonstrates an exponential increase

Study	n	Distance (km)	$\dot{V}O_{2max}$	RE	$v\dot{V}O_{2max}$	LT	LTP
McLaughlin et al. 2010	17	16	-0.91	0.81	-0.97	-0.85	-0.89 3 mMol.L^{-1}
Jones & Doust 1998	13	8	-0.69	-	-0.93	-0.93 (INFL)	-0.81
Noakes et al. 1990	43	10	-0.55	0.41	-0.94 (PTV)	-	-0.91 (INFL)
Stratton et al. 2008	39	5	-0.55	0.20	-0.89 (PTV)	-0.73 (INFL)	-
Morgan et al. 1989	13	10	-0.55	0.30	-0.78	-	-0.85 -
Yoshida et al. 1993	57	3	-0.51	0.24	-0.75	-0.77 (INFL)	-0.60
This thesis (hot)	**17**	**5**	**-0.70** r^2**=0.49** p**=0.003**	**0.36** r^2**=0.11** p**=0.205**	**-0.83** r^2**=0.69** p**<0.001**	**-0.77** r^2**=0.59** p**<0.001**	**-0.78** r^2**=0.62** p**<0.001**
This thesis (cool)	**17**	**5**	**-0.67** r^2**=0.46** p**=0.004**	**0.62** r^2**=0.38** p**=0.011**	**-0.90** r^2**=0.82** p**<0.001**	**-0.79** r^2**=0.63** p**<0.001**	**-0.80** r^2**=0.64** p**<0.001**

(LTP).

9.4 Discussion

When measured in the heat, $v\dot{V}O_{2max}$, $\dot{V}O_{2max}$, LT and LTP accounted for 72% of the total variance in 5 km performance. Therefore, these relationships indicate endurance performance in the heat is still underpinned by the traditional physiological determinants. However, the strength of the overall model reveals greater unexplained variance than has previously been reported between the relationship between laboratory variables and 16 km running performance (R^2=0.978, McLaughlin et al. 2010). However it should be acknowledged that there is likely a greater anaerobic contribution during a 5 km time trial rather than 16 km

(Londeree 1986), whilst the study of McLaughlin et al. (2010) also involved over-ground, rather than treadmill running. Indeed, as shown in Table 30 (above), the strength of the relationship between individual predictors and endurance performance is also lower than previously reported, indicating other factors elicit a greater influence on endurance performance in the heat. Finally, the SEE of the derived equations is of sufficient magnitude that predictions would not be meaningful, given the typical variation observed in this population during time trial performance in Study 4 (Chapter 7). Therefore, the traditional determinants of performance may be considered prerequisites for success in the heat. However, there is far greater unexplained variance than in normothermic conditions, indicating thermal specific factors also influence performance. It should also be considered that these data are only specific to 5 km, as for example, RE may exert a greater influence during longer events in the heat (Saunders et al. 2004a), whilst the decrement to $\dot{V}O_{2max}$ would progressively increase (Racinais et al. 2015b). Furthermore, these relationships are derived from treadmill running, which has been modified to replicate the increased energy expenditure of outdoor running (Jones & Doust 1996), but may remain insensitive to small, intuitive changes in running speed (St Clair Gibson et al. 2006).

It is interesting to observe the apparently stronger model from the variables measured in cool conditions (R^2 hot = 0.72, R^2 cool = 0.82), indicating time trial performance in the heat is not as closely associated to the physiological responses during a GXT in the heat, as when the determinants are assessed in the cool. One explanation for this may be that the fixed exercise intensities during the GXT not eliciting analogous decrements on the determinants across participants, possibly because of differences between individuals in terms of body composition (Cramer & Jay 2015) and/or training status (Jay et al. 2011). The differences between hot and cool GXTs cannot be explained by the addition of RE to the model, because whilst RE strengthens the prediction of performance, the smaller model derived from $\dot{V}O_{2max}$, $v\dot{V}O_{2max}$, LT and LTP already explained a greater proportion of variability and demonstrated a lower SEE when measured in the cool.

Figure 56: Relationships between the determinants of endurance performance when measured in a hot environment and 5 km time trial performance in the heat. A- $\dot{V}O_{2max}$, B - $v\dot{V}O_{2max}$, C – Lactate Threshold, D – Lactate Turnpoint, E – Running economy.

There is generally considered to be a strong inverse relationship between $\dot{V}O_{2max}$ and performance (r=-0.81-0.91, Brandon 1995; Costill et al. 1973; Farrell et al. 1979; McLaughlin et al. 2010, Noakes et al. 1990). As shown in Figure 56, the relationship between $\dot{V}O_{2max}$ and performance in the heat is weaker than these reported values, but very similar to other studies utilising individuals who display both heterogeneous $\dot{V}O_{2max}$ and running performance (Stratton et al. 2008; Yoshida et al. 1993), as shown in Table 30. Appropriately, given the -7.7%

impairment in $\dot{V}O_{2max}$ under heat stress observed in Study 4 (Chapter 7), $\dot{V}O_{2max}$ in the heat demonstrated a slightly stronger relationship than when measured in the cool. A high $\dot{V}O_{2max}$ has long been considered a pre-requisite for elite endurance performance, not least as a consequence of the estimated energy requirements necessary to sustain running velocities observed in high level competition (Bassett & Howley 1997). However, the strength of the relationship between $\dot{V}O_{2max}$ and performance may be weaker within elite athletes of similar $\dot{V}O_{2max}$ (Morgan et al. 1989). Notwithstanding, at sub-elite levels, such as the cohort within this thesis, certain individuals may be genetically predisposed to demonstrate a high $\dot{V}O_{2max}$ (Bouchard et al. 1999), but yet produce slower running performance due to a reduced training regularity or an inability to maintain an appropriate fractional utilisation of $\dot{V}O_{2max}$. Whilst variation in training status, and the relatively small sample size may help to explain the weakened predictive power of $\dot{V}O_{2max}$, it is also likely that specific thermal factors exert a greater influence on performance, given the improvements in $\dot{V}O_{2max}$ in the control group of Study 4 (Chapter 7), but no improved time trial performance.

Running economy data, derived from a hot, incremental exercise test, revealed no relationship with endurance performance (R^2=0.11, p=0.205), unlike in cool conditions (R^2=0.38, p=0.011), which could indicate the assessment of RE during a hot incremental test to be inappropriate. As discussed in Study 4 (Chapter 7), the influence of heat stress on RE is equivocal, which may be a consequence of differences in exercise protocols and progressive onset of heat strain and associated thermoregulatory responses. The GXT, starting at relatively low intensities, may therefore initially afford a benefit to RE through a more efficient muscle (De Ruiter & De Haan 2000), which is in contrast to the time trial that demonstrates a high, sustained, intensity from the outset (~94% maximum heart rate). Consequently, despite being matched for heat stress, the time trial results in a faster and heightened thermoregulatory response, relative to the GXT. Accordingly, a stronger relationship was observed with the RE measured in cool conditions, but the relationship remains relatively weak, reaffirming RE to be a poor predictor of running performance of 5 km or similar (Yoshida et al. 1993, Stratton et al. 2008). This is unsurprising, given that RE is most related to longer distance events than 5 km (Londeree 1986) and would appear to exert the greatest influence within a group of athletes with relatively homogenous $\dot{V}O_{2max}$ (Morgan et al. 1989), which was not the case in this sample ($\dot{V}O_{2max}$ range 51-75 mL.kg.$^{-1}$min.$^{-1}$).

Figure 57: Relationships between the determinants of endurance performance when measured in a cool environment and 5 km time trial performance in the heat. A- $\dot{V}O_{2max}$, B - $v\dot{V}O_{2max}$, C – Lactate Threshold, D – Lactate Turnpoint, E – running economy.

The relationships between endurance performance and both LT and LTP are stronger than those for $\dot{V}O_{2max}$ or RE, but remain weaker than previous research in normothermic conditions, as shown in Table 30. Furthermore, it is notable that the relationships are also weaker than those reported by Lorenzo et al. (2011), who conducted a comprehensive assessment of a range of blood and ventilatory methods of assessing the aerobic-anaerobic transition, both in hot and cool conditions. Lorenzo et al. (2011) demonstrated relationships of r= 0.87, 0.86 and

0.86 for the lactate inflection point (increase >0.2 mMol.L^{-1}), 1 mMol.L^{-1} and 4 mMol.L^{-1} predicating performance in very hot (38°C, 30%) conditions respectively and r= 0.89, 0.91 and 0.91 for the lactate inflection point, 1 mMol.L^{-1} and 4 mMol.L^{-1} in cool conditions (13°C, 30%), ultimately recommending thresholds to be assessed in the respective environmental conditions the performance will take place in. The current data may therefore, indicate a lessened ability of blood lactate indices to predict performance in shorter, endurance running events, that are completed at a greater exercise intensity to the 1 hour cycling time trial adopted by Lorenzo et al. (2011). This may reflect both the higher intensity of 5 km running, which is thought to represent 94-98% of $\dot{V}O_{2max}$ in elite athletes (Londeree 1986) as well as the shorter duration of the protocol, which lasted 23 min on average in the heat. The training status of participants should also be considered, as Lorenzo et al. (2011) used a highly trained cohort, who typically display a less varied response than lesser trained athletes (Malcata & Hopkins 2014), whilst Lorenzo et al. (2011) also pre-warmed individuals for 30 min to elevate T_{CORE} in a controlled manner prior to exercise. The weakness of the relationships between blood-based markers and endurance performance again alludes to alternative factors influencing performance outside of the traditional model in this time trial.

As a stepwise approach did not yield the best predicting model, the relative contribution of each variable must be inferred from the respective coefficient of determination (R^2) for each variable. This indicates the strongest predictor to be $v\dot{V}O_{2max}$, reaffirmed by this being the only variable accepted into stepwise models of regression, both in the heat and in the cool. This observation is in broad agreement with previous literature that suggests $v\dot{V}O_{2max}$ to be the best predictor of endurance performance across a range of distances, as shown in Table 30. Despite the limitations of the relationship between RE and endurance performance, $v\dot{V}O_{2max}$ remains the strongest predictor, both when derived from hot and cool GXTs. This observation reaffirms the importance of training the parameters that determine $v\dot{V}O_{2max}$ for improving 5 km performance in the heat. Accordingly, Morgan et al. (1989) has highlighted the intuitive notion that a direct link may exist between $v\dot{V}O_{2max}$ and determinants of blood lactate indices such as capillary density, fibre-type distribution, respiratory capacity and muscle enzyme activity, suggesting that training of either $\dot{V}O_{2max}$, RE or lactate thresholds may provide mutual benefits.

10 General Discussion

10.1 Thesis aims

This thesis sought to optimise preparation strategies for endurance runners competing in a hot environment. Both the multidisciplinary approach, as well as the adopted exercise protocols, are novel in the context of evaluating endurance performance in the heat. Experimental chapters incorporated both short and long-term strategies, and consideration was not limited to thermal interventions, as shown by the inclusion of ischaemic preconditioning. A multidisciplinary intervention approach to alleviating the detrimental consequences of heat strain would seem appropriate given the multidimensional nature of the cardiovascular, metabolic, neuromuscular and perceptual perturbations that collectively impair endurance performance in the heat (Nybo et al. 2014). Moreover, endurance performance is typically replicated through time trial or time to exhaustion trials, that evaluate either free-paced exercise performance or the underpinning physiological mechanisms respectively, but not both (Stevens & Dascombe 2015). Therefore, this thesis utilised a novel, holistic approach involving a well-established incremental exercise protocol (Jones 2006a, Saunders & Green 2013) to encapsulate both the physiological responses and yield a valid estimate of performance within a single test (Bassett & Howley 2000; Denadai et al. 2004; Joyner & Coyle 2008), in order to optimise interventions that athletes may wish to adopt.

This thesis also investigated the association between traditional markers of endurance physiology; $\dot{V}O_{2max}$, running economy and blood lactate indices, and 5 km running performance in a hot environment. Previous research indicates the lactate turnpoint remains a strong predictor of endurance cycling performance in both hot and cold environments (Lorenzo et al. 2011) and that pacing is maintained as a relative percentage of $\dot{V}O_{2max}$ during a 43 km cycling time trial across different environmental conditions (Périard & Racinais 2015), indicating this to be a sensitive and valid model for holistically evaluating endurance performance under heat stress. However, these markers and their utility, have yet to be assessed individually, or as a model, during running in the heat. The relative dearth of research on endurance running in the heat, compared with cycling, may reflect the difficulties presented by the greater metabolic heat production in running, given the prevalence of ethical institutional T_{CORE} termination boundaries that persist, despite routinely being exceeded when exercising in the field (Nybo & Gonzalez-Alonso 2015). Therefore, there was significant novelty in the exercise modality used, as it was unclear if previous effects from interventions such as precooling and heat acclimation observed in cycling, transfer to running.

The first investigation (Chapter 4) was conducted to identify a reliable and valid measure of T_{SKIN}, that could be adopted for subsequent studies, highlighting the need for a robust measure that did not suffer from long, trailing wires. Experiments then focussed on acute interventions, providing novel insights into the effectiveness of cooling internally versus externally, as well as whether previously observed benefits of ischaemic preconditioning transferred to a hot environment. Acute interventions were selected based upon published evidence for influencing endurance performance, either in hot or normothermic conditions, as well as the practicality for use at a competition. Study 4 (Chapter 7) introduced a chronic approach, adopting a simple and novel method of exercise prescription within a widely utilised short term heat acclimation (STHA) model, in order to quantify the effect of STHA on the determinants and time trial performance. Finally, Study 5 (Chapter 8) provided a direct comparison of the best supported acute technique versus both heat acclimation and a combined strategy. This reflected the relative dearth of direct comparisons between acute and chronic interventions, and will inform athletes and coaches of the worthiness of completing time-consuming and expensive acclimation or acclimatisation, compared with a cooling manoeuvre in the 30 min prior to performance.

This General Discussion will firstly summarise the findings of individual experimental chapters, before discussing the efficacy of the adopted interventions for enhancing the determinants of endurance performance in the heat. A retrospective analysis will then be presented, exploring the factors that best characterise and predict endurance running in the heat. Based on these conclusions, practical recommendations arising from the thesis will be provided, as well as acknowledging the limitations of the work within this thesis and areas upon which future research can extend.

10.2 Summary of findings

Study 1 (Chapter 4) investigated the validity and reliability of T_{SKIN} measurements using hard-wired thermistors, a telemetry thermistor system and thermal camera. Relative to hard-wired thermistors, telemetry thermistors demonstrated good validity (typical error [TE] = 0.18°C) and reliability (TE = 0.31°C) throughout rest and exercise. Poor validity (TE = 0.35°C) and reliability (TE = 0.52°C) was observed for a thermal camera, indicating use may be limited to controlled, static situations as it was inappropriate for safe monitoring of T_{SKIN} at rest and during exercise testing in the heat. Based on waterbath and exercising comparisons, telemetry thermistors appear more valid and reliable than the traditional criterion measure of hard wired thermistors, and are more convenient for treadmill running. Consequently, a telemetry thermistor system was validated for measuring T_{SKIN} in the heat throughout subsequent studies.

Study 2 (Chapter 5) compared internal (ice slurry ingestion) and external (multiple cooling garments) precooling strategies on the determinants of endurance performance in the heat. Neither technique influenced $\dot{V}O_{2max}$, however both techniques demonstrated modest effects on blood lactate concentration, which may help prevent impairments of LT and LTP in the heat. This may occur through a reduction in body temperature preserving splanchnic circulation, thereby maintaining pyruvate conversion in the liver. An independent effect on lactate removal was supported by the maintenance of oxygen consumption at the muscle, based on RE, as well as the unchanged RER. Changes in blood lactate concentration were at the upper end of day-to-day variation, but may be more pronounced under greater heat strain. Internal cooling did not elicit greater effects than external cooling. However, external cooling demonstrated greater effects on T_{SKIN}, PSI and thermal sensation. Finally, the rate of change in T_{CORE} was reduced in external cooling, compared with internal, alluding to an increased core:skin gradient.

Study 3 (Chapter 6) involved the second investigation of acute methods. Ischaemic preconditioning (IP) may afford an ergogenic effect independently of alleviating thermal strain, through haemodynamic (Bailey et al. 2012b) and/or metabolic mechanisms (Andreas et al. 2011). Ischaemic preconditioning did not proffer any benefit across the determinants of endurance performance in the heat, with metabolic alterations under heat stress apparently insensitive to IP signalling. Furthermore, IP did not influence deep muscle temperature, but elicited a modest influence on T_{CORE} (-0.1°C) during subsequent exercise. Following Study 3 (Chapter 6), of the three acute interventions investigated, external precooling was considered the most effective and most suitable to complement a chronic heat-alleviating strategy.

Study 4 (Chapter 7) investigated the effect of a STHA strategy on the determinants of endurance performance and 5 km performance. Both hot and temperate GXTs indicated the decrement across the determinants afforded by heat stress, to which intervention's effects were interpreted. Compared with cool conditions, heat stress impaired $\dot{V}O_{2max}$ (-7.7%), LT (-4.4%), LTP (-4.8%), $v\dot{V}O_{2max}$ (-4%) and running time during GXT 2 (-19%), but improved RE (5.3%). A control group (CON) was adopted to differentiate between the contributions of thermal and exertional strain during STHA on the subsequent exercise performances. The independent effect of thermal strain, above that of normothermic high intensity training, accounted for improvements in running time to exhaustion (STHA 20.8%, CON 9.8%), TT performance (STHA 6.2%, CON 0.6%) and $\dot{V}O_{2max}$ (STHA 7.3%, CON 3.8%). Heat acclimation was attained through high intensity cycling (87% maximum HR), prescribed relative to body mass (2.7 W.kg^{-1}), which maintains thermal strain both between and within individuals. The disparity

between the respective improvements determinants of endurance performance and time trial performance indicates specific thermal are necessary in order to improve performance.

Study 5 (Chapter 8) provided a direct comparison of precooling (acute), heat acclimation (chronic) and a combined (HA+PC) intervention during 5 km time trial performance in the heat. The adopted interventions addressed previous concerns that previous cooling techniques may have been insufficient (Schmit et al. 2015), that precooling may be unnecessary following long term heat acclimation (Castle et al. 2011). However, external precooling provided no further benefit to performance following acclimation, despite modest alleviation of physiological strain. No statistical difference was observed in 5 km performance following precooling and heat acclimation (STHA +6.6%, precooling +3.7%) however this likely represented a meaningful improvement following STHA. individuals should prioritise a HA strategy, however when this is not possible, precooling remains beneficial. Combined HA and PC appears to elicit a better maintained core:skin gradient, which did not transfer into improved time trial performance. Consideration should be given to familiarising with pacing following precooling alone and when following acclimation.

Study 6 (Chapter 9) presented a retrospective analysis of how the determinants of endurance performance predict 5 km time trial performance in the heat, both when measured in the heat and in cool conditions. Multiple, linear regression indicated the physiological determinants of endurance performance do not accurately predict time trial performance when measured in the heat (R^2=0.72, standard error of the estimate =105.6 s), or in the cool (R^2=0.86, standard error of the estimate =77.8 s).

10.3 Efficacy of acute and chronic interventions for enhancing the determinants of endurance performance

Traditionally, the determinants of endurance performance may be enhanced through chronic (>4 weeks) endurance training (Midgely et al. 2007), with training volume, intensity and frequency prescribed specific to an individual's needs and the event in question. This is befitting with training programmes that routinely overload specific physiological mechanisms in order to exceed a critical intensity to maintain chronic adaptation (Hellebrandt & Houtz 1956). However, as an individual's training status improves following a large volume of training, diminishing returns may be observed, resulting in a plateau of adaptation (Midgley et al. 2007). This complicates interpreting the *true* response of training when the training history of a cohort is unknown or heterogeneous, but also supports the investigation of alternative interventions

to which individuals may not be desensitised to, or at a plateau phase, in order to influence the underpinning physiology of a specific determinant. In the following section, the efficacy of the adopted interventions within this thesis will be discussed relative to their influence on each individual determinant of endurance performance in the heat, and how this compares to improvements that are often observed from traditional endurance training. An overview of the effect of all interventions on the determinants of endurance performance is provided below in Table 31.

Table 31: Improvements in dependent variables derived from graded exercise tests from interventions investigated in this thesis. All data are presented as means (±SD) and represent within group changes, relative to the control condition of the respective chapter.

	LT	LTP	RE	$\dot{V}O_{2max}$	$v\dot{V}O_{2max}$	Running time GXT 2
Internal precooling	+2.1 (2.7) % d_{av}= 0.22	+1.2 (1.4) % d_{av}= 0.16	-1.5 (6.7) % d_{av}= 0.23	+0.7 (8.7) % d_{av}= 0.05	+0.2 (7.4) % d_{av}= 0.02	+5.0 (18.6) % d_{av}= 0.11
External precooling	+2.2 (2.8) % d_{av}= 0.23	+1.1 (1.9) % d_{av}= 0.15	-1.3 (7.1) % d_{av}= 0.17	+2.3 (8.1) % d_{av}= 0.23	+3.2 (10.9) % d_{av}= 0.26	+6.4 (21.4) % d_{av}= 0.17
Ischaemic preconditioning	-0.4 (4.4) % d_{av}= 0.04	+0.3 (3.0) % d_{av}= 0.02	+1.4 (3.0) % d_{av}= 0.20	+1.1 (3.9) % d_{av}= 0.17	+2.6 (3.4) % d_{av}= 0.33	+10.0 (21.4) % d_{av}= 0.58
Heat acclimation	+4.0 (6.0) % d_{av}= 0.24	+2.5 (2.9) % d_{av}= 0.20	-3.5 (3.5) % d_{av}= 0.59	+7.3 (4.0) % d_{av}= 0.47	+3.5 (5.3) % d_{av}= 0.24	+20.8 (12.7) % d_{av}= 2.09

10.3.1 $\dot{V}O_{2max}$

The single most important physiological characteristic for an endurance athlete is purported to be $\dot{V}O_{2max}$ (Saltin 1967). $\dot{V}O_{2max}$ represents the functional capacity of the cardiovascular system to oxygenate the blood, as well as transport and consume oxygen at the muscle, during maximal exercise that utilises a large muscle mass (Bassett & Howley 2000). A plateau in oxygen consumption during a GXT is often, but not always, observed (Edvardsen et al. 2014), as venous return becomes insufficient to maintain cardiac output and oxygen delivery to sustain exercise (Cerretelli & Di Prampero 1987). The increase in cutaneous blood flow under heat stress exacerbates exertional cardiovascular strain, typically impairing $\dot{V}O_{2max}$ by 5-20% (Nybo et al. 2014), whilst an impairment of -7.7% was observed in Study 4 (Chapter 7). Within this thesis, all interventions displayed the potential to mediate the primary systemic limitation to $\dot{V}O_{2max}$, albeit via different approaches. Precooling affords individuals a reduced core or peripheral body temperature, thus reducing skin blood flow requirements (Price & Maley

2015). Accordingly, this would be expected to maintain cardiac output and mean arterial pressure for longer, in accordance with the maintenance of $\dot{V}O_{2max}$ in thermo-neutral, versus hot environments (Périard et al. 2011). The underpinning mechanisms of ischaemic preconditioning remain a source of contention (Salvador et al. 2015; Marocolo et al. 2015; Sharma et al. 2015; Barbosa et al. 2015) however, previous improvements in maximal aerobic performance have lead authors to propose an improved muscular blood flow of peripheral vasculature arising from nitric oxide release (Bailey et al. 2012b). From a chronic perspective, heat acclimation is well supported for maintaining $\dot{V}O_{2max}$ in the heat through plasma volume expansion (Sawka et al. 1985, Lorenzo et al. 2010), whilst medium and long-term programmes may also induce further localised cardiac adaptations such as a decreased myocardial $\dot{V}O_2$ (Horowitz et al., 1986).

However, despite these potential mechanisms, no acute interventions elicited an enhanced $\dot{V}O_{2max}$. In Study 2 (Chapter 5) it should be noted that thermometric effects of both cooling had likely dissipated during GXT 2 and thus at the point of $\dot{V}O_{2max}$, supported by the similarity in T_{CORE} and T_{SKIN} across cooled and non-cooled trials, thereby reinforcing the transient nature of cooling. Therefore, the independent effect, or indeed the lack of effect of precooling techniques on $\dot{V}O_{2max}$ cannot be discerned from these data. Despite differences in exercise intensities, the time-course of the adopted protocol is not dissimilar to athletic events whereby a precooling intervention would occur prior to a warmup, before a competition of 24-32 min of exercise, which may culminate in an all-out end-spurt. Therefore, whilst a greater cooling impulse and/or a shorter duration protocol may reveal an influence on $\dot{V}O_{2max}$, the ecological validity of assessing $\dot{V}O_{2max}$ and precooling in isolation is questionable, given the effects will remain transient in competition. Whilst precooling techniques did not enhance $\dot{V}O_{2max}$, they did prolong running during GXT 2 by ~5%, which is in keeping with previous evidence indicating precooling may continue to influence exercise performance after thermometric effects have dissipated, due to a reduced perceived strain (Duffield et al. 2010). Therefore, precooling may increase the propensity to elicit a $\dot{V}O_{2max}$, rather than $\dot{V}O_{2peak}$, given the necessity to be highly motivated to achieve $\dot{V}O_{2max}$, which is likely heightened under such extreme environmental conditions (Levine 2008), although this cannot be discerned from the data of Study 2 (Chapter 5) due to the shorter data averaging procedure adopted.

In Study 3 (Chapter 6), the apparent insensitivity of $\dot{V}O_{2max}$ under heat stress to IP was consistent with the submaximal blood lactate and oxygen uptake responses in the heat, but also reflects the wider literature and the variability within $\dot{V}O_{2max}$ effects (Marocolo et al. 2015). Methodological differences between studies prevent synthesis of results, as the relatively small

number of studies conducted on ischaemic preconditioning and exercise performance vary with regard to; the time of exercise following preconditioning, the type of placebo/control condition, information provided to participants, exercise mode, cohort training status and data analysis techniques (Salvador et al. 2015). Notwithstanding the lack of consensus, the data of Study 3 indicates the influence of heat stress to supersede the purported mechanisms that may enhance $\dot{V}O_{2max}$. Consequently, the acute interventions within this thesis are not well-supported for enhancing or maintaining $\dot{V}O_{2max}$ under heat stress.

From a chronic perspective however, STHA afforded consistent and meaningful increases in the heat for all but one participant for $\dot{V}O_{2max.}$ (+7.3%, d=0.47), such that no difference was observed relative to $\dot{V}O_{2max}$ under cool conditions. Although $\dot{V}O_{2max}$ in the control group increased by 3.8% (d=0.30), this did not constitute a meaningful change (>2 mL.kg^{-1}.min^{-1}, Tanner & Gore 2013) and remained lower than $\dot{V}O_{2max}$ in the cool (d=0.56). However, it should be acknowledged that these inferences are made in the absence of a Group*Time interaction effect for $\dot{V}O_{2max}$, likely reflecting the small sample of the control group (n=7) and within the STHA group for this measure (n=8). Hypervolaemia is advocated as the primary mechanism facilitating an increased $\dot{V}O_{2max}$ following heat acclimation within this timescale (Sawka et al. 2011). Plasma volume expansion of 5.7% was observed following STHA, indicating an enhanced delivery of haemoglobin carrying O_2 to the target tissue as a consequence of increased stroke volume for matched heart rate. Alongside an increased ability to deliver oxygen to the muscle, hypervolaemia may also facilitate a reduced blood temperature, in turn promoting a leftward shift in the oxyhaemoglobin saturation curve, thereby improving the oxygen carrying capacity of the blood (Barcroft & King 1909). The relatively high training intensity (~81% maximum heart rate control, ~87% maximum heart rate STHA) is associated with faster and larger improvements in $\dot{V}O_{2max}$ than moderate intensity training under normothermic conditions (<80% $\dot{V}O_{2max}$, Helgerud et al. 2007; Montero et al. 2015; Milanović et al. 2015). Therefore, high intensity training may have also contributed to the plasma volume expansion, given that haemodynamic adaptations are inducted first, following high intensity training (Montero et al. 2015). A stimulative role for high intensity exercise would also seem appropriate following recent evidence suggesting permissive dehydration may not always elicit plasma volume expansion following similar STHA protocols (Neal et al. 2015). Whilst high intensity training may enhance $\dot{V}O_{2max}$ through a multitude of adaptations aside of plasma volume expansion (Bacon et al. 2013), many of these adaptations would not be expected to present within this timescale or from the current exercise stimulus. For example, cardiac factors such as increased left ventricular size, myocardial contractility and end-diastolic volume, that collectively increase

stroke volume and greater systemic blood flow, are generally associated with at least 4 weeks of training (Jones & Carter 2000; Midgely et al. 2007) and appear to necessitate a mechanical myocardial overload that normally involves training above $\dot{V}O_{2max}$ for adaptation (Cooper 1997). Similarly, peripheral adaptations such as increased capillary density (Ingjer 1979) and mitochondrial enzyme content (Green et al. 1992) collectively improve oxygen extraction and therefore enhance the arteriovenous oxygen difference (a-$\dot{V}O_{2diff}$) component of the Fick equation, but require over eight weeks of training (Montero et al. 2015). Furthermore, the recent meta-analysis of Montero et al. (2015) indicates the strongest relationship between improvements in $\dot{V}O_{2max}$ and maximal cardiac output, rather than the a-$\dot{V}O_{2diff}$ following endurance training.

In conclusion, acute strategies do not appear to influence $\dot{V}O_{2max}$, and in the case of precooling, not within the timescale of the adopted protocol or likely competition event. Conversely, chronic heat acclimation affords adaptations that increase $\dot{V}O_{2max}$, which will likely benefit endurance performance in the heat in accordance with the traditional model (Bassett and Howley 2000).

10.3.2 Blood lactate response

Blood lactate indices, the lactate threshold (LT) and the lactate turnpoint (LTP), denote the respective initial, and exponential, increases in blood lactate concentration during incremental exercise. These markers therefore demarcate an aerobic to anaerobic transition (Faude et al. 2009), as the proportion of ATP production via anaerobic processes progressively increases to complement oxidative phosphorylation in order to meet the energetic demand of the exercise intensity. Exercise above these thresholds is associated with an increased respiratory, perceptual and metabolic strain (Jones & Carter 2000), leading to expedited fatigue and/or exercise termination from metabolic acidosis (Sahlin 1992), as well as accelerated muscle glycogen depletion, that may be accentuated during prolonged or repeated exercise in the heat (Boyd et al. 1974). The exercise intensities corresponding to blood lactate thresholds are reduced in the heat (Powers et al. 1985; Smolander et al. 1986; Lorenzo et al. 2011), in accordance with elevated body and muscle temperatures mediating a shift to glycogenolysis (Febbraio et al. 1994) and reduced splanchnic blood distribution to facilitate peripheral vasodilation (Rowell et al. 1968), but appears independent of hypohydration (Papadopoulos et al. 2008). Consequently, the decrement to the thresholds is progressive and proportional to heat strain, with reductions of 4.4% and 4.8% for LT and LTP observed within this thesis. The LTP appears to remain a valid predictor of one-hour endurance cycling performance in the heat

(*r*=0.86-0.89, Lorenzo et al. 2011), despite a 5% reduction at 38°C and 30% relative humidity. Experimental chapters revealed a relative insensitivity of the blood lactate response to acute interventions within this thesis. Small, albeit consistent, improvements in thresholds were observed in Study 4 (Chapter 7), as shown in Table 30 (above), potentially arising from reduced core or skin temperatures maintaining lactate-pyruvate conversion in the liver. Whether these effects would be enlarged following a more aggressive combined internal and external technique, or under more severe heat strain warrants consideration. Retrospective analysis of these data alludes to a relationship between ice slurry ingestion enhancing the lactate thresholds to a greater extent in individuals with greater body mass (LT; *r*=0.64, *p*=0.03, LTP; *r*=0.76, *p*=0.01) and body surface area (LT; *r*=0.60, *p*=0.05, LTP; *r*=0.63, *p*=0.04), whereas the improvements following external cooling appeared to be independent of anthropometric characteristics (all comparisons *r*=0.22-0.41, *p*>0.05). As ice slurry ingestion was delivered relative to body mass, future external cooling techniques may consider adjusting the cooling volume of external garments to cover a greater surface area for larger athletes, or for a longer duration, in order to elicit changes in lactate thresholds.

Following IP, Bailey et al. (2012a) reported a reduced increase in blood lactate concentration during a GXT, that was not replicated within a similar protocol under heat stress, in Study 3 (Chapter 6). Bailey et al. (2012a) attributed the mediated blood lactate response and prolonged time to reach OBLA (4 mMol.L^{-1}) to improvements in vascular function and muscle blood flow, that may have enhanced the removal and transport of lactate. The same authors subsequently reported the maintenance, rather than reduction, of flow-mediated dilation within the brachial artery following preconditioning (Bailey et al. 2012a), reaffirming a role for haemodynamic changes following IP. Alongside this mechanism, increased mitochondrial lactate oxidation has also been proposed to contribute to a reduced blood lactate concentration, either independently (Pang et al. 1995; Andreas et al. 2011), or concomitantly with the haemodynamic changes arising from increased endothelial nitric oxide release that attenuates sympathetic vasoconstriction (Kimura et al. 2007). Assuming an increased muscle blood flow was achieved, the data from Study 3 (Chapter 6) would appear supportive of previous observations that increased blood lactate accumulation under heat stress does not occur due to ischaemia, but may be better explained by alternative explanations, such as plasma-adrenaline levels (Febbraio 2001). It would appear that the purported underpinning mechanisms of peripheral vasodilation and/or altered mitochondrial function for reducing blood lactate concentration following IP do not supersede the influence of heat strain. Therefore, as shown in Table 30, the largest improvements occurred following STHA, of 4% and

2.5% to the LT and LTP respectively, although this was not statistically greater than the normothermic control training group, that improved LT and LTP by 3.4 and 1.8% respectively. Furthermore, both LT and LTP remained impaired in the heat, relative to cool conditions following STHA. Unlike the chronic approach of STHA, acute interventions such as precooling and IP would only afford transient blood lactate dynamics, rather than a prolonged effect that is sustained across subsequent days arising from physiological adaptation.

Notwithstanding the lack of statistical difference relative to the control group in Study 4 (Chapter 7), the improvement from STHA remains comparable to the 5% increase reported by Lorenzo et al. (2010) following a two-week long term heat acclimation programme. The physiological adaptations from STHA that enhance the thresholds may differ from those following typical endurance training (Jones & Carter 2000). STHA resulted in a reduced body temperature and hypervolaemia, both of which likely contribute to a mediated splanchnic haematic redistribution (Rowell et al. 1968), whilst a reduced body temperature may proffer reduced plasma adrenaline levels, affording a relative maintenance of fat oxidation, supported by the lower RER observed (Febbraio et al. 1994). However, these apparently thermal-related mechanisms cannot wholly account for the improvements, given the change in the control group of Study 4 (Chapter 7) following normothermic training. This indicates a contribution from alternative mechanisms, possibly due to the consistently high training intensity, an observation that is supported by recent data demonstrating no effect on the lactate threshold following 5 days of STHA and variable intensity exercise (Chalmers et al. 2016). The control training was completed at a mean intensity of ~81% of maximum heart rate, that may have recruited type 2 muscle fibres (Gollnick et al. 1974), as well as affording a consistently high blood lactate level that appears to promote adaptation in clearance mechanisms (Weltman et al. 1992). However, to what extent these non-thermal alternative mechanisms contribute is unclear, as traditionally improvements to the lactate thresholds arise following long-term training, with a 50-100% increase in the number, size and enzyme content of mitochondria, enhancing mitochondrial respiration capacity and the ability of skeletal muscle to oxidize pyruvate following weeks or months of training (Holloszy & Coyle 1984). Similarly, extracellular adaptations such as increased capillary density that increase the transit time for red blood cells to complete metabolite exchange with working muscles, are observed across similar timescales (Ingjer 1979). Despite the lack of statistical difference, the mean response of the STHA group may reflect a meaningful improvement following STHA above that of the control group, as shown below in Figure 58.

Figure 58 Top: Blood lactate response to 5 days short term heat acclimation training. Bottom: Blood lactate response to 5 days of normothermic training. X axis represents stage number during incremental running test (GXT 1).

In accordance with the determinants of endurance performance model (Bassett & Howley 2000), improvements to the thresholds will afford not only a higher sustainable fractional utilisation of $\dot{V}O_{2max}$ under heat stress, but a reduced rate of glycogen utilisation and potentially improved oxygen uptake kinetics that is less reliant on anaerobic metabolism as exercise intensity changes (Carter et al. 1999; MacRae et al. 1985).

In conclusion, acute interventions of precooling and ischaemic preconditioning afforded small or no effect on blood lactate thresholds respectively in the heat, whilst the chronic approach of STHA provided the largest effects, with these benefits arising through a combination of specific thermal and traditional endurance training adaptations.

10.3.3 Running economy

Running economy (RE), a representation of the energy demand for a given velocity of submaximal running, combines with $\dot{V}O_{2max}$ and the sustainable fractional utilisation of $\dot{V}O_{2max}$,

to determine running performance for a given distance (Foster & Lucia 2007). Appropriately, an economical runner demonstrates a lower oxygen consumption for a given velocity, utilising a lower percentage of their $\dot{V}O_{2max}$, thereby preserving fuels, minimising metabolic heat production and necessitating a smaller contribution from anaerobic metabolism (Saunders et al. 2004a; Jones 2006b). Running economy is multifactorial, combining biomechanical, physiological and neuromuscular variables (Saunders et al. 2004a; Barnes & Kilding 2015). Traditionally, improved RE has been associated with a high training volume and distance (Morgan et al. 1995), attributed to neuromuscular and gait adaptations (Cavanagh & Williams 1982) that result in improved motor unit synchronisation (Barnes & Kilding 2015). However, a dearth of longitudinal studies with appropriately controlled potential co-variates, such as training status and training intensity, confounds an accurate quantification of the strength of this relationship (Midgely et al. 2007). In keeping with the multifactorial nature of running economy, multidisciplinary acute interventions have been shown to enhance running economy. For example, resistance training increases muscle strength and the musculoskeletal unit stiffness (Barnes et al. 2013), enhancing stored elastic energy in the lower limbs, improving a runner's biomechanics. Similarly, plyometric training provides neuromuscular improvements, such as improved motor unit recruitment (Paavolainen et al. 1999). From a physiological perspective, the most common, initial adaptations that improve RE following a high training volume appear to be haematological changes, notably increased plasma volume and red blood cell mass (Sjödin et al. 1982). Subsequently, central and peripheral cardiovascular adaptations may present, such as increased stroke volume and increased capillarisation respectively, which may enhance all of the determinants of endurance performance, not just running economy (Bassett and Howley 2000).

In keeping with the multidisciplinary approach of this thesis, running economy would appear to be sensitive to improvement from a range of different perspectives, however the adopted interventions within this thesis were all orientated around the physiological parameters that influence running economy. The influence of heat stress on running economy is contentious, with both enhanced (MacDougal et al. 1974) and reduced (Rowell et al. 1967) submaximal $\dot{V}O_2$ reported in the heat. Energy demanding thermoregulatory responses and a reduction in the efficiency of oxidative phosphorylation in mitochondria may occur under heat stress (Brooks et al. 1971), however oxygen kinetics remain unchanged (Koga et al. 1997; Nybo et al. 2001; Burnley et al. 2002), avoiding complications arising from the $\dot{V}O_2$ slow component within GXT 1. Notwithstanding these divergent responses, poor running economy elicits a greater MHP and therefore risks an expedited onset of heat strain (Bergeron 2014). The mixed

results may reflect an energetic demand proportional to heat strain and the thermoregulatory responses at the time of measurement, such as sweat gland activation and hyperthermic hyperventilation (Sancheti & White 2006). Hyperventilation alone has been shown to increase $\dot{V}O_2$ by 31–50 ml (0.4-0.6 mL.kg^{-1}. min^{-1}) when ventilation is in the range of 117-147 L.min^{-1} (Aaron, Johnson, et al. 1992; Aaron, Seow, et al. 1992). However, when heat strain is modest, an additional energy demand may not be apparent and physiological perturbations may be offset by biomechanical alterations, such as a warmer muscle possessing increased mechanical efficiency (De Ruiter & De Haan 2000; Racinais & Oksa 2010). Therefore, it is interesting to note Study 4 (Chapter 7) did not reveal an impairment to running economy under heat stress, but rather a relative improvement of 5.3%, indicating any metabolic perturbations arising from ~24 min of incremental running within GXT 1 to be subordinate to the beneficial mechanical effects. This is supported by the lack of observable increase in ventilation in the heat during GXT 1, which may be a consequence of the modest differences in the rate of T_{CORE} increase between hot and cold conditions (GXT 1 finishing T_{CORE} hot 38.7°C, cool 38.5°C), given the close relationship between T_{CORE} and hyperthermic hyperventilation (Cabanac & White 1995).

Accordingly, it is unsurprising that cooling afforded no improvement to running economy in Study 2 (Chapter 5). However, given that a lowered ventilation rate has been shown to improve running economy by 3% (Franch et al. 1998), it is possible precooling would have provided a benefit under greater heat strain where a cooler body would mediate an elevation in hyperthermic hyperventilation. Despite suggestions that ischaemic preconditioning may afford reduced mitochondrial oxygen consumption as a consequence of nitric oxide release (Cooper & Brown 2008), a reduction in oxygen uptake during submaximal exercise has yet to be widely observed (De Groot et al. 2010; Crisafulli et al. 2011; Clevidence et al. 2012). The results of Study 3 (Chapter 6) are in keeping with these observations, demonstrating IP does not reduce oxygen consumption during submaximal running in the heat. From a chronic perspective, no apparent benefit from STHA was observed, with differences not statistically different from the control training group. However, the 7.3 mL.kg^{-1}.km^{-1} (3.5%, d_{av}=0.59) increase in $\dot{V}O_2$ following STHA, compared with 2.4 mL.kg^{-1}.km^{-1} (1.1%, d_{av}=0.12) following control, indicates meaningful differences in the change in running economy between training types. Indeed, an elevated submaximal $\dot{V}O_2$ would appear detrimental to endurance performance, but an increased oxygen uptake is not uncommon when $\dot{V}O_{2max}$ and/or the fractional utilisation have increased (Midgely et al. 2007), with some previous studies suggesting there may be an inverse relationship between RE and $\dot{V}O_{2max}$. Accordingly, Study 4 (Chapter 7) demonstrated increases in both $\dot{V}O_{2max}$ and the LTP, which may explain this finding.

In conclusion, running economy was unchanged following precooling and ischaemic preconditioning and apparently impaired by STHA, although this may be an artefact of improvements to the LTP and/or $\dot{V}O_{2max}$. (Pate et al. 1992; Morgan & Daniels 1994).

10.3.4 v$\dot{V}O_{2max}$

v$\dot{V}O_{2max}$ may be calculated from extrapolating the regression line of an individual's running velocity and $\dot{V}O_2$ relationship to the measured $\dot{V}O_{2max}$ (Daniels 1985) or, as appears to be more commonly adopted, by dividing an individual's $\dot{V}O_{2max}$ by the mean running economy (Jones 2006a). v$\dot{V}O_{2max}$ is a simple performance metric, easily interpretable by both athletes and coaches, and appears to be the strongest predictor of running performance at both 8 km (Jones & Doust 1998) and 16 km (McLaughlin et al. 2010). Conceptually, v$\dot{V}O_{2max}$ demonstrates how an athlete with good running economy, but the same $\dot{V}O_{2max}$ as a competitor, will run at a greater velocity as they approach maximal intensities. The strong relationship between v$\dot{V}O_{2max}$ and endurance performance represents the integration of two primary determinants of endurance performance into one variable, however running performance will also be largely influenced by the lactate turnpoint determining the fractional utilisation of $\dot{V}O_{2max}$ an individual can sustain. By combining two physiological variables, v$\dot{V}O_{2max}$ may also reveal differences that arise from subtle changes in either $\dot{V}O_{2max}$ or running economy, which individually do not appear meaningful or reach statistical significance, but combine to represent meaningful performance differences. Despite the prevalence of v$\dot{V}O_{2max}$, it does not appear to have previously been investigated under heat stress. Study 4 (Chapter 7) revealed a 4% reduction in v$\dot{V}O_{2max}$ under heat stress, which will have been predominantly determined by the reduction in $\dot{V}O_{2max}$, given the improvement to running economy afforded by the hot environment. In keeping with the lack of effects on running economy and $\dot{V}O_{2max}$, neither internal nor external cooling elicited a statistical effect on v$\dot{V}O_{2max}$. Whilst a ~3% improvement following external cooling appears notable, the response was highly variable, as demonstrated by the high standard deviation of the percentage change (10.9%). Moreover, the 0.4 km.h^{-1} change is below what appears meaningful (0.5 km.h^{-1}, Billat & Koralsztein 1996). Study 3 (Chapter 6) revealed a similar magnitude of improvement in v$\dot{V}O_{2max}$ (2.6%), which did elicit a statistically significant effect (d=0.33), however it is again unclear how meaningful this is, given the 0.4 km.h^{-1} mean difference. The effect following IP would appear to be the result of small changes in both running economy and $\dot{V}O_{2max}$ that individually are not apparent, but yield an effect when combined. In accordance with the magnitude of effects on the other determinants of endurance performance, STHA demonstrated the greatest effect on v$\dot{V}O_{2max}$, although the change in speed (0.5 km.h^{-1}) and percentage change (3.4%) remain modest. The large (-7.7%)

increase in $\dot{V}O_{2max}$ would appear to have been attenuated by the greater oxygen uptake observed following STHA.

In conclusion, despite the novel application of a range of thermal and non-thermal based interventions, no intervention yielded a meaningful and/or consistent improvement in $v\dot{V}O_{2max}$.

10.4 Unexplained variance during endurance performance in the heat

In addition to investigating a range of interventions that seek to improve endurance performance in the heat, a secondary aim of the thesis was to characterise the physiology underpinning endurance running performance in the heat better. Free-paced exercise represents the observable, behavioural consequence of collective physiological, psychological and tactical feedback. As previously discussed, under normothermic conditions, this behavioural response is highly associated with physiological markers of endurance performance (Bassett and Howley 2000). However, the findings of this thesis indicate the relationship between running performance and the overall model of the physiological determinants to be weakened when in the heat, relative to normothermic conditions (Table 30, Chapter 9), whilst improvements to these determinants following high intensity normothermic training in the control group of Study 4 (Chapter 7), did not elicit improved time trial performance in the heat. This alludes to a greater role for specific thermal factors influencing endurance performance. Considerable evidence documents the marked influence that perceived thermal strain and/or exertional strain can exert on self-selected exercise intensity, in advance of changes in body temperature, through to moderate levels of hyperthermia (Ely et al. 2010; Périard et al. 2011; Schlader, Simmons, et al. 2011b). Such adjustments to self-selected exercise intensity represent a behavioural response, and in the heat such alterations to exercise intensity are thought to reflect behavioural attempts to thermoregulate and/or alleviate perceived thermal strain (Flouris & Schlader 2015). Therefore, behavioural thermoregulation, i.e. a reduction in the self-selected running speed is a likely candidate to contribute to the unexplained variance role in determining endurance performance in the heat, and has previously been suggested to be a determinant of exercise performance under heat stress (Flouris & Schlader 2015). Alterations to the exercising intensity, and therefore metabolic rate, serve as primary defence mechanisms in clinical populations who are at risk of heat illness due to a reduced thermoregulatory ability (Nielsen 1966), or when protective clothing impairs typical heat loss mechanisms (Cheung et al. 2010). In high level athletes, the trend towards a flatter pacing profile following familiarisation to endurance exercise in the heat (Schmit, Duffield, et al. 2015), represents a form of behavioural thermoregulation as individuals seek to

avoid beginning exercise at an intensity that may yield a subsequent disadvantage due to excessive heat storage. Therein, the consequential metabolic heat production would appear to become a regulated variable and a determinant of endurance performance during exercise in a hot environment (Flouris & Schlader 2015). Such thermoregulatory practices arise from the perceived thermal strain, which can be differentiated into thermal sensation, the relative intensity of the temperature being sensed (Attia 1984) and thermal comfort, which reflects the subjective indifference with the thermal environment (Mercer 2001). Thermal comfort, and specifically discomfort, appears more strongly linked with behavioural changes than thermal sensation (Gagge et al. 1969; Schlader, Simmons, et al. 2011b). Accordingly, individuals within this thesis may have altered running speed in accordance with their perceived discomfort, rather than in accordance with the quantified physiological markers established during the GXTs.

Thermal comfort appears to be primarily driven by changes in T_{SKIN} (Flouris 2011), but as hyperthermia progresses, the influence of T_{CORE} increases (Cabanac 1971), as may the relative contribution of skin wetness (Fukazawa & Havenith 2009). Mirroring the response of thermal comfort, the holistic measure of perceived exertion, RPE, is also initially influenced by T_{SKIN}, however T_{CORE} exerts a greater influence as hyperthermia progresses. Accordingly, as T_{CORE} elevation and hyperthermia becomes more pronounced, physiological strain will influence performance to a greater extent both directly, through limiting the aerobic capacity, but also indirectly by enhancing RPE (Marino 2004; Périard et al. 2011). Therein, RPE has been suggested to be the ultimate moderator of behavioural thermoregulation, to which thermal comfort may feed into, as thermal comfort alone may be misinterpreted during strenuous exercise that is by nature, uncomfortable (Flouris & Schlader 2015). As shown below in Figure 59, a theoretical representation of this integrated response has been proposed by Flouris and Schlader (2015), demonstrating that as T_{SKIN} increases, there is a concomitant effect on both perceived strain i.e. thermal discomfort and/or RPE, and cardiovascular strain, indicating performance to be influenced by both behavioural consequences as well as systemic physiological limitations.

↓ **Exercise Work Rate**

↑ **Perceived Exertion**

Thermal Perception
(↑ warmth discomfort)

Cardiovascular Strain
(↓ peak oxygen uptake)

↑ **Ambient Temperature** → ↑ **Skin Temperature**

Core Temperature

Figure 59: Model of behavioural thermoregulation (Flouris & Schlader 2015). Perceived thermal strain and/or cardiovascular strain may predominantly mediate reductions in exercise work rate in the heat through their impact on RPE (perceived exertion). The reductions in exercise work rate are modulated predominantly via increased skin temperature and ultimately control the rate of rise in T_{CORE}.

Although RPE was measured during the GXTs, it has been shown that RPE measured during an incremental exercise test may not replicate the RPE in more prolonged exercise protocols, especially under heat stress (Potteiger & Weber 1994) and may also be influenced by prior knowledge of the protocol length, which would be apparent in a discontinuous GXT (Albertus et al. 2005; Joseph et al. 2008). Therefore, it was unsurprising to observe no differences in RPE during the GXT in Study 4 (Chapter 7) following STHA, despite the large improvement in time trial performance. However, STHA attenuated the peak thermal sensation, indicating an improved perceptual response to heat stress following HA. Thermal comfort was not measured, so any influence is inferred from changes in T_{SKIN} and/or thermal sensation. Similarly, Study 2 (Chapter 5) demonstrated marked improvements in thermal sensation following precooling techniques, whilst external cooling also dramatically lowered T_{SKIN}. However, it should be acknowledged the lack of suitable representative airflow within studies 4 and 5 (Chapters 7 & 8) may have exaggerated thermal discomfort, by preventing a sensation of cooling that may lower thermal comfort (Morrison et al. 2014), thereby overestimating the contribution of behavioural thermoregulation, relative to a similar outdoor environment. However, this

limitation is a systematic error across all experimental chapters of this thesis, and would also have served to attenuate the influence of beneficial interventions such as precooling and heat acclimation.

Whilst the model of Flouris and Schlader (2015) encapsulates the interactions between variables that determine behavioural thermoregulation responses, this model remains broad, and is not specific to the exercise type or informing of training priorities. A modified model, integrated with the known physiological determinants of endurance performance is therefore presented below, demonstrating the main factors that appear to combine to determine self-selected running speed in the heat (Figure 60).

Figure 60: Proposed theoretical model of the determining factors for self-selected running speed in the heat. The physiological determinants of endurance performance (encircled bottom right), will directly influence both the magnitude of cardiovascular strain and T_{CORE} elevation, therefore influencing perceptual strain that appears to determine running speed. Behavioural framework is modified from Flouris and Schlader (2015). Individual characteristics such as Heat Acclimation State (HA state) and previous experience may modify the relationship between self-selected running speed and physiological determinants. Novel variables added to the model based on data within this thesis are shown in *italics*.

The model demonstrates the interrelation of both the physiological strain from the exercise intensity, as well as the heightened perceptual strain, arising from the environmental conditions in determining running speed. For example, the magnitude of cardiovascular strain under heat stress may be influenced by both the fractional utilisation, as well as the elevation

of T_{SKIN} and/or T_{CORE} at a given point in time. The model demonstrates perceptual adaptation should be prioritised equally with physiological adaptation before competing in the heat. Theoretically, heat acclimation strategies adopting far greater heat stress than will be experienced during competition may be appropriate in order to facilitate greater improvements in perceived strain. The model also demonstrates how familiarisation with the competition conditions will be important to an athlete, due to the influence of prior familiarisation of a given task on RPE (Lamb et al. 1999), which may independently serve to reduce the perceived exertion and therefore enable a greater running speed. Heat acclimation state appears central to the model, given heat acclimation affords cardiovascular, metabolic, perceptual and thermoregulatory adaptations (Périard et al. 2015). Therefore, the use of heat acclimation state tests would appear important to quantify an individual's thermosensitivity. Such tests derive the linear relationship between sweat setpoint and sweat gain, in relation to changes in core temperature during submaximal exercise under a fixed heat stress (Willmott et al. 2015). The model therefore demonstrates how heat acclimation state directly influences T_{SKIN}, cardiovascular strain and perceived strain.

Although the model does not specifically define all physiological perturbations under heat stress that may influence performance, such as metabolic, respiratory and neuromuscular alterations (Nybo et al. 2014), it helps to define the priorities for variables that training should aim to improve, prior to competing in the heat. For example, the data from this thesis would indicate strategies that seek to enhance the lactate thresholds and $v\dot{V}O_{2max}$ should be prioritised. However, as when preparing for normothermic conditions, the relative importance and prioritisation of each variable will be specific to an individual's strengths and weaknesses (Jones & Carter 2000), as well as acclimation state (Gonzalez & Gagge 1976). Therefore, the model remains broad, and does not attribute relative importance to specific variables, because the relative importance of each variable will not only change based on an individual's strengths and weaknesses, such as training status, but also on the event length. Future research may consider developing specific models to inform training prioritisation for elite and non-elite individuals, as well as shorter (~5 km) and much longer (~half marathon) endurance events where the relative importance of individual variables may change.

Therefore, in conclusion, behavioural factors arising from thermal discomfort may play a greater role in determining endurance exercise performance.

10.5 Practical applications

The studies conducted within this thesis were intended to inform athletic preparation for competing in the heat and as such, many findings naturally transfer to the applied environment. The hot and humid conditions adopted appear to be above the anticipated mean heat stress expected at the 2016 Rio de Janeiro Olympics (ambient temperature; 26°C), but not disproportionate to the peak temperatures that may reach 38°C and exceed 80% relative humidity (National Institute of Meteorology Brazil 2015). Consequently, the physiological impairments observed in the heat in Study 4 (Chapter 7), relative to cool conditions, within the moderately trained cohort in this thesis, may serve as a useful index across endurance-based sports to gauge the impact of heat stress for the 2016 Olympics or other major championships with similar environmental challenges. While this thesis indicates that the determinants of endurance performance do not explain performance to the same extent in the heat as has been reported in cool conditions, it is unclear how this relationship would transfer to an elite population. Moreover, the event characteristics such as distance and duration may determine whether it is appropriate to conduct a laboratory test in representative environmental conditions, due to the potential for heat stress to afford a transient improvement to running economy that does not appear to replicate time trial exercise. Therefore, completing time trials in the heat that also encompass self-selected exercise intensity under heat stress, may be appropriate for assessing training status in competitive athletes prior to competing in the heat. When laboratory testing does take place, the best single predictor from an incremental test would appear to be $v\dot{V}O_{2max}$, measured in cool laboratory conditions. Notwithstanding the problems with predicting performance in the heat when derived from laboratory testing, the traditional determinants appear to remain prequisites, accounting for 82% of variance in time trial performance, emphasizing the importance of continuing to train these areas. However, data from this thesis also indicates individuals should prioritise a reduced perceived thermal and/or exertional strain, in order to minimise impairments arising from behavioural attempts to thermoregulate. Therefore, it would appear to be important to integrate indices of thermal comfort and RPE into athlete's training programmes in order to track improvements in these areas.

The data within this thesis also supports the traditional approach of adopting chronic heat acclimation prior to endurance running competitions. The STHA protocol used in Studies 4 and 5 (Chapters 7 & 8) would appear to be easily adopted by athletes and coaches. Totalling 5 days, it encompassed high intensity exercise (~87% heart rate maximum) to expedite the elevation of T_{CORE} and onset of sudomotor output, with such higher intensity exercise more sport specific than traditional fixed intensity training (Houmard et al. 1990; Sunderland et al. 2008). This

exercise intensity would be expected to complement, rather than compromise a training taper phase (Spilsbury et al. 2015). The prescription of exercise relative to body mass (W.kg^{-1}) negates additional visits to establish relative intensities and reduces time demands on athletes and practitioners alike. In turn, this simple prescriptive approach also facilitates the simultaneous training of multiple individuals in the heat, although care must be taken to maintain appropriate measures of T_{CORE}, which can be expensive. Although the 5 days of STHA within this thesis was effective, the length of the heat acclimation programme warrants further consideration, given the tendency for thermal sensation to continue to improve during days 5-10 of controlled hyperthermia acclimation, despite the absence of further large physiological adaptations during this same timescale (Mee et al. 2015b; Gibson et al. 2015a). Emerging evidence also suggests completing two heat exposures each day elicits greater adaptation than a single exposure, indicating an alternative approach to yield greater adaptation within a 5 day timescale (Willmott et al. 2016). However, consideration should also be given to ensure individual's recover sufficiently and do not experience a performance decrement, or suffer infection from repeated heat exposures (Gleeson 2007). To this end, acclimating in environmental conditions that far exceed the expected competition conditions, as occurred in this thesis (STHA: 40°C, 60%, Experimental trials: 32°C, 60%), may promote greater perceptual benefits, by increasing thermal discomfort, relative to acclimating under cooler conditions, even when this is an uncompensable thermal environment. Furthermore, the use of conditions involving high humidity may be appropriate, in order to enhance perceived strain, due to the potential link between thermal comfort and skin wetness (Fukazawa & Havenith 2009).

In keeping with research conducted on cycling performance (Castle et al. 2011; Brade et al. 2012; Schmit, Le Meur, et al. 2015), this thesis also demonstrates that the addition of a precooling technique to the acclimated individual does not immediately provide an additive benefit, despite an apparent initial alleviation of physiological and perceptual strain. This highlights the need for athletes to familiarise with interventions in advance of a competition, as the optimum pacing strategy when an individual is both acclimated and precooled, or indeed following acclimation or precooling alone, may be very different to pacing under normothermic conditions. The transient nature of precooling presents a tactical challenge, as theoretically it affords athlete's the ability to undertake a faster start, but this is atypical of 5 km pacing (Abbiss & Laursen 2008), and the benefits may have gone by the latter parts of a competitive race, where tactical pacing adjustments may be most important. From a practitioner perspective, it may be advisable to cool both prior to, and following the warmup, in order to maintain a lower T_{SKIN} and therefore promote improved thermal comfort, whilst concurrently

alleviating skin blood flow requirements. Finally, whilst the external precooling technique from Study 2 (Chapter 5) remains practical for use in the field, preparation will be necessary in order to ensure multiple garments remain cool.

Finally, whilst preparation and practising appear essential to implementing acclimation and precooling strategies effectively, these interventions must coexist with other practices that were not included within experimental studies of this thesis. Notably, athletes tend to adopt longer and higher intensity warm-ups (Randall et al. 2015), that may result in greater heat storage than the warm-ups induced for this thesis. Within this thesis, the warm-up was attenuated relative to current competition practices to ensure participants did not exceed the ethical T_{CORE} limit. Therefore, athletes and coaches should practice interventions in accordance with other race-day routines.

10.6 Limitations

Despite upmost caution being taken to ensure valid and robust measures were taken throughout all experimental chapters, the findings of this thesis should be considered in light of the following primary limitations:

- Neither graded exercise tests, nor time trials, were conducted with representative air flow. This will likely impair convective cooling (Morrison et al. 2014), and in turn may have enhanced thermal discomfort, relative to outdoor running. Whilst all interventions were always compared against a control condition under the same conditions, this may have influenced the relationship between physiological markers and time trial performance, relative to experiments adopting outdoor running, thus exaggerating the contribution of perceptual strain and behavioural thermoregulation adjustments.

- Throughout this thesis thermal sensation was taken as a measure of perceived thermal strain, however this may be independent of both RPE and thermal comfort (Gagge et al. 1969), which appear more closely linked to changes in behaviour. Consequently, the true relationship between thermal comfort and changes in free paced exercise cannot be quantified. However, thermal comfort is modulated through changes in skin temperature, therefore changes to thermal comfort can be inferred.

- A 5 km time was not completed in cool conditions and therefore, the efficacy of determinants of endurance performance for predicating time trial performance for this cohort outside of heat stress is unknown. Similarly, this would have informed whether the impairment to the determinants under heat stress, as observed in Study 4 (Chapter 7), was associated with an impairment to time trial performance of a similar magnitude when heat

stress was present. Therefore, inference is taken from the wealth of research that states strong relationships between these variables and endurance performance for participants of similar fitness.

- Time trial performance was assessed on a motorised treadmill which, although valid and reliable for assessing endurance running performance (Stephens & Dascombe 2015), may result in a relative insensitivity to small, intuitive pacing changes, relative to over-ground running, as individuals must adjust stride pattern in order to select a higher or lower speed (St Clair Gibson et al. 2006). To mitigate against this, participants were asked to practice both small and large adjustments in treadmill speed during their familiarisation, as well as being reminded they were free to adjust the speed as much or as little as they liked prior to every trial. Furthermore, despite best efforts to create a simulated competitive environment, motivational issues from exercising in a laboratory may attenuate the true performance of an individual (Stephens & Dascombe 2015).

10.7 Future directions

A theoretical model has been proposed to demonstrate the interrelation of the physiological underpinnings of endurance running and behavioural decisions that determine the self-selected running speed in the heat (Figure 60). Within the model, the relative contribution from each interacting factor is likely to demonstrate significant individual variability, depending not least upon the individual's underlying cardiovascular fitness, the given environmental conditions, how much prior exercise has occurred and acclimation status. Therefore, there appears limited value in attempting to differentiate the precise role of each contributing factor, given how these relationships may be different between individuals. Rather, this model may provide a broad framework from which intervention strategies and training can be focussed upon, with a priority to further differentiate the relative contributions of physiological strain, relative to perceived thermal strain in determining running speed. Therefore, future research should firstly seek to replicate the findings of this thesis under conditions incorporating suitable airflow.

Subsequently, to disentangle the physiological and perceptual influences, the use of interventions such as menthol that provide a sensation of cooling, without influencing body temperature (Schlader, Simmons, et al. 2011b; Barwood et al. 2015), would appear appropriate. Existing research has demonstrated that menthol can influence behaviour to a similar magnitude as skin cooling, affording improved thermal comfort (Schlader, Simmons, et al. 2011b; Barwood et al. 2015). Under such circumstances, it would appear pertinent to

investigate whether a mediation of thermal discomfort following menthol application results in the physiological variables explaining performance to a greater degree, reducing the unexplained variance observed in this thesis. Such research may be particularly relevant for populations who experience reduced thermoregulatory sensation (Price 2006). The effectiveness of menthol application may be dependent upon event distance and the duration of the protocol, given the influence thermal perception prior to the onset of severe physiological strain (Tucker et al. 2004; Ely et al. 2010). Therefore, interventions that alleviate thermal discomfort may be most effective during shorter duration events that still display impairments to performance under heat stress, in the absence of severe hyperthermia (Guy et al. 2015). One practical consequence of differentiating the roles of physiological strain and thermal comfort would be improved prescription of precooling techniques. For example, precooling techniques across different events may be optimised between absolute cooling volume to alleviate physiological strain, and maximising thermal comfort, which is pertinent given the potential for precooling to continue to aid performance once the thermometric effects appear to have gone (Duffield et al. 2010).

Theoretically, menthol application could also complement an existing heat acclimation strategy to provide further alleviation of thermal discomfort. Notwithstanding the potential for yielding an additive effect through combining these interventions, the decay of alleviated perceptual strain following heat acclimation alone is unknown, as previous research has focussed on the induction and decay of physiological adaptations (Garrett et al. 2009; Ashley et al. 2015; Poirier et al. 2015). Finally, attention should be given to understanding how training status may modulate the relationship between perceived strain and exercise performance given elite runners have an altered perception of their physiological thermal strain during exercise (Tikuisis et al. 2002), and therefore may demonstrate a higher pain tolerance (Astokorki & Mauger 2016). Therefore, the relative importance of behavioural thermoregulation may be different within elite runners, in comparison to the conclusions drawn from a heterogeneous group of moderately trained runners within this thesis.

The data within this thesis also alludes to the importance of practicing pacing strategies when adopting interventions. As shown in Study 5 (Chapter 8), an additive effect apparently mediated physiological strain at the start of the time trial, but this was not matched by an increase in running speed. Therefore, individuals may have adopted a predetermined pacing strategy, not utilising the potentially beneficial additive effect from the two interventions. Similar effects have previously been reported following combined heat acclimation and precooling (Schmit, Le Meur, et al. 2015) as well as following menthol application (Barwood et

al. 2015). Consequently, future research should seek to identify the optimal pacing strategy following interventions that takes advantage of possibly transient alleviation of physiological or perceptual strain, without a subsequent performance decrement from beginning a trial at too great an intensity. However, despite the need to practice pacing following precooling, it may be necessary to balance this against the propensity for humans to develop a habituation to repeated cold exposures in accordance with acclimatization to cold environments (Young 2011), thereby attenuating the ergogenic benefits of precooling. This is another avenue that research should consider for those athletes who regularly compete in the heat and repeatedly adopt precooling techniques.

The findings of Study 4 (Chapter 7), alongside previous research, present a mixed picture on the influence of heat stress on running economy. If indeed it is that running economy initially benefits from elevated muscle temperature in advance of the onset of energy demanding thermoregulatory processes, then trials that manipulate starting muscle temperature through passive heating or cooling, may help to understand this relationship better. Furthermore, running protocols that involve longer continuous exercise in the heat greater than the 3 min stages of the GXT in this thesis, may help to establish the degree of heat strain where running economy benefits are lost and oxygen consumption increases, i.e. identify the point of best performance. However, whilst such questions are pertinent to understand from a research perspective, the practical application of such findings may be limited, given the modest relationship demonstrated between running economy and time trial performance.

The application of non-sport-specific, cycling training during STHA in Study 4 (Chapter 7) would appear counter-intuitive within this running cohort. However, a cycling model afforded many advantages that cannot currently be realised using running training, given a dearth of research pertaining to running acclimation protocols (Costa et al. 2014). Logistically, cycling allowed multiple individuals to train simultaneously in an environmental chamber, but also permitted simple and precise quantification of the completed mechanical work, allowing the training load to be matched between groups, independently of potentially confounding factors such as body mass differences. Furthermore, prescribing cycling exercise relative to body mass eliminates the need for participants or athletes to complete an additional laboratory test in order to derive relative intensities (Gibson et al. 2015a). The importance of efficient exercise prescription is not only to minimise exercise volume to complement a training taper (Spilsbury et al. 2015), but also ensures the major potentiating stimuli for heat adaptation are achieved and maintained across a training programme, namely the rapid elevation of T_{CORE} and initiation of profuse sweating (Sawka et al. 2011). Therefore, that significant adaptation can be achieved

through an alternative, and potentially more convenient, exercise mode is highly meaningful for many individuals preparing to compete in the heat and supports previous suggestions that heat acclimation is not modality specific (Taylor 2014). Notwithstanding these benefits, elite athletes will require sport-specific conditioning in weeks prior to a competition, so acclimation protocols facilitating practical and robust acclimation procedures for weight-bearing sports such as running are warranted. In this instance, the application of mechanical work, relative to body mass, would appear inappropriate, given the inverse relationship between running performance and body mass (Lucia et al. 2006). Greater consideration should also be managing the overall training load if weight-bearing training is adopted, as this type of exercise may be associated with a heightened risk of injury and/or cumulative fatigue (Vleck & Garbutt 1998, Millet et al. 2009). Emerging evidence indicates intensity may be effectively prescribed based on a fixed RPE (Neal et al. 2015; Gibson et al. 2015a; Chalmers et al. 2016). However, research should seek to establish the influence of training status on the change in T_{CORE} at a given RPE, as large differences in absolute MHP between fit and unfit individuals may present (Jay et al. 2011), not least due to differences in perceived exertion (Astokorki & Mauger 2016).

10.8 Conclusion

This thesis has investigated acute and chronic strategies that may enhance endurance performance in the heat. The most effective strategy, short term heat acclimation, appears to enhance endurance running performance through both the traditional model of endurance physiology, as well as promoting specific thermal adaptations. Accordingly, traditional markers of endurance performance, the lactate thresholds, $\dot{V}O_{2max}$, running economy and $v\dot{V}O_{2max}$, explain less of the variance in time trial performance than is often reported in normothermic conditions. The greater unexplained variance may be a consequence of an altered pacing, as individuals behaviourally thermoregulate, due to heightened perceived thermal discomfort and exertion. As a consequence, preparation for competition in the heat should prioritise improving thermal comfort and perceived exertion under heat stress, alongside improvements to traditional physiological markers. Theoretically, a combined heat acclimation and precooling strategy would yield physiological adaptations alongside a mediated perceived strain, predominantly through a reduced skin temperature. However, this technique failed to demonstrate meaningful benefits above those arising from heat acclimation alone, despite indications of a greater alleviation of physiological and perceptual strain. Consequently, this thesis emphasises the importance of familiarising individuals with precooling and/or heat acclimation in order to optimise the most effective pacing strategy. Future research should consider other methods through which thermal discomfort can be improved, and whether

interventions, such as menthol application, can enhance performance in acclimated individuals further.

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Appendix 1 – informed consent form

University of Brighton

SCHOOL OF SPORT AND SERVICE MANAGEMENT

INFORMED CONSENT FORM

Project Title (*example*)

The effect of acute, chronic and combined strategies on endurance running performance in hot and humid conditions.

DECLARATION

I ………………………………………………… hereby volunteer to take part in this research, which is to investigate ischemic preconditioning during a running protocol.

The principal investigator has explained to my satisfaction the purpose of the experiment and the possible risks involved.

I have had the principles and the procedure explained to me and I have also read the participant information sheet. I understand the principles and procedures fully.

I am aware that I will be required to:

- Exercise to volitional exhaustion during each visit.
- Exercise in a hot and humid environment
- Have my core temperature measured using a rectal thermometer
- Provide fingertip blood samples on each occasion.

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

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

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

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

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

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

Signature of participant: ....   Date: ....

Signature of Investigator: ....   Date: ....

University of Brighton

Appendix 2 – medical questionnaire

SCHOOL OF SPORT AND SERVICE MANAGEMENT
MEDICAL QUESTIONNAIRE

Name: ....

Age: ....

Are you in good health ?   Yes/No
If no, please explain:
How would you describe your present level of activity?
Vigorous: < once per month
                             once per month
                             2-3 times per week
                             4-5 times per week
                             > 5 times per week

Have you suffered from a serious illness or accident ? Yes/No
If yes, please give particulars:

Do you suffer, or have you ever suffered from:

Asthma	Yes	No
Diabetes	Yes	No
Bronchitis	Yes	No
Epilepsy /Convulsion/Seizure	Yes	No
High blood pressure	Yes	No

Fainting/Syncope	Yes	No

Are you currently taking medication ?   Yes/No
If yes, please give particulars:

Have you taken a long-haul flight in previous 2 weeks? Yes/No

Are you currently attending your GP for any condition or have you consulted your doctor in the last three months?   Yes/No

If yes, please give particulars:
Have you, or are you presently taking part in any other   Yes/No
laboratory experiment ?

PLEASE READ THE FOLLOWING CAREFULLY

Persons will be considered unfit to participate in the study if they:
- are unsure of the test protocol and the possible risks and discomforts designated on the subject information sheet;
- the answers given on the medical questionnaire or informed consent form do not meet the required criteria;
- do not complete running training at least 3 times per week or are unable to attain 5 km within 22 minutes;
- are pregnant;
- have any medically inserted plates or pins, including pace makers, aneurysm clip, heart/vascular clip, prosthetic valve, or intracranial metal prosthesis;
- have suspended training due to joint or muscle injury;
- have been verified, or documented as having any blood carried infections (Hepatitis, HIV), are diabetic or obese (Body Mass Index>30), have a known history of haematological, cardiac, respiratory, or renal disease; or had a head injury, brain infection (meningitis) or brain tumour;
- have a known history of severe headaches, fainting, dizziness, heat stroke or other heat induced illness;
- have symptoms of nausea or light-headedness to needles, probes or other medical-type equipment;
- have known anal problems such haemorrhoids, fissures and anal bleeding

DECLARATION

I ……………………………………………… hereby volunteer to be a participant in experiments/investigations during the period commencing February 2015.
My replies to the above questions are correct to the best of my belief and I understand that they will be treated with the strictest confidence. The experimenter has fully informed me of, and I have understood, the purpose of the experiment and possible risks involved.
I understand that I may withdraw from the experiment at any time and that I am under no obliga

tion to give reasons for withdrawal or to attend again for experimentation.

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

Signature of Subject ………………………………………………….. Date……………

Signature of Experimenter ……………………………………….. Date   …………