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Post-exercise hot water immersion
a novel approach to heat acclimation

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POST-EXERCISE HOT WATER IMMERSION: A NOVEL APPROACH TO HEAT ACCLIMATION

by

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Summary

Hot environmental conditions can reduce work productivity/exercise performance and increase the incidence of exertional heat illness. In preparation for hot environments, individuals commonly complete heat acclimation protocols that initiate physiological and perceptual adaptations to reduce thermal strain and improve physical capabilities. However, conventional exercise-heat acclimation interventions can be costly and impractical, as they require access to an artificial hot environment and often require control of core temperature during exercise. As such, there is a demand for a flexible heat acclimation intervention that can be easily incorporated into the daily training of athletes and military/occupational personnel. Therefore, the aim of this thesis was to develop a novel and practical post-exercise hot water immersion heat acclimation protocol.

Firstly, 6-days of submaximal exercise in temperate conditions followed by a hot water immersion induced hallmark heat acclimation adaptations during submaximal exercise in temperate and hot conditions and improved endurance exercise performance in the heat (**Chapter 4**). The initiated adaptations were also not specific to the clock-time of when heat exposures occurred (**Chapter 5**). Post-exercise hot water immersion also initiated hallmark heat acclimation adaptations in both endurance trained and recreationally active individuals (**Chapter 6**). In addition, the decay of the induced adaptations following post-exercise hot water immersion is slow, with no observable loss of heat acclimation two weeks following the cessation of the protocol (**Chapter 7**).

The studies presented in this thesis demonstrate that the novel post-exercise hot water immersion intervention provides heat acclimation and reduces thermal strain during exercise in the heat. Future research is required to optimise this technique to improve its incorporation into different military/occupational or athlete scenarios and assess the impact of the intervention on the incidence of exertional heat illness.

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Publications

I was fully involved in all aspects of protocol design, data collection and analyses and preparation of manuscripts for publication. However, I acknowledge input from other named authors for each publication.

Full papers

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Thesis Format

This thesis contains a critical literature review that outlines the main research aims (**Chapter 2**) and a general methods section (**Chapter 3**) that provide information on procedures and measures adopted in experimental chapters. The thesis consists of four experimental chapters. The first of which investigates whether a post-exercise hot water immersion can provide heat acclimation adaptations and improvements in endurance performance in temperate and hot conditions (**Chapter 4**). **Chapter 5** investigates if this intervention initiates clock time dependent adaptations and **Chapter 6** examines the effect of training status on adaptation from the intervention. The final experimental chapter investigates the timeframe of adaptation decay from the post-exercise hot water immersion intervention (**Chapter 7**). The general discussion (**Chapter 8**) summarises the main findings of the thesis, recognising the limitations of the research and suggested potential areas for future research. Lists of abbreviations, tables and figures appears prior to **Chapter 1** and **bold type** is used to refer to chapters and sections within this thesis.

List of Tables

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List of Abbreviations

°C	degrees Celsius
ANOVA	analysis of variance
AUC	area under the curve
cm	centimetre
CO	carbon monoxide
COHb	carboxyhaemoglobin
d	Cohen's *d* effect size
dL	decilitre
EDTA	ethylenediaminetetraacetic acid
EHI	exertional heat illness
EHS	exertional heat stroke
g	gram
h	hour
Hb	haemoglobin
HR	heart rate
Hct	haematocrit
km	kilometre
kg	kilogram
L	litre
LTHA	long-term heat acclimation
µL	microlitre
min	minute
MJ	megajoules
mL	millilitre
MTHA	medium-term heat acclimation
O_2	oxygen
ppm	parts per million
RER	respiratory exchange ratio
RH	relative humidity
RPE	rating of perceived exertion
s	second

SD	standard deviation
STHA	short-term heat acclimation
T_{es}	oesophageal core temperature
T_{re}	rectal core temperature
T_{sk}	mean skin temperature
TTE	time to exhaustion
TT	time trial
USG	urine specific gravity
$\dot{V}O_2$	oxygen uptake
$\dot{V}O_{2max}$	maximal oxygen uptake
WBSR	whole body sweat rate

CHAPTER ONE

General Introduction

The ability to maintain a thermal steady state is fundamental to the maintenance of endurance exercise performance and occupational work capacity in hot environments (Galloway and Maughan, 1997). Athletes, military personnel, miners and firefighters are regularly exposed to this stress when travelling to areas of high ambient temperatures. In the heat, the relative intensity of activities increases, which augments metabolic heat production and places a greater strain on heat loss mechanisms (Arngrimsson *et al.*, 2003). Heat that cannot be dissipated is stored, increasing body temperatures which is a known risk factor for exertional heat illness (EHI), characterised by the occurrence of muscle cramps, syncope and exhaustion (Binkley *et al.,* 2002). Larger elevations in core body temperature (≥ 40 °C) and central nervous system dysfunction characterise more serious heat illness, otherwise known as exertional heat stroke (EHS; Casa *et al.,* 2012), which can lead to systemic inflammatory response syndrome, organ failure and death (Sawka *et al.,* 2011). Individuals exposed to the heat therefore seek strategies to reduce thermal strain. Heat acclimatisation, which occurs following exposure to a naturally occurring hot environment and conventional exercise-heat acclimation, which is experimentally induced in an artificial environment (Pandolf, 1998) and/or physical training protocols in temperate conditions are widely used methods that aim to reduce thermal strain in the heat.

Habitual endurance exercise in temperate conditions increases parameters of physical fitness and initiates thermoregulatory adaptations and tolerance to the heat akin to 'partial' heat acclimation (Piwonka *et al.*, 1965; Strydom *et al.*, 1966; Gisolfi and Robinson, 1969; Shvartz *et al.*, 1974). Additionally, improving physical fitness increases the speed of adaptation from conventional exercise-heat acclimation interventions (Pandolf *et al.*, 1977). The completion of endurance exercise protocols cannot however wholly replace the benefits acquired from heat acclimation. To induce heat acclimation adaptations, recommendations suggest to complete submaximal exercise, which initiates sweating responses and maintains an elevation in core temperature, in hot environmental conditions for 60-90 min on 5 to 14 consecutive days (Armstrong and Maresh, 1991; Garrett *et al.*, 2011; Taylor, 2014). The phenotypic adaptations induced following conventional exercise based protocols improve exercise

capabilities (Shvartz *et al.*, 1973) and performance (Lorenzo *et al.*, 2010) in the heat and minimise the threat of thermal injury (Cheung *et al.*, 2000). However, the completion of exercise-heat acclimation can be costly, as they require access to a hot climate (environmental chamber or hot natural environment). In addition, recommendations that aim to increase the benefits of heat acclimation, for example, through matching the time of day of heat acclimation sessions with the anticipated time of exercise-heat stress are impractical and can disrupt training (Shido *et al.*, 1999; Garrett *et al.*, 2012).

Interestingly, conventional exercise-heat acclimation may also provide ergogenic benefits (increased; $\dot{V}O_{2max}$, plasma volume, stroke volume and cardiac output) that improve endurance exercise capabilities/performance in thermoneutral conditions (Lorenzo *et al.*, 2010; Neal *et al.*, 2016a). Therefore, heat acclimation protocols may have the potential to be utilised as a training intervention to improve exercise performance in sea level, thermoneutral conditions (Lorenzo *et al.*, 2010). This idea however, is yet to be universally accepted (Minson and Cotter, 2016; Nybo and Lundby, 2016).

Information regarding the induction of heat acclimation through conventional methods is extensive. Phenotypic adaptations are however transient and will gradually disappear following the removal of the heat stimulus (Armstrong and Maresh, 1991). Unlike knowledge regarding the induction of heat acclimation, research concerning the decay of adaptations remains equivocal and is limited by inconsistencies between experimental designs (Pandolf, 1998; Garrett *et al.*, 2009; Taylor, 2014). The timeframe of heat acclimation decay was previously defined as the loss of 1-day of adaptation every 2-days without heat exposure (Givoni and Goldman, 1973). This philosophy is however now questioned, with the retention of a portion of adaptation thought to last up to 1-month (Weller *et al.*, 2007). Due to the limited understanding of the timeframe of adaptation decay, individuals are advised to complete heat acclimation protocols in the days prior to movement to the heat. For an athlete however, the physiological cost of repeatedly exercising in the heat during a taper may have a detrimental effect on competition performance. Therefore, a greater understanding of adaptation retention is required to improve the scheduling and implementation of heat acclimation protocols.

One method of initiating heat acclimation adaptations that is readily available and has practical applications is through immersion in hot water. Although passive heat exposures are thought to initiate a smaller magnitude of heat acclimation adaptation than exercise based protocols, evidence suggests that the combined elevation in core and skin temperatures during immersion in hot water may provide a substantial stimulus for adaptation (Brebner *et al.*, 1961; Brazaitis and Skurvydas, 2010). Additionally, taking a hot bath following exercise could be easily incorporated into daily training routines when washing after exercise; presenting a practical, cost effective protocol for athletes/military personnel prior to movement to areas of high ambient temperatures.

The primary aim of this thesis was to develop a novel post-exercise hot water immersion heat acclimation intervention; to investigate the induction and decay of phenotypic adaptations and examine its effect on endurance exercise capabilities and performance in hot and thermoneutral conditions. This thesis also questions established heat acclimation recommendations by investigates whether the greatest heat acclimation benefits occur at the same clock-time as heat acclimation sessions and what effect training status has on adaptation from post-exercise hot water immersion.

CHAPTER TWO

Literature Review

Hot environmental conditions can have detrimental effects on work productivity and exercise performance. This decrease in work productivity was first observed and examined in the hot-humid conditions in South African gold mines. Between 1924 and 1932, exposure to these conditions reportedly caused one death every month through EHS (Cluver, 1932). Unacclimatised workers were more susceptible to EHI and EHS as the majority of fatalities occurred within the worker's first four shifts (Cluver, 1932). Researchers therefore developed and implemented heat acclimatisation/acclimation strategies to initiate the adaptations acquired during these first four working shifts under safe, controlled conditions (Dreosti *et al.*, 1935; Wyndham *et al.*, 1969). The success of these protocols in reducing fatality numbers and increasing work capacity has led to the development and optimisation of heat acclimation protocols over the past century (Dreosti *et al.,* 1935; Fox *et al.*, 1963).

2.1 Acute responses to exercise-heat stress

Metabolic heat produced from physical activity or exercise must be dissipated to maintain homeostasis. The heat balance equation demonstrates that when heat loss matches heat production, a thermal steady state is maintained. However, when metabolic rate exceeds the rate of heat dissipation, heat is stored and core temperature increases. Behavioural and/or physiological temperature regulation responses can alter the rate of heat storage and heat dissipation. Behavioural temperature regulation operates through conscious behaviour to alleviate heat strain (Sawka *et al.*, 2011b) whereas, physiological regulation of heat balance is controlled by the autonomic nervous system (Morrison and Nakamura, 2011). Afferent and efferent feedback provides information to the hypothalamic thermoregulatory centre to initiate effector responses that aim to maintain optimal body temperatures (Sawka *et al.*, 2011b).

Metabolic heat is dissipated through the activation of evaporative and non-evaporative (radiation, conduction and convection) heat loss mechanisms (Kenny and Jay, 2013). A large transfer gradient between the core and skin, and skin and ambient environment facilitates non-evaporative mechanisms that are controlled through the adjustment in skin blood flow

and skin temperature (Sawka *et al.*, 1993). Exercising in a hot environment augments heat production and reduces the heat transfer gradient between the skin surface and the surrounding environment (Gagge and Gonzalez, 2011). A reduction in the heat transfer gradient places substantial strain on heat loss pathways and places a greater reliance on sweat evaporation from the skin surface to maintain heat balance (Sawka *et al.*, 2011a). Aerobic fitness, acclimatisation and hydration status, clothing, exercise duration and intensity also influence thermoregulation in the heat (Pandolf, 1979; Nadel *et al.*, 1980; Cadarette *et al.*, 1984; Sawka *et al.*, 1993).

Metabolic rate and relative exercise intensity (% $\dot{V}O_{2max}$) increases when exercising in the heat compared to exercise in temperate conditions (Gonzalez *et al.*, 1978; Maw *et al.*, 1993). In an attempt to maintain homeostasis during exercise in hot conditions, skin blood flow and evaporative sweating work in tandem to dissipate heat and maintain exercise capabilities, with blood flow redistributed from the viscera to the periphery and working skeletal muscles (Rowell, 1974; González-Alonso *et al.*, 2008). However, during prolonged exercise-heat stress sweat evaporation reduces central blood and plasma volume (hypovolemia), further increasing competition between metabolic and thermoregulatory demands for circulating blood (Montain and Coyle 1992; Sawka *et al.*, 1993). The competition for circulating blood reduces venous pressure, cardiac filling and stroke volume and in an attempt to maintain cardiac output and meet metabolic and thermoregulatory demands, heart rate (HR) progressively increases (Fritzsche *et al.*, 1999; Trinity *et al.*, 2010; Montain and Coyle, 1992; Lafrenz *et al.*, 2008). If cardiac output is not maintained, heat is stored and exercise capabilities/performance progressively diminish (Sawka *et al.*, 1985b).

The optimal ambient environment to maximise endurance exercise capacity/performance is 11 °C (Galloway and Maughan, 1997). As ambient temperatures increase beyond this, relative exercise intensity progressively increases, augmenting physiological and perceptual strain, shortening the time for fatigue onset and reducing endurance exercise performance and maximal oxygen uptake ($\dot{V}O_{2max}$) irrespective of aerobic fitness (Sawka *et al.*, 1985a; Galloway and Maughan, 1997; Arngrimsson *et al.*, 2003; Periard *et al.*, 2011; Periard *et al.*, 2012). For example, marathon times are 5 % slower in races completed in a temperate (20-25 °C) environment, compared to cool conditions (5-10 °C; Montain *et al.*, 2007).

The reduction in exercise performance is, in part, due to aforementioned rise in physiological strain during exercise in the heat. However, negative perceptions associated with the elevation in physiological strain initiate behavioural changes to reduce and/or terminate exercise in the heat in order to protect against excessive heat accumulation (Tucker *et al.,* 2006). For example, the elevation in skin temperatures, which necessary to dissipate heat, and the storage of heat during exercise in hot conditions has been associated with an increase in thermal sensation (Gagge *et al.,* 1969) and reduction in work rate during exercise in the heat at a fixed rating of perceived exertion (Tucker *et al.,* 2006).

2.2 Adaptations to the heat

The repeated application of a heat stimulus, that disturbs homeostasis, initiates complex integrated central and peripheral physiological and perceptual adaptations that can reduce the detrimental effect of a hot environment on work capacity and exercise performance (**Section 2.1**; Taylor, 2014). Commonly reported markers of adaptation to the heat include; a lower HR and body temperatures and a higher sweat rate during exercise in the heat. These physiological adaptations are paralleled with improvements in perceived exertion, thermal sensation and an improvement in exercise capacity/performance (Gagge *et al.*, 1967; Sawka *et al.*, 2011a). The acquisition of these physiological adaptations has also been linked to the reduction in the number of heat stroke cases in South African gold mines (Cluver, 1932). Over the past century, strategies to acclimatise to the heat have been adapted from rudimentary protocols and optimised to suit the demands of numerous industrial, occupational, military and sporting settings (Dreosti *et al.*, 1935; Cheung and McLellan, 1998; Weller *et al.*, 2007; Garrett *et al.*, 2011; Schmit *et al.*, 2017).

2.2.1 Heat adaptation continuum

The process of adaptation to the heat is sequential, with different adaptations thought to occur following a different number of heat exposures (Armstrong and Maresh, 1991). Adaptations that are linked to the cardiovascular system including an expansion in plasma volume and reduction in exercising HR occur first (≈ 3-days; Armstrong and Maresh, 1991). These are followed by reductions in body temperatures (≈ 5-days), improvements in sweating responses (≈ 8-days) and an increase in thermal tolerance (> 10-days; McClung *et al.*, 2008; Sandström *et al.*, 2008), although increases in sweating rate have been noted following fewer heat exposures (Neal *et al.*, 2016a; Neal *et al.,* 2016b; Rendell *et al.,* 2017). Factors that affect the

speed and extent of adaptation include; the size of the daily adaptation/thermal impulse, the duration of the heat acclimation protocol and the training status of the individual (**Sections 2.3, 2.4.1 and 2.4.3**). Recent literature has however questioned the timeframe of the aforementioned adaptations. The suggestion being that an artefact of the conventional, exercise based constant work-rate heat acclimation protocol, where the stimulus for adaptation diminishes with ensuing adaptation, may elicit these timeframe differences in adaptation (**Section 2.3;** Taylor, 2014). Therefore, it may be more accurate to describe the adaptation continuum in terms of the size of the thermal stimulus rather than the number of heat exposures, this however is yet to be quantified (Taylor, 2014).

2.2.2 Physiological and perceptual adaptations to the heat

Irrespective of the method employed to adapt to the heat, an expansion in blood and plasma volumes are thought to appear first on the adaptation continuum (Shvartz *et al.*, 1973; Senay *et al.*, 1976; Harrison *et al.*, 1981; Garrett *et al.*, 2012; Patterson *et al.*, 2014). The post-exercise restoration of blood volume, which is compromised through prolonged exercise in the heat, is initiated through a rapid increase in total circulating proteins, namely an increase in plasma albumin content (Fortney *et al.,* 1981; Convertino, 1991). This increase in circulating proteins increases oncotic pressure, causing a shift in fluid from the extravascular to intravascular space, which leads to hypervolemic haemodilution (Fortney *et al.*, 1981; Nose *et al.*, 1988; Goto *et al.*, 2010). This process of expanding plasma volume is assisted by an increase in thirst and fluid intake following exercise in the heat and a reduction in urine output, through an increase in sodium reabsorption (Convertino, 1991). The expansion in plasma volume following heat acclimation increases ventricular filling pressure and stroke volume, which allows cardiac output to be maintained with a reduction in HR during exercise in the heat (Rowell *et al.*, 1967; Wyndham *et al.*, 1968; Senay *et al.*, 1976; Gledhill *et al.*, 1994; Wyndham *et al.*, 1976; Goto *et al.*, 2010). The size of expansion in plasma volume is however variable and dependent upon the technique and timing of the measurement, the population, the number of heat exposures and the size of the thermal impulse (Harrison, 1985; Gore *et al.*, 2005; Periard *et al.*, 2015).

Another adaptation associated with heat acclimation is a reduction in body temperatures during exercise-heat stress (Shvartz *et al.*, 1973; Nielsen *et al.*, 1993; Garrett *et al.*, 2009). The reduction in core and peripheral body temperatures is initiated through an attenuation in

metabolic heat production and an improvement heat dissipation through a widening of the core-to-skin temperature transfer gradient (Chaffee and Roberts, 1971; Sawka *et al.*, 1983; Assayag *et al.*, 2012). Reducing body temperatures during exercise-heat stress attenuates the demands placed on the cardiovascular and thermoregulatory systems to support skin blood flow and evaporative sweating. As such, blood flow can be redistributed to active tissues to assist in the improvement in exercise capacity and performance in the heat. The reduction in exercising core temperature may also be attributed, in part, to a reduction in core temperature at rest that can occur following heat acclimation (Fox *et al.*, 1963; Cotter *et al.*, 1997; Weller *et al.*, 2007; Brazaitis and Skurvydas, 2010) and physical training programmes (Fox *et al.*, 1963; Shvartz *et al.*, 1974; Baum *et al.*, 1976). External factors such sleep, fluid and food intake can affect circadian rhythm therefore, experimental control is required when assessing resting core temperature (Waterhouse *et al.*, 2005). In a well-controlled experiment, Buono *et al.* (1998) first highlighted this reduction in resting core temperature following exercise-heat acclimation. They reported that 50 % of the reduction in end-exercise core temperature (-0.6 °C) was initiated through a reduction in core temperature at rest (-0.3 °C; Buono *et al.*, 1998; Tyler *et al.*, 2016). However, this meaningful reduction in core temperature at rest (≥ 0.20 °C; Tyler *et al.,* 2016) is not commonly observed following conventional short-term exercise-heat acclimation (≤ 5 exposures; STHA; **Section 2.3.2**; Garrett *et al.*, 2012; Willmott *et al.*, 2016). The initiation of this adaptation may therefore require exposure to a larger thermal stimulus. However, the mechanism for the reduction resting core temperature following heat acclimation is currently unknown. Elucidation of this mechanism is required but previously described methods suggest that an increase in resting skin blood flow or reductions in thermoregulatory set point, basal metabolic rate and/or thyroid activity may initiate this adaptation (Collins and Weiner, 1968; Chaffee and Roberts, 1971; Aoyagi *et al.*, 1997; Taylor, 2014).

The reduction in resting core temperature is also a major contributing factor for the reduction in core temperature at sweating onset reported following heat acclimation. The reduced threshold for sweating is one of three reported heat acclimation induced alterations in the sweat response; the other two include an increase in sweating sensitivity and an increase in production of a more dilute sweat (Dill *et al.*, 1938; Wyndham, 1967; Fox *et al.,* 1967; Allan and Wilson, 1971). The combined effect of these adaptations is an enhancement in evaporative cooling and a greater capacity for heat dissipation. Previous research has

however questioned whether exercise-heat acclimation improves the sensitivity of the sweating response (Nadel *et al.,* 1974; Cotter *et al.,* 1997; Patterson *et al.,* 2004). For example, Nadel and colleagues (1974) suggest that heat acclimation and physical training programmes completed in temperate conditions, initiate different sudomotor adaptations, with a reduction in core temperature at sweating onset following heat acclimation and an increase in sweating sensitivity following physical training. However, within this research heat acclimation followed physical training without a washout period therefore, an increase in sweating sensitivity may follow heat acclimation that improves endurance capacity and initiates partial heat acclimation (Strydom *et al.*, 1966; Nadel *et al.*, 1974; Taylor, 2014). Additionally, examinations of thermoregulatory adaptations following endurance training in temperate conditions also demonstrated a reduction in the core temperature threshold for sweating (Nadel *et al.*, 1974; Henane *et al.*, 1977). This information may suggest that the reported improvements in sweating sensitivity following heat acclimation may in part be initiated through an improvement in physical fitness (Sawka *et al.,* 1985a; Lorenzo *et al.,* 2010; James *et al.,* 2017).

2.2.3 Heat acclimation and endurance performance

The physiological and perceptual adaptations described above reduce thermal strain, prolong tolerance to exercise-heat stress and increase $\dot{V}O_{2max}$ in the heat (Sawka *et al.*, 1985a; Nielsen *et al.*, 1997; Lorenzo *et al.*, 2010; James *et al.*, 2017). More recently, due to the global locations of numerous sporting events, the effect of heat acclimation/acclimatisation on endurance athletic performance has become a focus for researchers. For example, Lorenzo *et al.* (2010) and Garrett *et al.* (2012) demonstrated that acclimating to the heat improves 60 min cycling (8 %; Lorenzo *et al.*, 2010) and 2 km rowing (4 s; Garrett *et al.*, 2012) time trial (TT) performance in the heat. As the 4 s improvement in 2 km rowing performance was greater than the reported variance of the test (1 % vs 0.6 %), this small performance improvement is considered beneficial (Schabort *et al.,* 1999; Garrett *et al.,* 2012). However, the comprehensive study completed by Lorenzo *et al.* (2010), further highlighted the benefit of acclimating to the heat on endurance performance in hot conditions, as an exercise matched control group demonstrated a 4 % reduction (12 % difference to heat acclimation) in performance following a 10-days of submaximal cycling in temperate conditions (13 °C). Racinais *et al.* (2015b) also report that two weeks of heat acclimatisation restored cycling TT performance and power output in 36 °C to the level observed in 8 °C. The extensive

investigation by Lorenzo and colleagues (2010) also highlighted that improvements in performance in the heat were observed alongside similar increases in $\dot{V}O_{2max}$ (8 %) and power output at lactate threshold (5 %) in hot conditions, suggesting that conventional exercise-heat acclimation protocols initiate multiple interlinked adaptations to improve performance in the heat.

Interestingly, Lorenzo *et al.* (2010) also completed a battery of exercise tests in a cool environment (13 °C) and observed comparable improvements to those noted in the heat in TT performance (6 %), $\dot{V}O_{2max}$ (5 %) and power output at lactate threshold (5 %). Improvements in aerobic capacity and in time to exhaustion (TTE) have also demonstrated the beneficial ergogenic effects of heat acclimation (Sawka *et al.*, 1985b; Scoon *et al.*, 2007). These findings suggest that the adaptations initiated through the completion of heat acclimation may improve exercise performance in thermoneutral conditions. Therefore, similarly to the concept of the 'live-high, train-low' philosophy, the incorporation of repeated heat exposures into regular training maybe utilised as a training aid to improve performance in cool conditions (Corbett *et al.*, 2014).

This beneficial ergogenic effect of heat acclimation is however not universally supported, and its existence has been debated (Minson and Cotter, 2016; Nybo and Lundby, 2016a). Doubts regarding the ergogenic effect of heat acclimation exist as TT performance improvements in temperate conditions are not always observed, even when performance and maximal aerobic power are improved in the heat (Karlsen *et al.*, 2015; Keiser *et al.*, 2015) or when determinants of performance (peak power output and lactate threshold) are improved in cool conditions (Neal *et al.*, 2016a). These differences within the current literature however, could be due the difficulty in ensuring maximal participant effort during each trial, or participants not being fully familiarised with experimental performance tests or due to the difficulties in blinding participants during heat acclimation (Hopkins *et al.*, 2001; Nybo and Lundby, 2016a). Therefore, future research assessing the ergogenic potential of heat acclimation should utilise applicable measurement tests, ensure adequate familiarisation and incorporate exercise-matched control groups. Such experiments may then detect whether the mechanism for performance improvements in thermoneutral conditions is underpinned by heat acclimation and/or due to normal training gains through an increase in training load during heat acclimation (Corbett *et al.*, 2014; Nybo and Lundby, 2016b).

2.3 Methods of heat acclimation

Dreosti (1935) first examined heat acclimatisation strategies in South African gold mine workers through the completion of heat-tolerance screening tests. These original protocols involved the completion of work related tasks within designated areas of the mine to acclimatise workers. Over time, with the increasing number of workers entering mines and with advancements in technology, artificial acclimatisation, or heat acclimation strategies were developed (Pandolf, 1998). These graded heat acclimation protocols, which exposed heat intolerant individuals for longer durations to reduce mortality numbers, replaced work-related tasks with bench stepping exercise in above ground heat chambers that replicated the environmental conditions within the mine (Wyndham *et al.*, 1969). At this time, the British military also completed numerous laboratory based heat acclimation investigations (Fox *et al.*, 1963). These studies progressed the understanding of the stimulus required to induce optimal adaptation. Over the past century, this understanding has further improved and led to the development of constant work-rate and controlled hyperthermia heat acclimation strategies that provide an endogenous thermal load to initiate adaptations to the heat (Fox *et al.*, 1963; Nielsen *et al.*, 1993; Taylor, 2000).

2.3.1 Constant work-rate heat acclimation

Heat acclimation guidelines recommend the completion of submaximal exercise in the heat (≈ 50 % $\dot{V}O_{2max}$; ≈ 40 °C) for 1-2 hours on 5-14 consecutive days to initiate adaptations (Pandolf, 1998; Gill and Sleivert, 2001; Tyler *et al.*, 2016). The exercise completed during heat exposures can either be of an absolute or a relative exercise intensity, completed for a fixed duration or to exhaustion (Pandolf *et al.*, 1977; Nielsen *et al.*, 1993; Nielsen *et al.*, 1997). However, to promote adaptive responses the thermal stress must be sufficient to elevate body temperatures and initiate sudomotor activity (Henane *et al.*, 1977). Advantages of this programme are that the environmental conditions, the number of heat exposures along with the type, intensity and duration of the exercise completed can be manipulated to suit different scenarios (Shvartz *et al.*, 1973; Wyndham *et al.*, 1973; Sawka *et al.*, 1985a). As the exercise intensity completed is submaximal and at a fixed intensity, physiological measurements are not necessarily required, other than to ensure safety and confirm physiological adaptation. Research concerning constant work-rate heat acclimation has demonstrated that this protocol induces adaptations that attenuate thermal strain and improve tolerance to exercise-heat stress (Wyndham *et al.*, 1973; Nielsen *et al.*, 1993). In addition,

this protocol is economic and simple to perform in high numbers. Due to the relative ease of the completion of this protocol and the adaptations it initiates, athletes and military personnel commonly adopt constant work-rate heat acclimation in preparation for competing or working in the heat. The improvement in endurance exercise performance in the heat and its potential ergogenic effect in improving performance in cooler conditions (**Section 2.2.3**) has also been quantified following constant work-rate heat acclimation, adding to its potential benefits (Lorenzo *et al.*, 2010).

Although the flexibility, ease of use and the induced adaptations make the constant work-rate protocol a very commonly used regime, it is not without its limitations (Taylor and Cotter, 2006). Access to an artificial hot environment is required and as improvements in heat dissipation occur over the course of the intervention, heat storage and the relative intensity of the exercise gradually decreases with adaptation (Senay and Kok, 1977). With this reduction in thermal strain, physiological overload will lessen or cease, resulting in habituation to the non-progressive heat challenge. In military and occupational settings, constant work-rate protocols enable individuals to become accustomed to a standard challenge however, for athletes, the reduction in thermal strain results in sub-optimal adaptation. As a result, the expansion in plasma volume, for example, will to return to pre-acclimated levels during the completion of the intervention (Shapiro *et al.*, 1981; Aoyagi *et al.*, 1995). Additionally, data acquired from constant work-rate heat acclimation also demonstrates high inter-individual and between protocol variability due to differences in the adaptation impulse which makes interpretation of the results difficult (Pandolf *et al.*, 1977). Therefore, for an athlete seeking optimal adaptation or when completing mechanistic research, it is necessary to expose individuals to a constant, standard thermal impulse to reduce this variability (Fox *et al.*, 1963; Taylor, 2014).

2.3.2 Controlled hyperthermia heat acclimation

Researchers now advocate the use of the controlled hyperthermia technique to combat the progressive reduction in thermal strain with ensuing adaptation observed during constant work-rate heat acclimation (Taylor and Cotter, 2006). Controlled hyperthermia heat acclimation exposes individuals to a constant thermal impulse during heat exposures, via the clamping of core temperature above sweating threshold (Taylor, 2000). In elegant, ground breaking research, this method was first offered by Fox *et al.* (1963) who demonstrated that

clamping rectal core temperature (T_{re}; 38.5 °C; 60 min) using passive heating on 12-days reduced thermoregulatory and cardiovascular strain during intermittent exercise in the heat. Exercise based controlled hyperthermia heat acclimation maintains progressive physiological overload throughout the intervention via an increase in exercise volume during exposure sessions (Gibson *et al.*, 2015a). This constant forcing function optimises adaptation and allows for the isolation and measurement of variables, in mechanistic research (Fox *et al.*, 1963; Taylor, 2014).

Controlled hyperthermia heat acclimation has been demonstrated to induce a similar extent of adaptation as the constant work-rate protocol (Tyler *et al.,* 2016), and similarly improves self-paced performance in the heat (Garrett *et al.*, 2012). However, as controlled hyperthermia maintains the thermal stimulus throughout the intervention the expansion in plasma volume is maintained, an adaptation that is lost during 10 to 12-days of constant work-rate exercise in the heat (Shapiro *et al.*, 1981; Aoyagi *et al.*, 1995; Patterson *et al.*, 2004; Patterson *et al.*, 2014; Gibson *et al.*, 2015a). Additionally, the physiological cost of acquiring this similar extent of adaptation from controlled hyperthermia is reduced, with a lower exercise duration (≈ -30 min) and lower mean exercise intensity (≈ -9 % $\dot{V}O_{2peak}$) completed during controlled hyperthermia sessions than that completed during constant work-rate exposures (Gibson *et al.*, 2015a).

Based on these findings, the completion of controlled hyperthermia may have a greater practical application and reduce disruptions to training/taper programmes compared to the implementation of constant work-rate protocols (Gibson *et al.*, 2015a). This method of acclimating to the heat is still however not without its limitations, namely that access to an artificial hot environment is still required and the completion of the protocol increases training load, which may overreach and/or negatively affect the quality of an athlete's taper (Meeusen *et al.*, 2013). This increase in training load initiated through the completion of heat acclimation contradicts current pre-competition taper recommendations that training load should decrease, fatigue should be avoided and hydration should be maintained to optimise subsequent exercise performance (Le Meur *et al.*, 2012; Meeusen *et al.*, 2013; Periard *et al.*, 2015). Dehydration is nevertheless inevitable from daily exercise in the heat and often encouraged (Garrett *et al.*, 2014; Akerman *et al.*, 2016). Progressive dehydration during heat acclimation sessions may improve the extent of adaptation (Garrett *et al*., 2014), but if

athletes are not adequately rehydrated following each heat exposure, exercise capacity/performance in the heat will be attenuated (Casa *et al.*, 2010; Garrett *et al.*, 2011). In addition, a measure of core temperature, which requires specialist equipment, is paramount for the completion of controlled hyperthermia heat acclimation, as are practitioners or trained staff who prescribe exercise intensity to maintain thermal strain during the heat exposures. This reduces the practicality of the protocols, which can affect throughput numbers and athlete engagement and/or increase the cost of completing the protocol (Periard *et al*., 2017).

2.3.3 Alternative methods of heat acclimation

The aforementioned methods of heat acclimation utilise exercise in a hot environment as a stimulus for adaptation to the heat. It is however, possible to apply a thermal stress, to initiate adaptive responses, without an exercise stimulus as Fox and collegues (1963) demonstrated when developing the controlled hyperthermia heat acclimation technique (Taylor, 2014). Passive heat acclimation uses exposure to a hot air environment (≥ 45 °C; Henane and Bittel, 1975; Hessemer *et al.*, 1986; Shido *et al.*, 1999; Beaudin *et al.,* 2009), sauna bathing (Scoon *et al.*, 2007; Stanley *et al.*, 2015) or by wearing encapsulating clothing (Fox *et al.*, 1963; Fox *et al.*, 1967) to induce heat acclimation adaptations. It is also possible to induce heat acclimation adaptations through immersion in hot water, which is explained in detail in **Section 2.6**. Methods of passive heat acclimation are however not widely investigated or commonly used in practical scenarios, as traditional views suggest that the lack of an exercise stimulus and discomfort to the heat, which may lead to participant withdrawal, reduces the size of the thermal impulse and attenuates adaptation (Taylor *et al.*, 1997; Taylor *et al.*, 2014).

Nevertheless, passive heat acclimation techniques have been demonstrated to initiate classical heat acclimation adaptations such as reductions in core temperature at rest, at sweating onset and following exercise in the heat (Fox *et al.*, 1963; Shido *et al.*, 1999). Additionally, resting plasma volume is expanded and cardiovascular strain during submaximal exercise in the heat is attenuated (Fox *et al.*, 1963; Fox *et al.*, 1967; Scoon *et al.*, 2007; Stanley *et al.*, 2015). These adaptations are likely achieved through the similar rises in core temperature (≈ 38.5 °C) during exposure sessions as that observed during exercise-heat acclimation (Hessemer *et al.*, 1986; Garrett *et al.*, 2009). The additional elevation in peripheral body temperatures, in response an extreme external thermal stress (≥ 45 °C; Henane and Bittel, 1975; Hessemer *et*

al., 1986; Shido *et al.*, 1999; Beaudin *et al.,* 2009), may also provide an additional stimulus for adaptation (Fox *et al.*, 1964; Regan *et al.*, 1996). Advantages of passive heat acclimation interventions are that they are economical and may integrate better into training/taper as they cause fewer training disturbances, do not increase training load and can be completed away from specialist laboratories. From a mechanistic standpoint, passive interventions may also provide clearer information regarding adaptation to the heat as minimal metabolic responses contribute to the observed thermal responses.

Despite the promising application of alternative heat acclimation methods, some of the protocols outlined above still suffer from the same practical restrictions as exercise-heat acclimation. An artificial hot environment or specialist clothing may be still necessary, as is a measure of core temperature and a practitioner to monitor the thermal impulse (Fox *et al.*, 1963; Fox *et al.*, 1967; Henane and Bittel, 1975; Hessemer *et al.*, 1986; Shido *et al.*, 1999). There is also the possibility of 'home-remedy' interventions causing heat causalities, through an individual's motivation to maximise the benefits of the protocol without trained supervision. The safe use of these alternative passive heat acclimation methods does however offer flexible strategies, that more readily fit around daily training however, the effect of passive interventions on endurance exercise performance in hot and temperate environments is still an area of little research. Future, well-controlled investigations are therefore required to elucidate the effects of passive and post-exercise passive interventions on training/taper quality, the extent of induced adaptation and subsequent exercise capabilities/performance in hot and thermoneutral conditions.

2.4 Factors that affect heat acclimation adaptation

Adaptations to the heat can be induced through the previously explained heat acclimation protocols (**Section 2.3**). Additional factors however, such as the length of the heat acclimation intervention, the time of day that the intervention takes place and the fitness of the individual can also effect the extent of adaptation. Recommendations state that these factors should be considered when attempting to acclimate to the heat (Taylor, 2014; Periard *et al.*, 2015).

2.4.1 Length of heat acclimation interventions

The number of days that heat acclimation protocols are completed over is currently used to categorise programmes into short (STHA; < 7-days), medium (MTHA; 8 to 14-days) and long-term (LTHA; > 15 days) interventions (Garrett *et al.*, 2011). Acquiring tolerance to the heat is a sequential process, with the rate of adaptation differing between different physiological and perceptual variables (**Section 2.2.1**; Armstrong and Maresh, 1991). Traditional views suggest that the extent of adaptation as well as the induction of additional heat acclimation adaptations such as, improvements in sudomotor responses are initiated following MTHA to LTHA, potentially due to an increased exposure to a thermal stimulus (Armstrong and Maresh, 1991; Cotter *et al.*, 1997; Pandolf, 1998; Patterson *et al.*, 2004). As such, research suggests that exercise based MTHA to LTHA provides optimal adaptation and improves exercise capacity/performance in the heat and reducing heat causality risks (**Section 2.3**; Gill and Sleivert, 2001; Sawka *et al.*, 2011a; Casa *et al.*, 2015; Periard *et al.*, 2015). However, most performance enhancing adaptations, such as an expansion in plasma volume and reductions in exercising HR and core temperature begin to occur during STHA (Armstrong and Maresh, 1991). In addition, recent evidence contradicts these traditional beliefs and suggests that extending the number of heat acclimation exposures may not increase the magnitude of induced adaptation (Gibson *et al.,* 2015a; Tyler *et al.,* 2016). Therefore, when the time to acclimate is limited, or when minimal disruptions to pre-competition training/taper is required, the completion of more than seven exposures may be unachievable, costly and unnecessary (Garrett *et al.*, 2011).

Due to the perceived negative impact of MTHA on training quality (Garrett *et al.,* 2011) and the rapid uptake of heat acclimation adaptations (≈ 75 %; Pandolf, 1998), STHA programmes are thought to be an economic and practical alternative, while still providing substantial adaptation and performance benefits in the heat (Shvartz *et al.*, 1973; Periard *et al.*, 2015). In a series of investigations, Garrett and colleagues (2009; 2012; 2014) demonstrated that 5-days of controlled hyperthermia heat acclimation initiated adaptations that reduced thermal and cardiovascular strain during exercise-heat stress. These investigations do not however initiate a meaningful reduction in resting core temperature (≥ 0.20 °C; Tyler *et al.,* 2016), an adaptation, along with improvements in sweating responses that are more commonly (Cotter *et al.*, 1997; Buono *et al.*, 1998), but not always (Neal *et al.*, 2016a; Neal *et al.,* 2016b; Rendell *et al.,* 2017), observed after six or more heat acclimation exposures. In addition, five

controlled hyperthermia heat acclimation sessions have however subsequently been demonstrated to initiate a moderate reduction in core body temperature a rest (≈ -0.2 °C; Gibson *et al.*, 2015a; Neal *et al.*, 2016a; Neal *et al.,* 2016b).

The moderate reduction in resting core temperature following short-term controlled hyperthermia heat acclimation provides the suggestion that when the thermal stress is high during heat acclimation exposures, the number of sessions that are required to elicit optimal adaptation may reduce (Gibson *et al.*, 2015a; Neal *et al.*, 2016a; Neal *et al.,* 2016b; Rendell *et al.,* 2017). In this respect therefore, adapting to the heat, is much like the acquisition of endurance training benefits and is not only dependent upon the frequency, but also the magnitude and duration of the adaptation stimulus (Taylor *et al.*, 2014). Fox *et al.* (1963) outlined these adaptation parameters through the demonstration that a larger elevation in core temperature (38.5 *vs* 37.3 °C), maintained for a longer duration (120 *vs* 30 min), initiates greater reductions in core temperature (-1.05 *vs* -0.35 °C) and HR (-27 *vs* -7 beats·min^{-1}) during exercise-heat stress. In addition, as heat acclimation adaptations can also be achieved through the completion of twice-daily exposures, estimating the extent of adaptation from the number of days that exposures take place on, may be too simplistic (Lind and Bass, 1963; Willmott *et al.*, 2016). The size of the cumulative thermal impulse, or the change in body temperatures with respect to time (°C·min^{-1}; Taylor 2014) during heat acclimation exposures may therefore be a better predictor of subsequent adaptation (Sawka *et al.*, 2011a; Tyler *et al.*, 2016).

2.4.2 Time of day heat acclimation adaptations

Current recommendations concerning the induction of heat acclimation state that consecutive daily heat exposures completed at the same time of day, optimises adaptations at the same clock-time on subsequent days (Shido *et al.*, 1999; Gill and Sleivert, 2001; Cable *et al.*, 2007; Beaudin *et al.*, 2009; Chalmers *et al.*, 2014; Periard *et al.*, 2015). Clock-time heat acclimation adaptations were originally reported in a series of investigations, where the scheduling of heat acclimation interventions on circadian rhythm and thermoregulatory responses in rats was examined (Shido *et al.*, 1989; Shido *et al.*, 1991b). Data from these investigations; where a variety of experimental models were employed, report reductions in core temperature and improvements in thermoregulatory responses throughout the day, but that these adaptations were augmented at the same clock-time as previous heat exposures (Shido *et al.*, 1991a;

Shido *et al.*, 1991b; Sakurada *et al.*, 1994; Hara *et al.*, 2001). It was hypothesised, that these time of day adaptations originate from the suprachiasmatic nucleus within the hypothalamus which memorises the heat exposure clock-time and establishes a new circadian rhythm in preparation for an anticipated heat stress (Maruyama et al., 2007). These clock-time adaptations were later investigated in humans using a 9 to 10-day, 4 h passive heat acclimation protocol. In support of the findings from the animal studies, heat acclimation reduced core temperature by a larger magnitude in humans at the same clock-time that previous heat exposures occurred (Shido *et al.*, 1991a; Shido *et al.*, 1991b; Sakurada *et al.*, 1994; Hara *et al.*, 2001). Additionally, sweating latency and core temperature at sweating threshold, during passive lower leg hot water immersion, were also reduced by a larger magnitude at the same time of day as the previous heat exposures (Shido et al., 1999).

The time of day adaptations outlined by Shido and colleagues (1999) provided the rationale to align heat acclimation sessions with the anticipated time of exercise-heat stress (Gill and Sleivert, 2001; Cable *et al.*, 2007; Beaudin *et al.*, 2009; Chalmers *et al.*, 2014; Periard *et al.*, 2015). However, upon analysis of the experimental protocol and data, it is apparent that much of this evidence is inconclusive. For example, the recommendation is based on core temperature and thermoregulatory data at rest, not during exercise-heat stress. Additionally, when an acute passive internal heat stress was applied to rats following a 14-day passive heat acclimation protocol, core temperature and skin temperature responses improved similarly throughout the day (Sugimoto *et al.*, 1995). The purported time of day reduction in resting core temperature also may not be evident > 3-days following heat acclimation, raising questions regarding the practical relevance of matching heat acclimation sessions with the anticipated clock-time of future exercise-heat stress (Shido *et al.*, 1991a; Sakurada *et al.*, 1994). Information concerning the number of heat exposures required to demonstrate the time of day heat acclimation adaptation is also contradictory. Sakurada *et al.* (1994) demonstrate that time of day adaptations are evident following fourteen heat exposures, whereas data from this groups original work suggests that this adaptation is observed after five exposures but is no longer present after ten (Shido *et al.*, 1989). Data concerning the thermoregulatory responses of humans during passive heating is also inconclusive. Shido *et al.* (1999) state that sweating responses demonstrated ‘time-memory’ adaptations however, the poor sample size, the absence of detailed sweating data and a meaningful reduction in resting core temperature, do not provide categorical evidence in support of this finding (Shido *et al.*, 1999).

Adherence to the recommendation to match the time of heat acclimation sessions with the clock-time of anticipated exercise-heat stress also has practical limitations and can only be followed if the time of exercise-heat stress is known and if individuals are to only be exposed to exercise-heat stress at that clock-time. For athletes and military personnel these conditions cannot always be met. These individuals are likely required to complete races at different times or complete operations and be exposed to exercise-heat stress throughout the day. However, even when the conditions can be met, following the recommendation may cause disturbances to training or sleep patterns, especially with movement across time zones. For example, if following this recommendation, UK based athletes who are to compete at 1200 h local time at the 2018 Gold Coast Commonwealth Games or 2020 Tokyo Olympics would be instructed to complete heat acclimation sessions at ≈ 0300 h.

Information regarding whether the presence of clock-time heat acclimation adaptations exist during exercise-heat stress therefore has practical relevance and will influence the integration of heat acclimation protocols into training regimes. Current heat acclimation research however, may provide some evidence to the contrary of the work of Shido and colleagues. For example, short-term controlled hyperthermia heat acclimation completed at the same, or at a different clock-time, have been shown to demonstrate similar reductions in cardiovascular and thermoregulatory strain following exercise-heat stress (Garrett *et al.*, 2009; Gibson *et al.*, 2015a). Although, in line with the work of Shido and colleagues (1999), when these times were matched, reductions in core temperature at rest were reported (Gibson *et al.*, 2015a), but when sessions were completed at a different clock-time (1500 – 1700 h) to exercise-heat stress (0900 – 1200 h), core temperature at rest remained unchanged (Garrett *et al.,* 2009). Therefore, research is required to establish whether circadian rhythm demonstrates clock-time heat acclimation adaptations and to establish if time specific adaptations are apparent during exercise-heat stress and the impact that this may have on exercise capabilities in the heat.

2.4.3 Training status and heat acclimation adaptations

An individual's aerobic fitness can influence physiological strain during acute exercise-heat stress and adaptation from heat acclimation (Pandolf *et al.*, 1977). The differences in physiological strain experienced between endurance trained and lesser trained individuals is, in part, attributable to endurance training providing greater stability in cardiovascular

function through an expanded in blood volume and improvements in ventricular filling and myocardial contractility (Senay *et al.*, 1976; Schaible and Scheuer, 1979; Gledhill *et al.*, 1994). Increases in aerobic capacity may also improve the sweat response during exercise-heat stress through an increase in sweating sensitivity and reduction in core temperature at sweating onset (Gisolfi, 1973; Nadel *et al.*, 1974; Cadarette *et al.*, 1984).

Habitual endurance exercise, much like heat acclimation exposure sessions, repeatedly elevates body temperatures, increases cutaneous blood flow and activates sweating responses (Cadarette *et al.*, 1984). Consequently, the aerobically trained demonstrate thermoregulatory adaptations and tolerance to the heat akin to 'partial' heat acclimation (Piwonka *et al.*, 1965; Strydom *et al.*, 1966; Wyndham *et al.*, 1968; Gisolfi, 1973). Therefore, the aerobically trained experience less cardiovascular and thermal strain during exercise-heat stress than the lesser trained at the same absolute work rate (Piwonka and Robinson, 1967; Gisolfi, 1973; Shvartz *et al.*, 1977; Cadarette *et al.*, 1984; Goto *et al.*, 2010). Although habitual endurance training initiates partial heat acclimation adaptations, exposure to an external heat stress is required to optimise adaptation to the heat (Fox *et al.*, 1964; Piwonka *et al.*, 1965; Strydom *et al.*, 1966; Regan *et al.*, 1996). Currently, there is a lack of heat acclimation research that provides information regarding the effect of the intervention specifically on elite athletes (Bergeron *et al.*, 2012). The present literature does however suggest that as aerobic fitness increases, the time or number of heat acclimation sessions required to attain full adaptation decreases, with a negative correlation (r = -0.68) existing between $\dot{V}O_{2max}$ and the number of sessions required for end-exercise core temperature to plateau (± 0.1 °C) during constant work-rate heat acclimation (Pandolf *et al.*, 1977).

The constant work-rate heat acclimation protocol utilised by Pandolf *et al.* (1977) to demonstrate the effect of aerobic fitness on adaptation to the heat, required participants to complete 100 min of treadmill walking at 4.8 $km.h^{-1}$ in the heat (49 °C, 20 % RH). As the exercise protocol was not adjusted for aerobic fitness, the plateau in core temperature is however more likely a measure of habituation to a standard heat challenge, rather than the attainment of optimal adaptation (**Section 2.3.1**; Shvartz et al., 1977; Cheung and McLellan, 1998; Taylor, 2014). Shvartz and colleagues (1977) demonstrated this through the completion of eight constant work-rate heat exposures. Data from this group demonstrate that as untrained individuals were exposed to a larger cumulative thermal stimulus during constant

work-rate heat acclimation sessions than endurance trained individuals, they experience larger attenuations in HR (-22 *vs* -12 beats·min^{-1}) and core temperature (-0.7 *vs* -0.4 °C) during exercise-heat stress. Additionally, exposure to the larger relative thermal stress also initiated a reduction in core temperature at rest in the untrained (-0.3 °C), which was not observed in the trained participants (-0.1 °C; Shvartz *et al*., 1977).

The literature regarding magnitude of induced heat acclimation adaptation in endurance trained and untrained individuals suggests that inter-individual differences in the thermal impulse or the lack of a constant forcing function may account for variance in the extent of adaptation. Controlled hyperthermia heat acclimation exposes individuals to a standard endogenous thermal impulse and thus may initiate a similar extent of adaptation in trained and untrained individuals (**Section 2.3.2**; Taylor and Cotter, 2006). This comparison is however yet to be made but, current research that utilises this heat acclimation technique suggests that similar reductions in resting core temperature (-0.2 °C) may occur in trained and lesser-trained individuals (Mee *et al.,* 2015; Neal *et al.,* 2016a; Neal *et al.,* 2016b; Rendell *et al.,* 2017). The extent of adaptation in aerobically trained and lesser/moderately trained individuals using alternative heat acclimation methods is also yet to be established. Although these strategies have been shown to initiate cardiovascular adaptations and improve performance in endurance trained individuals (**Section 2.3.3**; Scoon *et al.*, 2007; Stanley *et al.*, 2015). One consideration however, of using alternative strategies is that these interventions can be self-regulated, which may influence the size of the thermal impulse and consequently the extent of induced adaptation.

2.5 Decay of heat acclimation adaptations

Information regarding the induction of physiological and perceptual heat acclimation adaptations is extensive (**Section 2.2**). Phenotypic heat acclimation adaptations are however transient and will disappear following the removal of a heat stimulus (Armstrong and Maresh, 1991). It is suggested that the adaptations that occur first, which are of a cardiovascular nature, such as an expansion in plasma volume and a reduction in exercising HR, demonstrate the most rapid decay (Williams *et al.*, 1967; Pandolf *et al.*, 1977; Armstrong and Maresh, 1991; Flouris *et al.*, 2014). Knowledge regarding heat acclimation adaptation decay is however limited and explanations for the differences in the literature are in part, due to a lack

of consistency between experimental designs, small sample sizes and/or inappropriate measures (Pandolf, 1998).

Original examinations of the decay of heat acclimation suggested that the loss of adaptation was as rapid as its acquisition, with the loss of 1-day of adaptation for every 2-days without a heat exposure (Givoni and Goldman, 1973). Subsequent work has however reported contrary findings, and demonstrated that phenotypic adaptations are retained for much longer (Pandolf *et al.*, 1977; Weller *et al.*, 2007; Poirier *et al.*, 2015). Pandolf and colleagues (1977) suggested that a degree of core temperature and HR adaptations are retained for 18-days, following a 9-day constant work-rate heat acclimation protocol. These findings were later substantiated by Daanen *et al.* (2011) and Weller *et al.* (2007), who concluded that adaptations may be observed up to a month after the completion of heat acclimation (Weller *et al.*, 2007). The heat acclimation protocols utilised in these investigations initiated meaningful reductions in resting and end-exercise core temperature. Following the respective decay protocols, they report either minimal decay in resting core temperature (2 %), or interestingly, a further attenuation in end-exercise (-9 %) core temperature (Weller *et al.*, 2007; Daanen *et al.*, 2011). In agreement with the popular consensus that those adaptations that are initiated first, are most rapidly lost, Weller *et al.* (2007) report a modest decay in HR (≈ 30 %) but again, Daanen *et al.* (2011) report no decay in end-exercise HR. Interestingly, the loss in sweating improvements was substantial in both investigations (Weller *et al.*, 2007; Daanen *et al.*, 2011). These findings contradict the heat acclimation decay continuum consensus, as the improvement in sweating responses are thought to occur later on the adaptation continuum (**Section 2.2.1**; Armstrong and Maresh, 1991).

STHA protocols that utilise the controlled hyperthermia technique are thought to induce a large portion of heat acclimation adaptations which is observed, in part, through a meaningful reduction in core temperature (≥ 0.20 °C; Tyler *et al.,* 2016) following exercise-heat stress (**Sections 2.3.2 and 2.4.1**; Pandolf, 1998; Gibson *et al.*, 2015a; Garrett *et al.*, 2009). Increasing the number of heat acclimation exposures is commonly thought to increase the extent in adaptation (Pandolf, 1998). It would therefore be expected, that STHA demonstrates a different pattern of decay in comparison to MTHA that utilise the constant work-rate technique (Weller *et al.*, 2007; Garrett *et al.*, 2009). Garrett and colleagues (2009) investigated adaptation decay from short-term controlled hyperthermia heat acclimation and

observed no decay in adaptations after 7-days however, these adaptations had completely disappeared after 14-days. Therefore, the extent of observed adaptation immediately following heat acclimation protocols may, in part, indicate the timescale of adaptation retention. Using the available information, adaptations such as the reduction in resting core temperature, that occurs following a greater number of exposures, or following exposure to a larger thermal impulse, could be an indicator that predicts the timeframe of adaptation retention (Weller *et al.*, 2007; Garrett *et al.*, 2009). However, other influences such as aerobic fitness and habitual endurance exercise could also be determining factors that may extend the timeframe for the retention of heat acclimation, but this is yet to be substantiated (Taylor, 2014).

Despite the benefits on the extent of adaptation and the duration of adaptation retention of MTHA (**Section 2.4.1**), STHA remains the preferred induction method, as the reduced number of exposures is considered to cause less disturbances to training and better maintain the quality of an athlete's taper (Garrett et al., 2011). However, due to the rapid decay of adaptations from STHA, these sessions are required to be completed in close proximity to competition/deployment. As cytoprotective molecular adaptations occur following MTHA (Tetievsky et al., 2014; Horowitz, 2016) and the retention of thermoregulatory and cardiovascular adaptations may remain for up to a month, it may therefore be more advantageous for athletes and military personnel to complete MTHA protocols 3 to 4-weeks before they are to be exposed to the heat. Individuals may then either have acquired the adaptation, or could 'top-up' adaptations during their taper/48 h prior to rapid deployment. Weller *et al.* (2007) outlined this possibility and observed full reinstatement of adaptations after 2-4 heat exposures following 4-weeks without a heat stimulus.

2.6 Hot water immersion as a potential method of heat acclimation

To acquire heat acclimation adaptations current recommendations, suggest the completion of exercise-heat acclimation protocols (**Section 2.3**; Pandolf, 1998; Tyler *et al.*, 2016). As previously described (**Section 2.3.3**), it is also possible to elevate body temperatures and initiate sweating responses without the use of an exercise-heat stimulus to initiate heat acclimation. However, many passive heat acclimation protocols do not alleviate all of the practical limitations associated with exercise-heat acclimation (**Section 2.3.3**). One passive intervention that may reduce these practical limitations is acclimating to the heat through

immersion in hot water (≥ 39 °C) following exercise in temperate conditions. Saturating the skin in hot water, following temperate exercise that causes a moderate rise in body temperatures, provides an exogenous heat stress that restricts heat loss pathways and further storage of heat. This concept of acclimating to the heat through immersion in hot water is not new, and has previously been used as either an initial stimulus prior to exercise-heat acclimation sessions or, in combination with an exercise-heat acclimation protocol (Turk and Thomas, 1975; Ruddock *et al.*, 2016).

Currently, no research has used a hot water immersion following exercise in temperate conditions as a stimulus for heat acclimation. Investigations that have used hot water immersion to initiate heat acclimation adaptation, without an additional exercise-heat stimulus, have received little attention, but the completed research has yielded encouraging results (Brebner *et al.*, 1961; Bonner *et al.*, 1976; Shin *et al.*, 2013). For example, a small (*n* = 4) R.A.F report originally assessed passive hot water immersion heat acclimation and observed that 10 immersions to the neck, in 40 °C water, reduced oral temperature and HR at rest and during bench stepping exercise in the heat as well as increasing sweat rate during exercise (Brebner *et al.*, 1961). Intermittent seated passive immersion in 41 °C hot water in five healthy males has also previously been utilised similarly to the controlled hyperthermia model, to maintain mild hyperthermia (aural core temperature; 38.5 °C) during heat acclimation, which initiated an expansion in plasma volume (+7 %), a hallmark of heat acclimation (**Section 2.3.2**; Bonner *et al.*, 1976). Brazaitis and Skurvydas (2010) have also used passive immersion in hot water to induce heat acclimation adaptations. Heat acclimation was initiated using a repurposed muscle warming protocol (Sargeant, 1987), with seven exposures, immersed to the waist (≈ 44 °C water for 45 min) completed over 14-days. Unfortunately, the effect of this protocol on thermal strain during exercise-heat stress was not assessed. Although, at rest, core temperature was reduced (-0.3 °C) and following the final hot water immersion, core temperature (-0.3 °C), HR (-12 beats·min^{-1}) and sweat loss (+10 % sweat loss) demonstrated heat acclimation adaptations (Brazaitis and Skurvydas, 2010).

Based on the presented findings, and information from other alternative heat acclimation strategies it may be hypothesised that hot water immersion heat acclimation protocols would reduce thermal strain during exercise-heat stress and improve endurance exercise performance in the heat (**Section 2.3.3**; Fox *et al.*, 1963; Fox *et al.*, 1967; Scoon *et al.*, 2007).

Another promising finding from hot water immersion heat acclimation is that the magnitude of core temperature reduction observed at rest, after only 7 exposures (Brazaitis and Skurvydas, 2010), is similar to that observed following longer duration exercise-heat acclimation (≥ 8 exposures; Nielsen *et al.*, 1993; Nielsen *et al.*, 1997; Weller *et al.*, 2007). The similarities in adaptation between these exercise based and hot water immersion protocols is likely attributable to the similar daily core temperature rise and/or the combined elevation of core and skin temperatures during immersion in hot water (Fox *et al.*, 1964; Regan *et al.*, 1996). The incorporation of a post-exercise hot water immersion into training may therefore provide a greater stimulus for heat acclimation or reduce the time required to be immersed in hot water to provide similar benefits. The similarity in the extent of resting core temperature reduction may also provide information regarding the retention of adaptations, which was hypothesised to be up to a month when large meaningful reductions in resting core temperature are initiated following exercise-heat acclimation, this however is yet to be substantiated (**Section 2.5**; Pandolf *et al.*, 1977; Weller *et al.*, 2007; Brazaitis and Skurvydas, 2010; Tyler *et al.,* 2016).

The current hot water immersion heat acclimation research is however limited by small sample sizes, a lack of physiological measurements and large differences in the extent of induced adaptation. In addition, the effect of repeated daily immersion in hot water following exercise in temperate conditions on hallmarks of heat acclimation is yet to be established. Also, thermoregulatory assessment of heat acclimation induction from immersion in hot water has either been made during immersion in hot water (Brazaitis and Skurvydas, 2010; Shin *et al.*, 2013) or during low intensity exercise (Brebner *et al.*, 1961; Bonner *et al.*, 1976). The induced adaptations from hot water immersion heat acclimation may have a beneficial effect on exercise capacity and/or performance in the heat; again, however, this requires investigation (Brebner *et al.*, 1961; Bonner *et al.*, 1976).

Hot water immersion heat acclimation protocols may also have practical advantages over exercise based protocols, through the potential to be easily incorporated into training regimes or taper (Brebner *et al.*, 1961; Bonner *et al.*, 1976; Brazaitis and Skurvydas, 2010; Shin *et al.*, 2013). It is also conceivable that much like the post-exercise sauna intervention described earlier, a novel protocol involving the completion of hot water immersion after exercise in temperate conditions could be incorporated into post-exercise washing regimes (**Section**

2.3.3; Scoon *et al.*, 2007; Stanley *et al.*, 2015). The integration of a hot water immersion following training may increase training stress however, unlike conventional exercise-heat acclimation the completion of these protocols would not increase training load, therefore possibly avoiding the performance decrements associated with overreaching (Meeusen *et al.*, 2013). Another potential advantage of a post-exercise hot water immersion heat acclimation is that this protocol could be easily adapted to suit a number of different situations. For example, when the aim is to improve tolerance to a standard heat challenge, the length of the immersion or water temperature could be reduced. Alternatively, for athletes wishing to optimise performance, completing a longer duration immersion may increase the thermal impulse and the magnitude of subsequent adaptation (Brebner *et al.*, 1961; Bonner *et al.*, 1976; Brazaitis and Skurvydas, 2010). It should be taken into consideration however that the perceptual discomfort associated with immersion in hot water could cause participants to remove themselves from the heat stress, in turn, reducing the thermal impulse and attenuating adaptation (Taylor *et al.*, 1997; Taylor *et al.*, 2014). On the other hand, highly motivated individuals driven to maximise adaptation should be aware of dangers such as burning the skin in very hot water, syncope when exiting the immersion and the development EHI, which may occur with large elevations in body temperatures. Accordingly, research is required to confirm the outlined benefits of a post-exercise hot water immersion protocol on the initiation of hallmark heat acclimation adaptations, the attenuation of thermal strain during exercise-heat stress and the improvement of exercise capacity/performance in the heat.

2.7 Thesis objectives

The present literature review has highlighted the practical difficulties regarding the completion of heat acclimation and interrogated current recommendations that are associated with acclimating to the heat. The aim of this thesis was to investigate the efficacy of a short-term post-exercise hot water immersion heat acclimation intervention. This thesis also investigates the effect of post-exercise hot water immersion on exercise capabilities and endurance performance in temperate and hot conditions. Additional objectives of this thesis include; establishing whether the intervention induces adaptations that are clock-time dependent, assessing if the intervention induces heat acclimation adaptations in both endurance trained and recreationally active males and to track the phenotypic decay of the induced adaptations.

CHAPTER THREE

General Methods

3.1 Ethical Approval

Each study received ethical approval from the School of Sports, Health and Exercise Sciences at Bangor University (**Chapters 4, 5, 6 and 7**) and was conducted in accordance with the Declaration of Helsinki (2013).

3.2 Participants

Following a full briefing of experimental procedures, all participants provided full written informed consent (**Appendix A**) and completed a medical screening questionnaire (**Appendix B**). Participants were physically active, healthy, non-smokers, free from any known cardiovascular or metabolic diseases and were not taking any medication. Additionally, prior to the commencement of their participation, all individuals were non-heat acclimatised (no heat exposure in the previous 3-months) and did not regularly take hot baths or saunas (≤ 1 per week).

3.3 Maximum oxygen uptake and speed verification

Maximum oxygen uptake ($\dot{V}O_{2max}$) was assessed by means of a continuous incremental exercise test on a motorised treadmill (**Chapters 4, 5, 6 and 7**). Participants began by running for 3 min at 8 $km \cdot h^{-1}$, 1 % gradient. Following this, running speed was increased by 2 $km \cdot h^{-1}$ every 3 min, with the gradient kept constant until 16 $km \cdot h^{-1}$. On the completion of the 16 $km \cdot h^{-1}$ stage, the gradient was increased by 2.5 % every 3 min until volitional exhaustion. Expired air was analysed continuously using an on-line breath-by-breath system (Cortex Metalyser 3B, Biophysik, Leipzig, Germany) to determine oxygen uptake ($\dot{V}O_2$). Using the interpolation of the running speed–$\dot{V}O_2$ relationship, the running speed that elicited 65 % $\dot{V}O_{2max}$ was then determined and verified 30 min later with a 60 s expired gas sample collected by Douglas bag method. This individualised running speed was used for both the submaximal exercise during experimental trials and intervention days (**Chapters 4, 5, 6 and 7**).

3.4 Physiological measurements

3.4.1 Body temperatures

Rectal (T_{re}; **Chapters 4, 5, 6 and 7**) and oesophageal core temperature (T_{es}; **Chapter 7**) were measured using flexible, sterile, disposable thermistors (Henleys Medical Supplies Ltd., Herts, UK) and recorded using a data logger (YSI model 4000A, YSI, Dayton, USA). Prior to insertion of the T_{re} thermistor, a bead was fixed 10 cm from the inserted end to ensure the thermistor remained inserted to the same depth. The T_{es} thermistor was inserted through the nasal fossae to a depth of 25 % of the participant's height (Ergonomics, 2004). Skin temperatures, were measured using stainless steel surface temperature thermistors (Grant EUS-U, Cambridge, UK) from four sites on the right side of the body (on the chest at a midpoint between the acromion process and the nipple, the lateral mid-bicep, the anterior mid-thigh and lateral calf) fixed using surgical tape and recorded using a portable data logger (Grant SQ2020, Cambridge, UK). Mean T_{sk} was calculated (**Chapters 4, 5, 6 and 7**) using a four-site weighted equation (Ramanathan, 1964).

3.4.2 Sweating responses

The change in dry nude body mass during experimental trials and intervention days was used to estimate whole body sweat rate (WBSR; **Chapters 4, 5, 6 and 7**). Local forearm sweating rate was measured by dew point hygrometry during experimental trials **(Chapters 4, 5, 6 and 7)**. To measure local sweating rate anhydrous compressed nitrogen was passed through a 5 cm^3 capsule placed on the lower arm ventral surface (halfway between the antecubital fossa and carpus) and connected to a hygrometry system (DS2000; Alpha Moisture Systems, UK). Local sweating rate was calculated using the difference in water content between effluent and influent air and normalised for the skin surface area under the capsule. Using GraphPad Prism (version 5.02, GraphPad Inc, San Diego, CA, USA) a segmented linear regression determined the active thermoregulatory sweating threshold for T_{re} (**Chapters 4, 5, 6 and 7**) and T_{es} (**Chapter 7**) and sweating sensitivity (**Chapter 7**) by plotting individual relationships between local forearm sweating rate and core temperature (Cheuvront *et al.*, 2009).

3.4.3 Heart rate

During all experimental trials and intervention days (**Chapters 4, 5, 6 and 7**) HR was monitored (Polar FT1, Polar Electro, Kempele, Finland) continuously and recorded every 5 min during all exercise, water immersion, rest and recovery periods.

3.4.4 Ventilatory responses

$\dot{V}O_2$ and respiratory exchange ratio (RER) were assessed from 60 s expired gas samples collected by Douglas bag method at 9-10, 19-20, 29-30 and 39-40 min of exercise during experimental trials (**Chapters 4, 5, 6 and 7;** James *et al.,* 2006).

3.4.5 Blood sample collection and analysis

Venous blood samples were collected following a 20 min seated rest, prior to exercise during experimental trials by a trained phlebotomist by venepuncture, without stasis and from an antecubital vein into a 6 mL EDTA (ethylenediaminetetraacetic acid) vacutainer (BD, Oxford, UK). Aliquots of whole blood were used for the immediate determination of haemoglobin (Hb) concentration (g·dL), in duplicate (Hemocue, Sheffield, UK) and haematocrit (Hct; capillary tube method; %), in triplicate (**Chapters 4, 5, 6 and 7**). The change in plasma volume was estimated by correcting the initial plasma volume for the percentage change in plasma volume between experimental trials (**Chapters 4, 5, 6 and 7**), as described (Dill and Costill, 1974).

3.4.6 Optimised CO rebreathing technique

Total Hb mass, blood and plasma volume were assessed using the optimised carbon monoxide (CO) rebreathing technique (**Chapter 7**; Schmidt and Prommer, 2005). In brief, following a 20 min seated rest, earlobe capillary blood samples (65 μL) were collected and assessed for carboxyhaemoglobin concentration (% COHb; ABL80 CO-OX Flex hemoximeter Radiometer; Copenhagen, Denmark). Participants were then instructed to exhale into a CO gas meter (Drager Pac 3500, Pittsburgh, Pennsylvania, USA), to provide baseline end-tidal CO concentration (ppm) from the lungs. Participants then rebreathed within a closed system a bolus (0.8-1.0 $mL \cdot kg^{-1}$ body mass) of CO (99.9 %) and oxygen (O_2; 3 L; 99.5%), that was mixed on the first inhalation, for 2 min. During rebreathing a CO meter (Drager Pac 3500, Pittsburgh, Pennsylvania, USA) was held close to the spirometer to ensure there were no leaks. After 2 min, participants exhaled to residual volume and were disconnected from the spirometer, this expired air sample was collected and CO content was measured. End-tidal CO concentration in the lungs was again assessed at 4 min, and earlobe capillary samples were taken at 6 and 8 min following the commencement of rebreathing and analysed for % COHb. The pre to post change in % COHb and the volume of CO distributed in the body was used to calculate total Hb mass (SpiCO calculation software, Version 2.0;

Blood tec, Bayreuth, Germany). The experimenter typical error of measurement for total Hb mass was 1.96 % (**Appendix C**). Total Hb mass, Hb concentration and Hct (%) was used to calculate blood volume (mL; [Hb mass / Hb concentration] × 100) and red cell mass (mL; blood volume × [Hct / 100]) for the calculation of plasma volume (mL; = blood volume – red cell mass). The change in plasma volume was estimated by correcting the initial volume for the percentage change.

3.4.7 Urine analysis

Prior to exercise on the day of experimental trials and intervention sessions a mid-flow urine sample was collected into a 25 mL universal container and immediately analysed for urine specific gravity (USG) using a handheld refractometer (Atago URC-Osmo refractometer, Japan) to confirm euhydration (< 1.03; **Chapters 4, 5, 6 and 7**; Armstrong, 2005). In the event USG > 1.03 participants consumed 500 mL of water and rested in the laboratory for 15 min, a USG measurement was then repeated to confirm euhydration.

3.5 Perceptual measurements

Rating of perceived exertion (RPE; 6-20 scale; Borg, 1970) and thermal sensation (0-13 point; Hollies and Goldman, 1977) were measured every 10 min during all submaximal runs in experimental trials and intervention sessions (**Chapters 4, 5, 6 and 7**). Additionally, thermal sensation was measured every 5 min at rest, during water immersions and during recovery from all exercise/immersion protocols (**Chapters 4, 5, 6 and 7**).

3.6 Statistical analysis

Data are presented as mean ± standard deviation (SD) and statistical significance was accepted at $P < 0.05$. Data were checked for normality and sphericity and Greenhouse-Geisser adjustments to the degrees of freedom were applied where necessary. The size of the within-subject and between group effect was also calculated using Cohen's effect size (d) with effect sizes greater than 0.2, 0.5 and 0.8 representing small, medium and large effects (Cohen, 1988). Sweating threshold was calculated by plotting individual relationships between local forearm sweating rate and T_{re} as described (**Chapters 4, 5, 6 and 7**; Cheuvront *et al.*, 2009). Sweating threshold and sensitivity was also calculated by plotting individual relationships between local forearm sweating rate and T_{es} as described (**Chapter 7**; Cheuvront *et al.*, 2009). An area under the curve (AUC) analysis was performed on T_{re} (time

T_{re} was >38.5 °C) during intervention sessions to serve as an indicator of the adaptation impulse (**Chapters 4, 5, 6 and 7**; Cheuvront *et al.*, 2008). All statistical analysis was conducted using SPSS software version 22 (IBM Corporation, New York City, New York, USA) and GraphPad Prism Version 5.02 (GraphPad Software Inc, La Jolla, California, USA).

CHAPTER FOUR

Post-exercise hot water immersion induces heat acclimation and improves endurance exercise performance in the heat

4.1 Summary

We examined whether a daily hot water immersion after exercise in temperate conditions induces heat acclimation and improves endurance performance in temperate and hot conditions. Seventeen non-heat acclimatised males performed a 6-day intervention involving a daily treadmill run for 40 min at 65 % $\dot{V}O_{2max}$ in temperate conditions (18 °C), followed immediately by either a hot water immersion (HWI; n = 10; 40 °C) or thermoneutral water immersion (CON, n = 7; 34 °C) for ≤ 40 min. Before and after the 6-day intervention, participants performed a treadmill run for 40 min at 65 % $\dot{V}O_{2max}$ followed by a self-paced 5 km treadmill TT in temperate (18 °C, 40 % RH) and hot (33 °C, 40 % RH) conditions. In HWI, heat acclimation was demonstrated by lower resting T_{re} (-0.27 °C, P < 0.01), and end-exercise T_{re} during submaximal exercise in 18 °C (-0.28 °C, P < 0.01) and 33 °C (-0.36 °C, P < 0.01). T_{sk}, T_{re} at sweating onset and RPE were lower during submaximal exercise in 18 °C and 33 °C after 6-days on HWI (P < 0.05) and thermal sensation was also lower during submaximal exercise in 33 °C after 6-days on HWI (P < 0.05). In HWI, TT performance improved in 33 °C (4.9 %, P < 0.01) but not in 18 °C. Thermoregulatory measures and performance did not change in CON. The 6-day post-exercise hot water immersion intervention presents a simple, practical and effective heat acclimation strategy to improve endurance performance in the heat.

4.2 Introduction

Athletes, military personnel and firefighters are often required to perform in the heat, which increases physiological demands and places substantial strain on heat loss mechanisms (**Section 2.1**; Cheung *et al.*, 2000). To reduce the risk of EHI and improve exercise capabilities in the heat, these individuals often prepare by completing conventional exercise-heat acclimation protocols (**Section 2.3**). The adaptive responses to exercise-heat acclimation have been widely documented and include, but are not limited to, earlier onset of sweating, increases in sweating rate, reduced cardiovascular strain and, in-turn, reduced core temperature and physiological strain and improved endurance capacity during exercise in the heat (**Section 2.2**; Taylor, 2014). Some evidence also supports the notion that the ergogenic benefit of exercise-heat acclimation extends to endurance performance in cool conditions (Lorenzo *et al.*, 2010) but recent evidence presents a mixed picture (**Section 2.2.3**; Neal *et al.*, 2016a).

Conventional exercise-heat acclimation protocols typically consist of a daily bout of exercise-heat stress over a 5 to 16-day period where core temperature is increased for 1-2 h in an artificial hot environment (Nadel *et al.*, 1974; Garrett *et al.*, 2009). Exercise intensity during these programmes is either fixed, self-regulated or manipulated to control a precise level of hyperthermia (**Section 2.3**; Fox *et al.*, 1963; Taylor, 2014). Owing to the ensuing adaptations, the training stimulus typically decreases during exercise-heat acclimation programmes (Taylor, 2014). The controlled hyperthermia technique has the advantage that the adaptive stimulus is kept constant, thus optimising adaptation and, via thermal clamping, affording greater insight into underlying mechanisms (**Section 2.3.2**; Fox *et al.*, 1963; Taylor, 2014). However, there are practical disadvantages to using these exercise-heat acclimation protocols. They can be costly and impractical for non-acclimated individuals residing in cooler climates, as their completion requires access to an environmental chamber or temporary relocation to a hotter climate. The controlled hyperthermia technique also requires measurement of core temperature and control of exercise intensity to maintain core temperature at 38.5 °C.

One novel, as yet unexplored, approach to these practical limitations may be to have non-heat acclimated individuals undertake hot water immersion immediately after daily exercise that is performed in temperate conditions (**Section 2.6**). Related support comes from studies showing thermoregulatory adaptations (Fox *et al.*, 1963; Beaudin *et al.*, 2009) and

performance benefits (Scoon *et al.*, 2007) of passive heat acclimation, but these methods are somewhat limited in terms of their practicality and no measures of thermoregulation were reported during exercise-heat stress (Scoon *et al.*, 2007; Beaudin *et al.*, 2009). Support also comes from studies in non-heat acclimated individuals showing that repeated hot water immersion over a 10 to 14-day period decreased core temperature at rest, during immersion in hot water (Brebner *et al.*, 1961; Brazaitis and Skurvydas, 2010) and during light exercise in the heat (Brebner *et al.*, 1961; Bonner *et al.*, 1976). More practical alternatives for heat acclimation would be welcome as these studies used a thermal clamp (Bonner *et al.*, 1976) and an unpleasant 44 °C hot water immersion protocol (Brazaitis and Skurvydas, 2010). Extending beyond the obvious practical advantages, as combined elevations in core and skin temperatures are important to achieve full heat acclimation (Fox *et al.*, 1964; Regan *et al.*, 1996), there is a reasoned physiological argument for why a hot water immersion (elevated skin temperature) immediately after daily exercise in temperate conditions (elevated core temperature) might elicit favourable heat acclimation responses (**Section 2.6**).

To date, no study has investigated whether a daily hot water immersion following exercise in temperate conditions induces heat acclimation and improvements in endurance exercise performance in hot and temperate conditions. To this end, we hypothesised that a short-term (6-day) protocol involving a 40 min hot water immersion each day after submaximal treadmill running in a temperate laboratory would induce heat acclimation and performance improvements during a 5 km treadmill TT in both temperate (18 °C) and hot (33 °C) conditions.

4.3 Methods

Participants: Seventeen physically active (≥ 2 h endurance exercise per week), non-heat acclimatised males were randomly assigned to either a 6-day hot water immersion (HWI: n = 10; mean ± SD, age: 23 ± 3 years; body mass: 69.5 ± 6.9 kg; $\dot{V}O_{2max}$ 60.5 ± 6.8 mL·kg^{-1}·min^{-1}) or control (CON: n = 7; age: 23 ± 3 years; body mass: 72.1 ± 5.8 kg; $\dot{V}O_{2max}$ 60.1 ± 8.9 mL·kg^{-1}·min^{-1}) intervention. There were no differences in the characteristics of participants in HWI and CON.

Study design: Prior to (days -3 to -1; Figure 4.1), and following (days +1 to +4) the 6-day HWI or CON intervention, experimental trials were completed in temperate (18.0 ± 0.1 °C, 42.5 ± 3.6 % RH) and hot conditions (33.0 ± 0.3 °C, 40.2 ± 0.7 % RH; Figure 4.1). Experimental trials included a 40 min submaximal run followed by 60 min rest, then a 5 km treadmill TT. A 6-day intervention was completed to align with other STHA protocols (Aoyagi *et al.*, 1995; Cotter *et al.*, 1997).

Preliminary measurements and familiarisation: Following the measurement of $\dot{V}O_{2max}$ and the verification of a running speed that elicited 65 % of $\dot{V}O_{2max}$ (**Section 3.3**), participants rested in the laboratory for 60 min. During this time, they were familiarised with the speed controls of the treadmill within the environmental chamber and with all instrumentation and procedures used in the experimental trials. Participants then entered the environmental chamber (18 °C, 40 % RH) and completed a maximal effort 5 km treadmill TT at self-selected intensities. One familiarisation was deemed sufficient to mitigate against learning effects (Laursen *et al.*, 2007). The chamber was silent and the only information the participant received was the distance covered displayed on a screen in front of them.

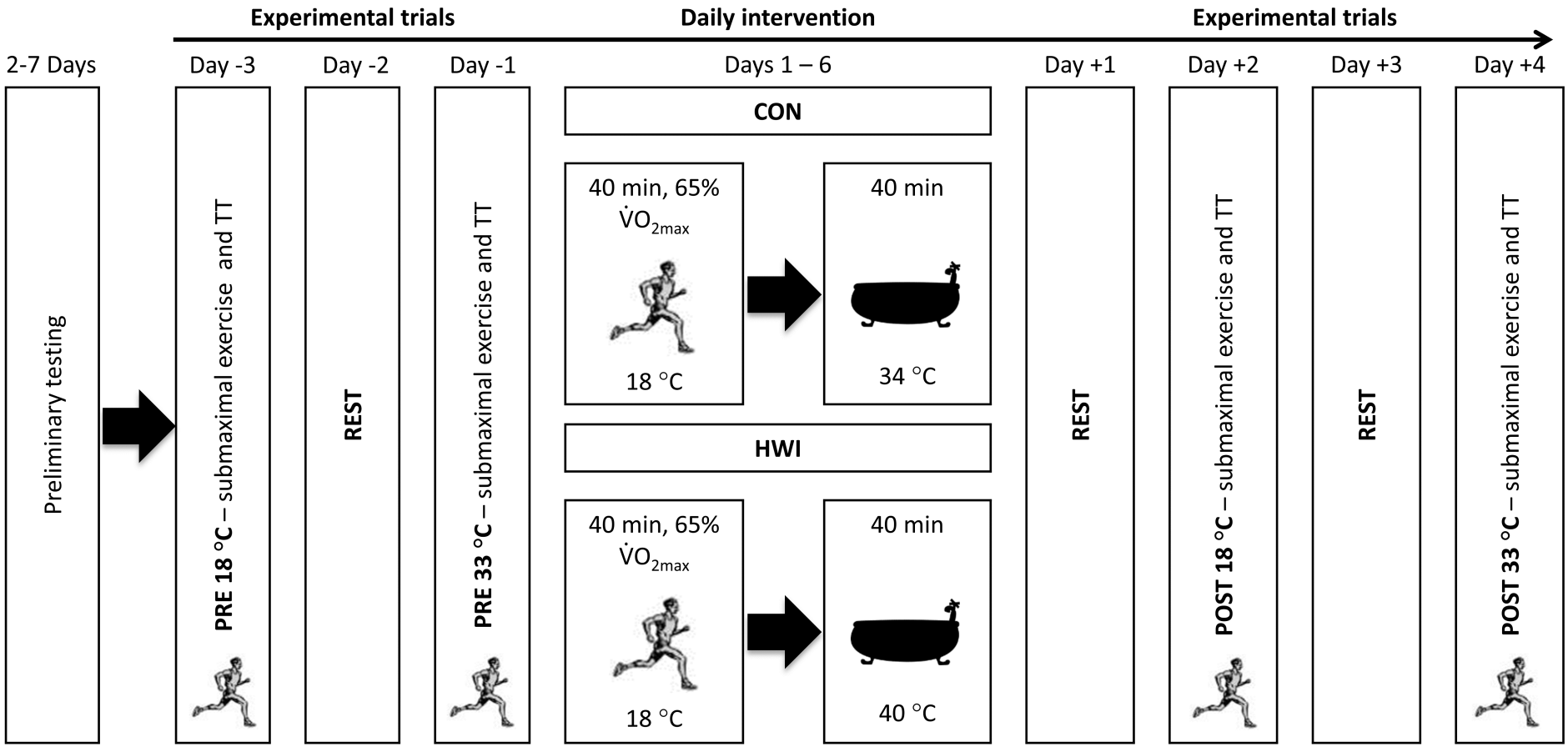

Figure 4.1 Schematic of study design.

Experimental trials: Participants completed a diet diary in the 24 h prior to their first experimental trial and were asked to replicate this prior to further experimental trials. No alcohol, any form of diuretics, caffeine or tobacco were consumed and no exercise was undertaken. On the day of each experimental trial, participants arrived at the laboratory at 0730 h fasted. They were provided with a standardised breakfast (0.03 $MJ \cdot kg^{-1}$) and a bolus of water equivalent to 7 $mL \cdot kg^{-1}$ of body mass. At 0800 h on days -1 and +2 a venous blood sample was taken without stasis following a seated rest (**Section 3.4.5**). Urine samples were then collected and analysed for USG (**Section 3.4.7**). A pre-exercise nude body mass was taken and then insulated thermistors were fixed using surgical tape as described (**Section 3.4.1**). Dressed in T-shirt, running shorts, socks and shoes participants then rested for 15 min in thermoneutral conditions (18 °C) to establish baseline measures.

Submaximal exercise: At 0900 h dressed in running shorts, socks and shoes the participant entered the environmental chamber that was maintained at either 18 °C, 40 % RH (day -3 and +2) or 33 ºC, 40 % RH (days -1 and +4) and completed a 40 min 65 % $\dot{V}O_{2max}$ treadmill run (1 % gradient). Body temperatures, sweating responses, HR and ventilatory responses were assessed as outlined (**Section 3.4**). Following the cessation of exercise the participant provided a nude body mass to estimate WBSR (**Section 3.4.2**) then sat quietly in the laboratory in temperate conditions (18 °C) dressed in T-shirt, running shorts, socks and shoes for 60 min. A single bolus of water (5 $mL \cdot kg^{-1}$ body mass) was consumed within the first 20 min of this rest period.

5 km treadmill time trial: The TT was completed immediately following a nude body mass after the 60 min rest period. The participant re-entered the environmental chamber dressed in running shorts, socks and shoes and completed the TT run on a motorised treadmill (1 % gradient) at self-selected speeds. Participants were instructed to run the 5 km TT as quickly as possible. No feedback other than the distance covered was provided. No fluids were consumed during the TT. T_{re} and HR were monitored continuously and on completion a nude body mass was recorded to estimate WBSR (**Section 3.4**). The participant was then provided with water equivalent to sweat losses and was free to leave the laboratory.

Post-exercise water immersion interventions: The 6-day HWI and CON interventions were completed over consecutive days (days 1 to 6; Figure 4.1). During the intervention,

participants were required to consume their normal diet and fluid intake, including caffeine and alcohol (≤ 3 units per day) and to reduce their regular training by the volume of endurance exercise completed during the intervention in the laboratory. Participants in HWI and CON completed the same submaximal exercise protocol on each of these days in temperate conditions (18 °C) which was followed by a 40 min, 40 °C hot water immersion in HWI or a 40 min, 34 °C water immersion in CON. The CON intervention controlled for any training and/or hydrostatic effects on subsequent thermoregulatory measures and endurance performance.

Submaximal exercise: On each day participants reported to the laboratory between 0600 h and 1000 h. A pre-exercise nude body mass after voiding was recorded and after fitting a rectal thermistor and HR monitor, participants rested in the laboratory for 15 min to establish baseline measures. Participants then ran for 40 min on a motorised treadmill at 65 % $\dot{V}O_{2max}$ in a temperate environment (18 °C) dressed in running shorts, socks and shoes. A bolus of water (5 $mL \cdot kg^{-1}$ of body mass) was consumed in the first 20 min of exercise, to replicate normal training procedures. T_{re} and HR were monitored continuously and RPE (Borg, 1970) and thermal sensation (Hollies and Goldman, 1977) were recorded every 10 min (**Sections 3.4 and 3.5**). At the cessation of exercise, participants undertook the water immersion (2-3 min transition).

Water Immersion: Following transition, participants were immersed to the neck in a water bath dressed in shorts. Those completing HWI were immersed in 39.9 ± 0.3 °C water while a thermoneutral water temperature of 34.1 ± 0.4 °C was used for CON. The water temperature was maintained during immersions by adding hot or cold water and allowing water to drain to maintain immersion to neck level, where necessary. The 34 °C water temperature in CON was chosen as pilot testing showed that T_{re} returned to baseline after exercise at a similar rate to sitting in temperate laboratory conditions (18 °C), and thus would not provide any additional cooling effect. During immersion, no fluids were consumed, T_{re} and HR were monitored continuously and thermal sensation was recorded every 10 min (**Sections 3.4 and 3.5**). Immersion ended after 40 min unless the participant removed themselves prior to this due to discomfort or T_{re} exceeded 39.5 °C in HWI, in which case immersion time was recorded. Following immersion participants sat in the laboratory for 15 min without fluids and a nude body mass was taken and adjusted for fluid intake during the submaximal exercise

in order to estimate WBSR (**Section 3.4.2**). Participants were free to leave the laboratory when $T_{re} \leq 38.5$ °C.

Measurement and instrumentation: T_{re}, T_{sk} calculated using a four-site skin temperature equation, local and WBSR were measured as outlined (**Section 3.4**). Initial plasma volume (day -1) was estimated from body mass, as described (Sawka *et al.*, 1992). On day +2 whole blood samples were used for the immediate determination of Hb and Hct (**Section 3.4.5**). The change in plasma volume (day -1 to +2) was estimated by correcting the initial plasma volume for the percentage change in plasma volume as described (Dill and Costill, 1974).

Statistical analysis: Two sample size calculations (G*Power 3.1.2) were performed using mean data taken from a 5-day heat acclimation study (Garrett *et al.*, 2009) and a 5 km treadmill TT reliability study (Laursen *et al.*, 2007). For a one-tailed *t*-test with alpha level set at 0.05 and power set at 0.8 a sample size of 9 participants was calculated to detect a meaningful end-exercise difference in T_{re} following STHA suggested to be -0.3 °C (Garrett *et al.,* 2009). To detect a meaningful improvement in 5 km treadmill TT performance it was estimated that a sample size of 7 participants was needed. A meaningful difference in 5 km treadmill TT performance was set at 3 %, which is 1 % greater than the day to day variance in this performance test (Laursen *et al.,* 2007) To ensure adequate power for both key variables, and allowing for dropout, a sample size of 10 participants was used for HWI. Data are presented as mean ± standard deviation (SD), or mean and 95 % confidence interval (CI) of the change for one-tailed tests where stated and statistical significance was accepted at $P < 0.05$. All data were analysed using *t*-tests or one-way repeated measure repeated measures ANOVA's. Pearson's correlations were performed to determine the strength of the relationship between the AUC and the change in hallmark heat acclimation variables e.g. change in resting T_{re}.

4.4 Results

4.4.1 Intervention

All participants in HWI and CON completed the 6-day intervention. T_{re} increased on average 1.13 ± 0.24 °C during 40 min of daily submaximal exercise. T_{re} increased a further 1.01 ± 0.31 °C during HWI and returned to the pre-exercise resting level during CON immersion (-1.10 ± 0.26 °C). Total AUC for the 6-day HWI intervention was 156 ± 83 °C·min^{-1} and for CON was 2 ± 4 °C·min^{-1}. Total AUC in HWI was greater on day 3 compared with day 1 (P = 0.05) but was not different on days 4 to 6 compared with day 1; indicating no significant reduction in the daily thermal impulse. No differences for daily AUC were observed in CON. Heat acclimation was demonstrated in HWI by an increase in WBSR by day 4 (P = 0.02) and an increase in immersion time by day 3 (P = 0.04; Table 4.1). By day 5, 9 out of 10 participants completed the full 40 min immersion in HWI: one participant was removed due to reaching the T_{re} safety limit (T_{re} 39.5 °C). On all other occasions when the 40 min immersion was not completed, participants removed themselves due to discomfort (Table 4.1). In CON, all participants completed all 40 min immersions and WBSR was unchanged from day 1 (0.39 ± 0.08 L·h^{-1}).

4.4.2 Experimental trials

Resting responses: Resting T_{re} was lower following HWI in 9 out of 10 participants with a mean change of -0.27 °C (CI: -0.16 to -0.39 °C, P = 0.001, d = 0.75; Figure 4.2 A). There was no change in resting T_{re} in CON (Figure 4.2 A). A moderate negative correlation (r = -0.39, P = 0.13) was observed between the total AUC for the 6-day HWI intervention and the decrease in resting T_{re}. USG was not different between experimental trials and there was a modest increase in plasma volume from day -1 to day +2 in HWI (3 ± 5 %, P = 0.05), with no change in CON (1 ± 3 %, P = 0.31).

Table 4.1 The influence of submaximal running at 65 % $\dot{V}O_{2max}$ for 40 min in 18 °C and post-exercise hot water immersion in 40 °C water immersed to the neck (HWI; n = 10) on daily thermoregulatory variables, heart rate and immersion time. Data displayed as mean ± SD.

	HWI intervention day					
	1	2	3	4	5	6
Submaximal exercise						
Change in T_{re} (°C)	1.11 ± 0.25	1.15 ± 0.23	1.15 ± 0.26	1.22 ± 0.17	1.12 ± 0.19	1.17 ± 0.23
Heart rate (beats·min^{-1})	142 ± 13	142 ± 15	142 ± 14	140 ± 12	139* ± 12	140 ± 11
Hot water immersion						
Change in T_{re} (°C)	0.95 ± 0.27	0.94 ± 0.33	1.04 ± 0.40	0.99 ± 0.31	1.08 ± 0.28	1.09 ± 0.30
Immersion time (min:s)	32:50 ± 07:14	35:18 ± 06:43	38:00* ± 03:30	39:21* ± 01:25	39:36* ± 01:16	39:45* ± 00:47
Participants completing 40 min immersion (n)	4	6	7	8	9	9
Submaximal exercise and hot water immersion						
WBSR (L·h^{-1})	0.89 ± 0.30	0.98 ± 0.33	1.03 ± 0.36	1.08* ± 0.30	1.08** ± 0.26	1.14** ± 0.31

T_{re}, rectal temperature; HR, heart rate; WBSR, whole body sweating rate. *P < 0.05, **P < 0.01 *vs.* day 1.

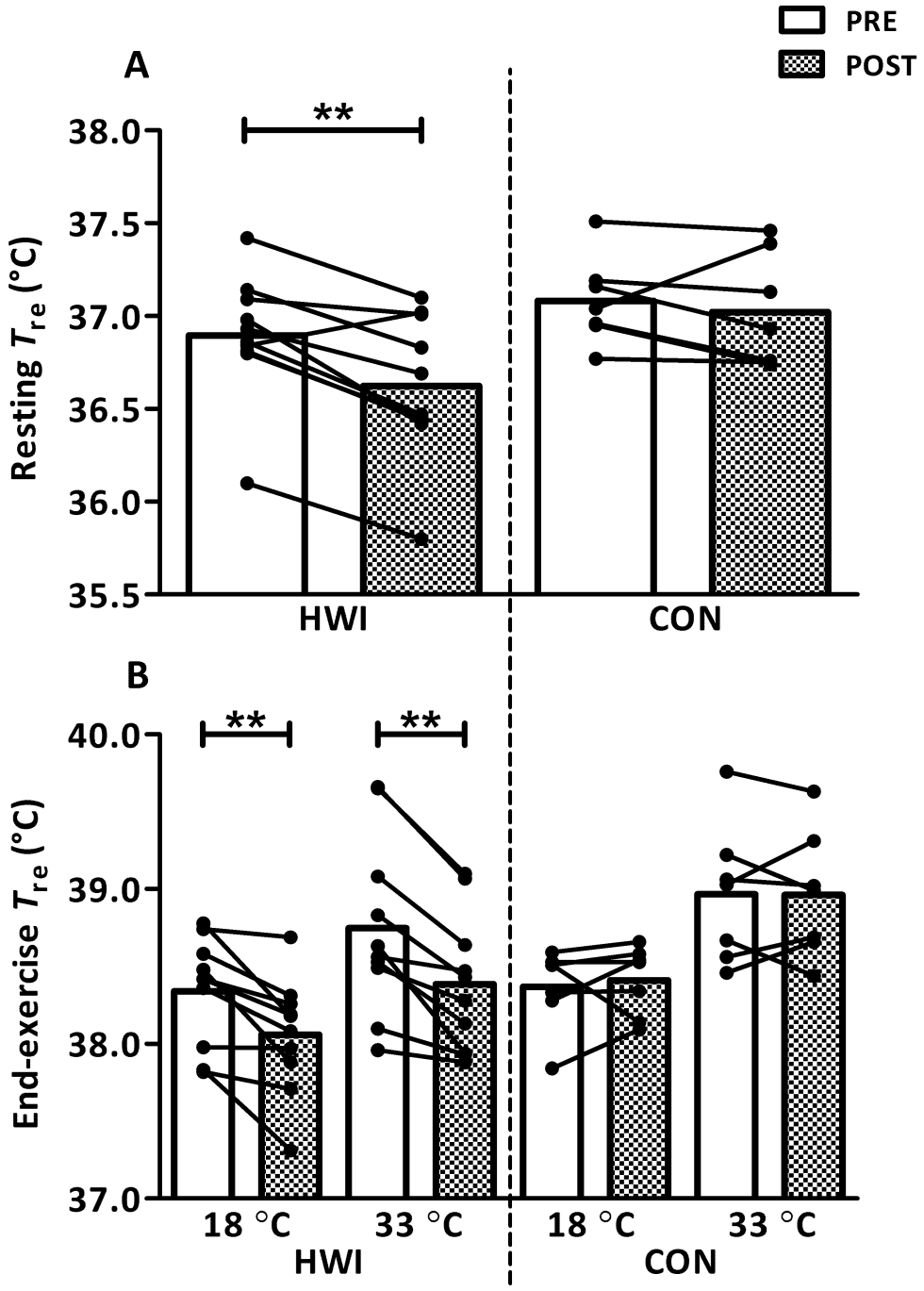

Figure 4.2 Influence of a 6-day post-exercise hot water immersion (HWI) or control (CON) intervention on resting rectal core temperature (T_{re}, A) and end-exercise T_{re} (B) assessed following 40 min running at 65 % $\dot{V}O_{2max}$ in 18 °C (40 % RH) and 33 °C (40 % RH). Bars show mean at PRE and POST. Lines represent individual participants. ** $P < 0.01$, POST less than PRE.

Submaximal exercise responses: After the 6-day HWI intervention, end T_{re} during submaximal exercise was lower in 9 of 10 participants in 18 °C and in all participants (n = 10) in 33 °C (Figure 4.2 B) where the change in end-exercise T_{re} was -0.28 °C (CI: -0.16 to -0.40 °C, P = 0.001, d = 0.78) in 18 °C and -0.36 °C (CI: -0.24 to -0.49 °C, P = 0.0001, d = 0.70) in 33 °C. A modest negative correlation (r = -0.45, P = 0.09) was observed between total AUC for the 6-day HWI intervention and the decrease in end-exercise T_{re} in 33 °C. CON demonstrated no change in end-exercise T_{re} in either 18 °C or 33 °C (Figure 4.2 B). Six days on HWI decreased end-exercise T_{sk} (18 °C: P = 0.001, d = 0.86; 33 °C: P = 0.001, d = 0.60; Figure 4.3 C) and decreased T_{re} at the onset of sweating in both 18 °C (P = 0.001, d = 0.86; Figure 4.3 A) and 33 °C (P = 0.02, d = 0.57). End-exercise RPE (18 °C: P = 0.01, d = 0.74; 33 °C: P = 0.04, d = 0.72; Figure 4.3 E) and HR were lowered in 18 °C (CI: -2 to -11 beats·min^{-1}; P = 0.02, d = 0.52) and 33 °C (-6, CI: -2 to -10 beats·min^{-1}; P = 0.01, d = 0.40; Figure 4.3 D) after HWI and thermal sensation was lower in 33 °C (P = 0.01, d = 0.70; Figure 4.3 F). HWI had no effect on $\dot{V}O_2$ and RER in 18 °C or 33 °C. There was no effect of CON on any of the above variables (Figure 4.3 A-F).

5 km treadmill time trial performance: Endurance exercise performance, assessed via a 5 km treadmill TT, was not altered in CON in either 18 °C (PRE: 1208 ± 191 s and POST: 1216 ± 167 s) or 33 °C (PRE: 1321 ± 219 s and POST: 1299 ± 207 s) indicating no training effect. One HWI participant did not complete the PRE 33 °C TT and another HWI participant's TT data was excluded from analysis due to obvious lack of effort on the POST 18 °C TT (mean % HR max was 82 % compared with 91 % for the group and 96 % for his PRE 18 °C TT). Endurance exercise performance was impaired in 33 °C compared with 18 °C before the intervention (P = 0.03, d = 0.40; Figure 4.4 A). The 6-day HWI intervention did not alter TT performance in 18 °C but improved TT performance in 33 °C (P = 0.01, d = 0.42; Figure 4.4 A and B). The 4.9 % improvement in TT performance in 33 °C in HWI restored performance to the level observed in 18 °C conditions (Figure 4.4 A). After the 6-day HWI intervention end-TT T_{re} was lower in 33 °C (-0.17 °C; CI: -0.04 to -0.30; P = 0.02, d = 0.49). There were no other PRE to POST differences in T_{re} in HWI or CON during the TT.

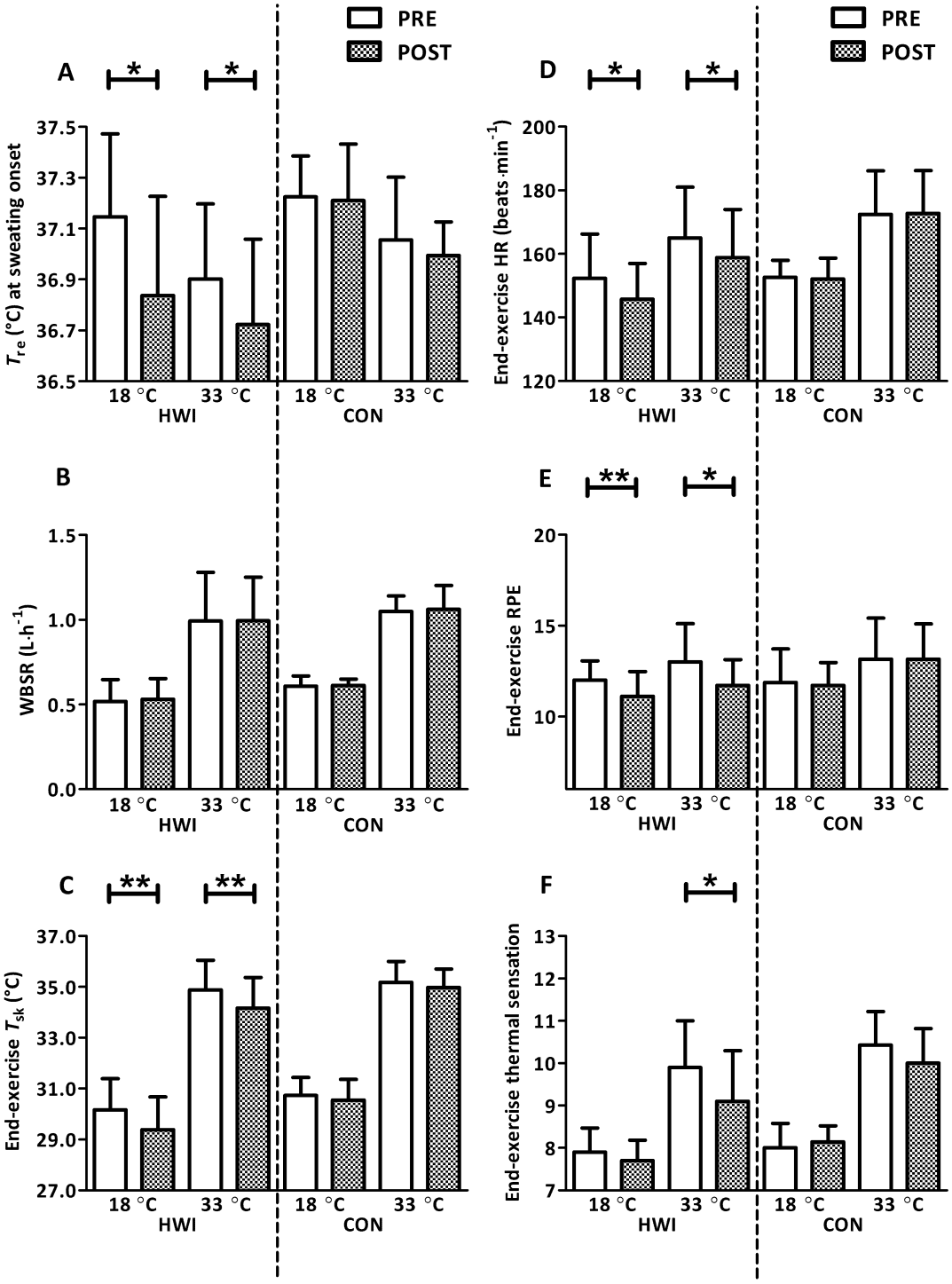

Figure 4.3 Influence of a 6-day post-exercise hot water immersion (HWI) or control (CON) intervention on rectal core temperature at sweating onset (T_{re}, A), whole body sweat rate (WBSR, B) and end-exercise responses for mean skin temperature (T_{sk}, C), heart rate (HR, D), RPE (E) and thermal sensation (F) following 40 min running at 65 % $\dot{V}O_{2max}$ in 18 °C (40 % RH) and 33 °C (40 % RH). Bars show mean and SD at PRE and POST. * $P < 0.05$ and ** $P < 0.01$, POST less than PRE.

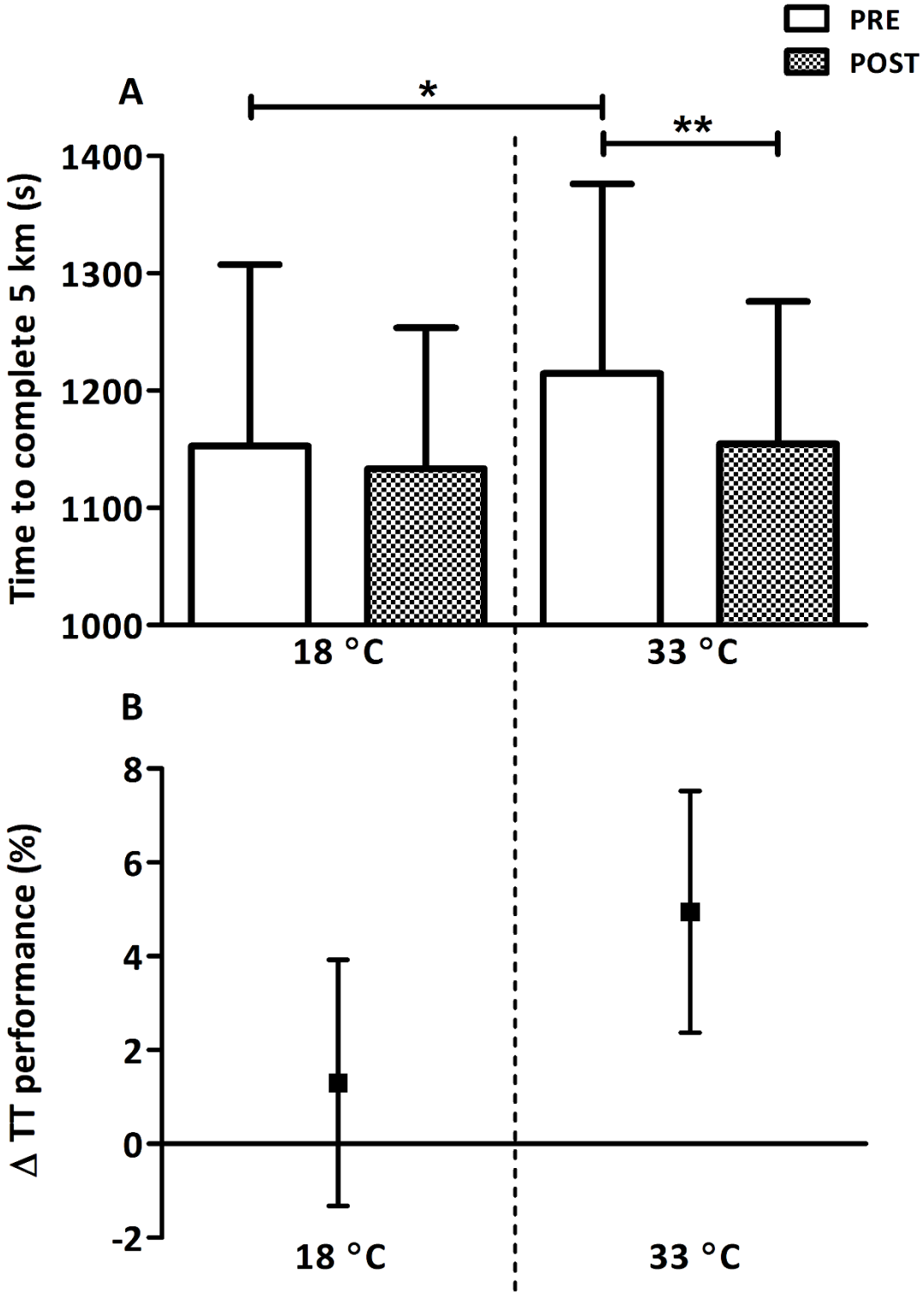

Figure 4.4 Influence of a 6-day post-exercise hot water immersion (HWI) intervention on 5 km treadmill time trial (TT) performance (A) and % change in 5 km treadmill TT performance (B) in 18 °C (40 % RH) and 33 °C (40 % RH). Shown are mean and SD (A) and mean and 95 % CI of the difference (B). ** $P < 0.01$, PRE to POST.

4.5 Discussion

These novel findings suggest that heat acclimation can be achieved by a hot water immersion after exercise in temperate conditions on 6-days and, as such, presents a practical strategy to improve heat dissipation and endurance performance in the heat. There are two principal findings in the present study that support this recommendation. Firstly, we observed clear evidence of heat acclimation after the HWI intervention demonstrated by lower resting T_{re} (-0.27 °C) and lower end-exercise T_{re} in both 18 °C (-0.28 °C) and 33 °C (-0.36 °C). Hallmark heat acclimation responses observed in this study also included a lower set point for sweating onset and reductions in T_{sk}, HR, RPE and thermal sensation during submaximal exercise in the heat after 6-days on HWI. Adaptations were evident sooner than day 6 of HWI; for example, WBSR was increased by day 4 of the intervention. Secondly, the HWI intervention improved 5 km treadmill TT performance in 33 °C (+4.9 %), restoring performance to the level achieved in 18 °C. Strengths of this study include control of the time of day for the intervention and experimental trials and the inclusion of a control group. Including CON provides confidence that the adaptations shown by HWI were attributed to bathing in hot water after exercise, since the daily exercise and thermoneutral water immersions completed by CON did not affect thermoregulatory or performance outcomes. We recognise that the addition of a traditional exercise-heat acclimation group would have enabled for comparisons with the HWI intervention. Additionally, though we observed a modest expansion of plasma volume in HWI we recognise the weakness of estimating this using Hb and Hct and recommend tracer techniques be used to verify this finding.

Current recommendations state that heat acclimation should comprise of repeated bouts of exercise in the heat over 1 to 2-weeks (**Section 2.3**; Racinais *et al.*, 2015). Our findings suggest that a hot water immersion after exercise in temperate conditions on 6-days presents an alternative strategy that elicits adaptation to a similar extent and overcomes some of the practical limitations of current exercise-heat acclimation protocols (Tyler *et al.*, 2016). Hallmarks of successful heat acclimation include a decrease in resting and exercising core temperature and an improved exercise capacity in the heat (**Section 2.2**; Nadel *et al.*, 1974). The utility of exercise based STHA protocols lasting 4 to 6-days has been investigated (Sunderland *et al.*, 2008; Garrett *et al.*, 2012), since most adaptations occur within the first 6-days of heat acclimation (Armstrong and Maresh, 1991) and because a shorter protocol is considered to integrate better into an athlete's training/tapering programme (**Section 2.4.1**;

Garrett *et al.*, 2011). In line with other short-term (Cotter *et al.*, 1997), and traditional, longer term exercise-heat acclimation protocols (Armstrong and Kenney, 1993), our 6-day post-exercise hot water immersion intervention, decreased end-exercise T_{re} (Figure 4.2 B), the T_{re} at the onset of sweating (Figure 4.3 A), T_{sk} (Figure 4.3 C) and restored exercise performance in the heat to the level achieved in 18 °C (Figure 4.4). Furthermore, the thermoregulatory benefits of HWI we observed during submaximal exercise in the heat were also apparent in temperate (18 °C) conditions (Figures 4.2 B and 4.3). There appear to be additional heat acclimation advantages of the post-exercise hot water immersion intervention because, unlike some (Sunderland *et al.*, 2008; Garrett *et al.*, 2009; Garrett *et al.*, 2012), but not all short-term exercise-heat acclimation studies (Gibson *et al.*, 2015a; Neal *et al.*, 2016a; Neal *et al.,* 2016b; Rendell *et al.,* 2017) we also demonstrate a reduction in resting T_{re} (-0.27 °C). The responsible mechanism requires elucidation but may include increased resting skin blood flow and sweating sensitivity (Taylor, 2014), and/or a potential decrease in thermoregulatory set point (Aoyagi *et al.*, 1997); although this concept is controversial (Romanovsky, 2007). The magnitude of adaptation demonstrated in HWI, where the total AUC for T_{re} > 38.5 °C was not different on day 6 *vs*. day 1, compares very favourably with exercise based STHA studies (Sunderland *et al.*, 2008; Garrett *et al.*, 2009; Garrett *et al.*, 2012), including those using controlled hyperthermia (Garrett *et al.*, 2012; Taylor, 2014), although research is required to confirm this. Elegant work by Fox *et al.* (1963) and later by Regan *et al.* (1996) demonstrated that whilst heat acclimation is dependent upon the degree of core temperature elevation, the elevation of skin temperature is important for full heat acclimation (Fox *et al.*, 1964; Regan *et al.*, 1996); therefore, indicating the importance of the exogenous thermal stress and a likely role for raised T_{sk} in the observed adaptations in HWI. Peripheral adaptations to local hot water immersion, with and without a rise in core temperature increased local sweating responses (Fox *et al.*, 1964), later coined "sweat gland training" (Avellini *et al.*, 1982), but the increase in local sweating was more dramatic when both core temperature and skin temperature were elevated (Fox *et al.*, 1964). Thus, the combined elevation of T_{re} (~39.3 °C after each HWI) and T_{sk} during daily HWI in 40 °C (where T_{sk} equilibrates with water temperature) after exercise likely accounts for the additional observed benefits shown compared with exercise based STHA.

The majority of studies investigating the effect of heat acclimation on endurance performance have used TTE protocols, e.g. $\dot{V}O_{2max}$ ramp protocols (Sawka *et al.*, 1985; Garrett *et al.*,

2009) or fixed intensity tests (Nielsen *et al.*, 1997; Scoon *et al.*, 2007). Whilst such tests have shown heat acclimation improvements of ≈ 14 to 32 % (Nielsen *et al.*, 1997; Scoon *et al.*, 2007; Garrett *et al.*, 2009), this magnitude of improvement needs to be considered in the context of the variability of TTE protocols, reported to be as high as 27 % (Jeukendrup *et al.*, 1996). Only a handful of studies have assessed the effect of heat acclimation on self-paced TT performance and these used daily exercise in the heat to induce heat acclimation (**Section 2.2.3**; Lorenzo *et al.*, 2010; Garrett *et al.*, 2012). To our knowledge, this is the first study to investigate the effects of a daily post-exercise hot water immersion intervention on TT performance and here we demonstrate an improvement in 5 km TT performance of 4.9 % in the heat, where performance was restored to the level achieved in 18 °C conditions (Figure 4.4 A). The magnitude of performance improvement exceeds the CV (≈ 2 %) for the 5 km treadmill TT (Laursen *et al.*, 2007), and thus, represents a meaningful performance improvement (Figure 4.4 B) attributable to the heat acclimation adaptations observed. The reduced heat strain after 6-days on HWI is also likely to benefit more prolonged endurance exercise performance in the heat, by blunting the rise in core temperature; though this requires investigation. The potential benefits of heat acclimation on endurance performance in cooler conditions received little attention until one study showed that 10 daily bouts of constant work rate exercise-heat stress improved cycling TT performance by 6 % in 13 °C conditions (Lorenzo *et al.*, 2010). Considering the decrease in thermal strain during submaximal exercise in 18 °C after 6-days on HWI (Figure 4.2 B) we might have anticipated, but did not observe, an improvement in 5 km TT performance in 18 °C. It is conceivable, but requires investigation, that the post-exercise hot water immersion intervention might improve endurance performance in temperate conditions that presents a greater thermal burden such as a 10 km TT (final T_{re} during 18 °C 5 km TT was only 38.6 °C).

This novel heat acclimation intervention overcomes a number of practical limitations with conventional exercise-heat acclimation protocols (**Section 2.3**). Not least, access to a hot training environment is not required, neither is precise control of exercise to ensure T_{re} at 38.5 °C is maintained, but also because a post-exercise hot bath does not interfere with daily training and might be incorporated into post-exercise washing routines. One rationale for exercise based STHA is the fit into an athlete's taper, but there are practical limitations requiring an athlete to perform 90 min of daily exercise in the heat clamped at T_{re} 38.5 °C at any time during training, not least during the specific requirements of the taper. Analogous to

the "live-high train-low" concept (Stray-Gundersen *et al.*, 2001) we contend that the findings of this study support the concept, 'train-cool bathe hot'. Although this alternative heat acclimation strategy appears to conflict with current athlete practice which includes post-exercise cryotherapy, with the purpose of improving recovery, the benefits of cryotherapy to improve recovery have been questioned (Leeder *et al.*, 2012). The benefits of post-exercise hot water immersion heat acclimation are likely greater when core temperature is elevated following exercise, but future research that is mindful of the prior exercise-heat strain, safety and real-world limitations is required to verify this and establish whether the intervention can be optimised for military/occupational or athlete scenarios. For example, in the military/occupational setting where habituation to a standard heat challenge has important benefits in terms of tolerating set working conditions and operational safety (reducing EHI risk), and simplifies implementation with large numbers of individuals (one-size-fits-all), it may be possible to reduce the water temperature (e.g. 39 °C), duration (e.g. 30 min) and frequency (e.g. 4 sessions). However, for the athlete wishing to optimise heat acclimation and exercise performance in the heat the emphasis of the post-exercise hot water immersion intervention should be on ensuring a constant adaptation impulse, which is important for this purpose (Taylor, 2014). Future studies are also required to investigate the decay of heat-acclimation following this intervention, in males and females, and to assess the purported benefits for cellular training adaptations (Tamura *et al.*, 2014) and immunity (Walsh *et al.*, 2011).

In conclusion, a hot water immersion immediately after exercise in temperate conditions on six consecutive days reduced heat strain during submaximal exercise in both temperate and hot conditions, and improved 5 km treadmill TT performance in the heat. For those residing and training in temperate conditions, incorporating a hot water immersion into the post-exercise washing routine on six consecutive days represents a simple, practical, economical and effective heat acclimation strategy to improve endurance performance in the heat.

CHAPTER FIVE

Heat acclimation by post-exercise hot water immersion in the morning reduces thermal strain during exercise-heat-stress in the morning and afternoon

5.1 Summary

Recommendations state that to acquire the greatest benefit from heat acclimation the clock-time of heat acclimation sessions should match the clock-time of expected exercise-heat stress. It remains unknown if adaptations by post-exercise hot water immersion demonstrate time of day adaptations. Thus, we examined whether adaptations following post-exercise hot water immersion completed in the morning were present during morning and afternoon exercise-heat stress. Ten males completed an exercise-heat stress test commencing in the morning (0945 h: AM) and afternoon (1445 h: PM; 40 min; 65 % $\dot{V}O_{2max}$ treadmill run) before (PRE) and after (POST) heat acclimation. The 6-day heat acclimation intervention involved a daily, 40 min treadmill run (65 % $\dot{V}O_{2max}$) in temperate conditions followed by ≤ 40 min hot water immersion (40 °C; 0630–1100 h). Post-exercise hot water immersion initiated reductions at POST in; resting T_{re} (AM; -0.34 ± 0.24 °C, PM; -0.27 ± 0.23 °C; P = 0.002), T_{re} at sweating onset (AM; -0.34 ± 0.24 °C, PM; -0.31 ± 0.25 °C; P = 0.001), and end-exercise T_{re} (AM; -0.47 ± 0.33 °C, PM; -0.43 ± 0.29 °C; P = 0.001), HR (AM; -14 ± 7 beats·min^{-1}, PM; -13 ± 6 beats·min^{-1}; P < 0.01), RPE (P = 0.01), and thermal sensation (P = 0.005). Adaptations by 6-day post-exercise hot water immersion in the morning were not different at AM compared to PM (P > 0.05). Morning heat acclimation by post-exercise hot water immersion induced adaptions at rest and during exercise-heat stress in the morning and afternoon.

5.2 Introduction

Prior to exercise-heat stress, athletes and military personnel are advised to complete a period of heat acclimation to alleviate thermal strain and improve exercise capacity in the heat (Periard *et al.*, 2015). The adaptive responses that improve exercise capacity in the heat include an earlier onset and an increase in sweating rate, a reduction in cardiovascular strain and improved thermal comfort (**Section 2.2**; Gonzalez and Gagge, 1976; Frank *et al.*, 2001; Lorenzo *et al.*, 2010). Despite practical limitations, heat acclimation recommendations state that individuals should exercise in the heat on 5–14 occasions, maintaining a specific degree of hyperthermia (T_{re}; ≥ 38.5 °C) for ≥ 60 min (**Section 2.3**; Taylor, 2014). To acquire the greatest benefit, consensus recommendations state that heat acclimation sessions should be scheduled at the anticipated time of day of future exercise-heat stress (**Section 2.4.2**; Cable *et al.*, 2007; Beaudin *et al.*, 2009; Chalmers *et al.*, 2014; Patterson *et al.*, 2014; Taylor, 2014; Periard *et al.*, 2015). The underpinning evidence for this recommendation stems from the observations that heat acclimation adaptations are clock-time dependent; albeit, this was shown in a passive model of heat stress (Shido *et al.*, 1999). It remains to be shown whether clock-time dependent adaptations extend to an exercise model of heat stress. From a practical standpoint, adhering to this recommendation without disturbing training or sleep patterns is problematic, since athletes and military personnel often move between time zones. Moreover, military personnel may not have pre-warning regarding the time of day when exertional-heat strain may occur, or they may be exposed to heat strain throughout the day.

The scheduling of passive heat acclimation on core temperature circadian rhythm and thermoregulatory responses was examined in a series of investigations in rats (Shido *et al.*, 1991b; Sakurada *et al.*, 1994), and then in humans (**Section 2.4.2**; Shido *et al.*, 1999). Six adult men and women heat acclimated via 9-10 daily, 4 h passive heat exposures commencing in the afternoon (46 °C, 20 % RH), achieved a reduced resting T_{re} and sweating onset (latency and core temperature threshold) during subsequent hot water immersion of the legs (42 °C). The relatively modest adaptations (e.g. reduction in resting T_{re} ≈ 0.2 °C) were only present at the clock-time of daily heat exposures (1500 h – 1700 h) but not in the morning (0900 h – 1100 h). The authors suggested that the clock-time dependent adaptations were due to circadian pattern changes in core temperature, associated with altered autonomic thermoregulatory function, and coined the term ‘time memory’ to describe their observations. Others support this concept, whereby the suprachiasmatic nucleus within the hypothalamus is

thought to retain the clock-time of previous heat exposures, establishing a new core temperature circadian pattern (Maruyama *et al.*, 2007). These findings inform the current recommendation that exercise-heat acclimation sessions should be scheduled at the anticipated clock-time of future exercise-heat stress (Cable *et al*., 2007; Beaudin *et al*., 2008; Chalmers *et al*., 2014; Patterson *et al*., 2014; Taylor, 2014; Periard *et al*., 2015). However, the findings of two studies where comparable exercise-heat acclimation protocols were performed either at the same clock-time as the exercise-heat stress test (Gibson *et al.*, 2015) or at a different time of day (Garrett *et al.*, 2009), show similar reductions in thermal (exercising T_{re}; ≈ -0.25 °C) and cardiovascular strain (exercising HR; ≈ -12 beats·min^{-1}) during exercise-heat stress. Despite not being designed to assess whether adaptations are clock-time dependent, the findings of these studies considered together provide evidence to challenge this notion (Garrett *et al.*, 2009; Gibson *et al.*, 2015).

Post-exercise hot water immersion completed on six consecutive days represents a practical, economical, and effective heat acclimation strategy, which elicits adaptations that may compare favourably to exercise-heat acclimation strategies, although this requires confirmation (**Chapter 4**; Tyler *et al.*, 2016). However, it remains to be shown whether post-exercise hot water immersion heat acclimation adaptations are present at a different clock-time to when the daily intervention occurs. Thus, the aim of the current study was to assess whether adaptations following 6-day post-exercise hot water immersion performed in the morning are observed during both morning and afternoon exercise-heat stress.

5.3 Methods

Participants: Ten physically active males (mean ± SD, age: 23 ± 4 years; body mass: 72.8 ± 7.8 kg; $\dot{V}O_{2max}$ 58.2 ± 8.4 $mL \cdot kg^{-1} \cdot min^{-1}$), who had not been exposed to hot environmental conditions in the 3 months prior to commencing testing, provided written informed consent to participate in the current study.

Study design: To assess whether morning heat acclimation improves thermoregulatory responses during morning (AM) and afternoon (PM) exercise-heat stress, participants performed two experimental trials on the same day, before (PRE) and after (POST) heat acclimation. The times selected for the experimental trials align with previous research showing the clock-time dependency for heat acclimation adaptations (Shido *et al*., 1999), where there is a meaningful difference in resting core temperature (≈ 0.3-0.4 °C between AM and PM; Aschoff, 1983). Heat acclimation involved the completion of six consecutive daily post-exercise hot water immersion sessions in the morning, between 0630 h and 1100 h, as described previously (**Section 4.3**). To control for any training and/or hydrostatic effects data from **Chapter 4** demonstrated that six consecutive daily post-exercise (18 °C) thermoneutral water immersion (34 °C) resulted in no effect on subsequent thermoregulatory measures at rest and during exercise-heat stress in seven males ($\dot{V}O_{2max}$ 60.1 ± 8.9 $mL \cdot kg^{-1} \cdot min^{-1}$).

Preliminary measurements: $\dot{V}O_{2max}$ was assessed using a continuous incremental exercise test on a motorised treadmill in temperate laboratory conditions (20 °C) as described previously (**Section 3.3**). Using the interpolation of the running speed – $\dot{V}O_2$ relationship, a running speed that elicited 65 % $\dot{V}O_{2max}$ was determined and verified 30 min later (**Section 3.3**).

Experimental trials: Participants completed a food diary 24 h prior to PRE and were instructed to replicate this diet 24 h prior to the POST. The food diary verified that no alcohol, diuretics, or caffeine, were consumed. Twenty-four hours prior to, and on the day of the experimental trials participants were also instructed to refrain from any additional exercise. As sleeping patterns can influence thermoregulation (Periard *et al.*, 2015), participants were instructed to sleep between 2200 h and 0700 h to ensure a similar circadian pattern prior to each experimental trial. This was confirmed by monitoring sleep, using an Actigraph (Actigraph GT3X Version 4.4.0, Actigraph, Pensacola, USA), worn on the non-

dominant arm with epoch length set to 1 min. Data was subsequently analysed for sleep efficiency (number of sleep min divided by total number of min in bed, multiplied by 100 to convert to percentage) and sleep duration using Actilife+Sleep Version 6 (Actigraph, Pensacola, USA).

On the day of each experimental trial, participants arrived at the laboratory at 0730 h. On arrival, they were provided with a standardised breakfast and a bolus of water as described (**Section 4.3**). At 0800 h dressed in a T-shirt, running shorts, socks and trainers, participants rested for 20 min in temperate laboratory conditions (20 °C). A venous blood sample was taken without stasis and assessed for Hb and Hct to determine changes is plasma volume (**Section 3.4.5**). A mid-flow urine sample was analysed for USG to confirm euhydration (**Section 3.4.7**; Armstrong, 2005). A rectal thermistor was fitted and T_{re} was recorded continuously between 0900 h and 1540 h. A pre-exercise nude body mass was recorded using digital platform scales and the participants were instrumented for the exercise protocol as described (**Sections 3.4.1 and 4.3**). To establish baseline measures participants rested for a further 30 min in temperate laboratory conditions (20°C).

At 0945 h dressed in running shorts, socks and trainers, participants entered the environmental chamber (33 °C, 40 % RH) to complete the AM trial which involved running for 40 min at 65 % $\dot{V}O_{2max}$ (1 % gradient) as previously described (**Section 4.3**). During this time, no fluids were consumed. T_{re}, T_{sk} and HR were monitored continuously and RPE (Borg, 1970) and thermal sensation (Hollies and Goldman, 1977) were recorded every 10 min (**Sections 3.4 and 3.5**). Local forearm sweating rate was measured every 20 s for the first 15 min of exercise to assess the onset of sweating as previously described (**Sections 3.4.2 and 4.3**). $\dot{V}O_2$ and RER were assessed from 60 s expired gas samples collected by Douglas bag method as described (**Sections 3.4.4 and 4.3**). On completion of the AM trial participants exited the environmental chamber. A nude body mass was taken 15 min following the cessation of exercise to estimate WBSR (**Sections 3.4.2 and 4.3**). Participants then rested in temperate laboratory conditions (20 °C) dressed in T-shirt, running shorts, socks and trainers during which fluid intake matched body mass losses during the AM trial. At 1230 h, participants were provided with a standardised lunch (0.03 $MJ \cdot kg^{-1}$) and a bolus of water (7 $mL \cdot kg^{-1}$ body mass). At 1330 h, participants were prepared for the PM experimental trial. At

1445 h, participants entered the environmental chamber to complete the PM trial, adopting identical procedures to the AM experimental trial.

Post-exercise hot water immersion heat acclimation: The post-exercise hot water immersion heat acclimation intervention was performed on six consecutive days as previously described (**Section 4.3**). Each day, Participants arrived at the laboratory between 0630 h and 0830 h.

Measurement and instrumentation: T_{re}, T_{re} at sweating threshold, T_{sk} calculated using a four-site skin temperature equation, local and WBSR were measured as previously outlined (**Section 3.4**). To assess cumulative hyperthermia, an AUC analysis (time T_{re} was ≥ 38.5°C) was performed on the daily T_{re} during the intervention as previously described (**Section 3.6**; Cheuvront *et al*., 2008). At PRE and POST, whole blood samples were used for the immediate determination of Hb and Hct (**Section 3.4.5**). The PRE to POST change in plasma volume was estimated by correcting the initial plasma volume for the percentage change in plasma volume as described (**Section 3.4.5**; Dill and Costill, 1974).

Statistical analysis: Using previous data (**Chapter 4**), a sample size estimation (G*Power 3.1.2) with an alpha level of 0.05 and power of 0.95, determined that eight participants were required to detect a significant difference in resting T_{re} (-0.27 °C) and end-exercise T_{re} (-0.36 °C) following post-exercise hot water immersion heat acclimation. To ensure adequate power and allowing for dropout, 10 participants were recruited. Data is presented as mean ± SD and statistical significance was accepted at $P < 0.05$. All data were checked for normality and sphericity. Two-way repeated measures ANOVA's with Greenhouse Geisser correction to the degrees of freedom (where necessary) were used to assess differences between the PRE and POST, AM and PM trials. Partial η^2 (ηp^2) were reported to analyse the magnitude of the effects. Cohen (1988) has provided benchmarks to define small ($\eta p^2 = 0.01$), medium ($\eta p^2 = 0.06$), and large ($\eta p^2 = 0.14$) effects. Paired sample *t*-tests were used to assess AM to PM differences in the magnitude of change from PRE to POST. All data was analysed using SPSS version 20 (IBM Corporation, NY, USA), or GraphPad Prism Version 5.02 (GraphPad Software Inc. La Jolla, USA).

5.4 Results

5.4.1 Post-exercise hot water immersion heat acclimation

All participants completed a 40 min treadmill run at 65 % $\dot{V}O_{2max}$, followed by hot water immersion (≤ 40 min) on six consecutive days. Hot water immersion time increased from 30 ± 6 min on day 1 to 40 ± 0 min on day 6 (Table 5.1). Daily end-immersion T_{re} averaged 39.34 ± 0.29 °C and daily AUC averaged 27 ± 13 °C·min^{-1}. No differences were observed for change in T_{re} or the AUC between the daily sessions, demonstrating a constant endogenous stimulus for adaptation during the 6-day intervention (Table 5.1: $P > 0.05$).

5.4.2 Experimental trials

There were no differences in sleep efficiency nor sleep duration the night before the experimental trials ($P > 0.05$). Resting T_{re}, T_{re} at sweating onset and end-exercise HR were higher at PM compared to AM ($P < 0.05$). Heat acclimation adaptations were observed during experimental trials between 0900 h to 1540 h evidenced by reductions in T_{re} (Figure 5.1). In both the AM and PM trials a reduction in resting T_{re} ($P = 0.002$, $np^2 = 0.68$; Figure 5.2A) and end-exercise T_{re} ($P = 0.001$, $np^2 = 0.75$; Figure 5.2B) were observed PRE to POST, with no difference in the magnitude of adaptation between AM and PM (resting and end-exercise T_{re}; $P > 0.05$). Control data from **Chapter 4** provides confidence that the adaptations shown are attributed to bathing in hot water after exercise, since daily exercise in temperate conditions followed by thermoneutral water immersion (34 °C) did not affect thermoregulatory outcomes (Figure 5.2; data shown for comparison only). Other hallmark adaptations of heat acclimation were not different between the AM and PM trials (Table 5.2: $P > 0.05$) including the decrease in: T_{re} at sweating onset ($P = 0.001$; $np^2 = 0.71$); end-exercise HR ($P < 0.001$; $np^2 = 0.85$); RPE ($P = 0.01$; $np^2 = 0.52$); thermal sensation ($P = 0.005$; $np^2 = 0.60$); T_{sk} ($P = 0.01$; $np^2 = 0.51$), and mean $\dot{V}O_2$ ($P = 0.02$; $np^2 = 0.46$) from PRE to POST. No differences were observed from PRE to POST in the AM or the PM trial for RER and WBSR (Table 5.2: $P > 0.05$). Furthermore, relative changes in plasma volume were not significant from PRE to POST (+2.6 %; $P > 0.05$).

Table 5.1 The influence of submaximal running at 65 % $\dot{V}O_{2max}$ for 40 min in temperate conditions (20 °C) and post-exercise hot water immersion in 40 °C on daily thermoregulatory variables, heart rate, and immersion time.

	Hot water immersion intervention day					
	1	2	3	4	5	6
Submaximal exercise						
Change in T_{re} (°C)	1.17 ± 0.28	1.19 ± 0.28	1.14 ± 0.26	1.13 ± 0.32	1.05 ± 0.24	1.11 ± 0.30
End HR (beats·min^{-1})	154 ± 7	150 ± 9	149 ± 8	146 ± 8	145 ± 8	143 ± 9**
Hot water immersion						
Change in T_{re} (°C)	0.84 ± 0.30	0.86 ± 0.16	1.05 ± 0.21	1.00 ± 0.20	0.92 ± 0.15	0.99 ± 0.16
Immersion time (min)	30 ± 6	37 ± 4	38 ± 4	38 ± 4	39 ± 2	40 ± 0**
Participants completing 40 min (n)	1 of 10	6 of 10	8 of 10	6 of 10	8 of 10	10 of 10
Submaximal exercise and hot water immersion						
AUC (°C·min^{-1})	27 ± 17	27 ± 16	30 ± 12	27 ± 15	23 ± 14	27 ± 14
WBSR (L.h^{-1})	0.94 ± 0.29	0.92 ± 0.20	0.97 ± 0.25	1.03 ± 0.27	1.04 ± 0.25	1.09 ± 0.23**

T_{re}, rectal temperature; HR, heart rate; AUC, area under the curve; WBSR, whole body sweating rate. ** $P < 0.01$ indicates a significant difference between day 1 and day 6. Data displayed as mean ± SD.

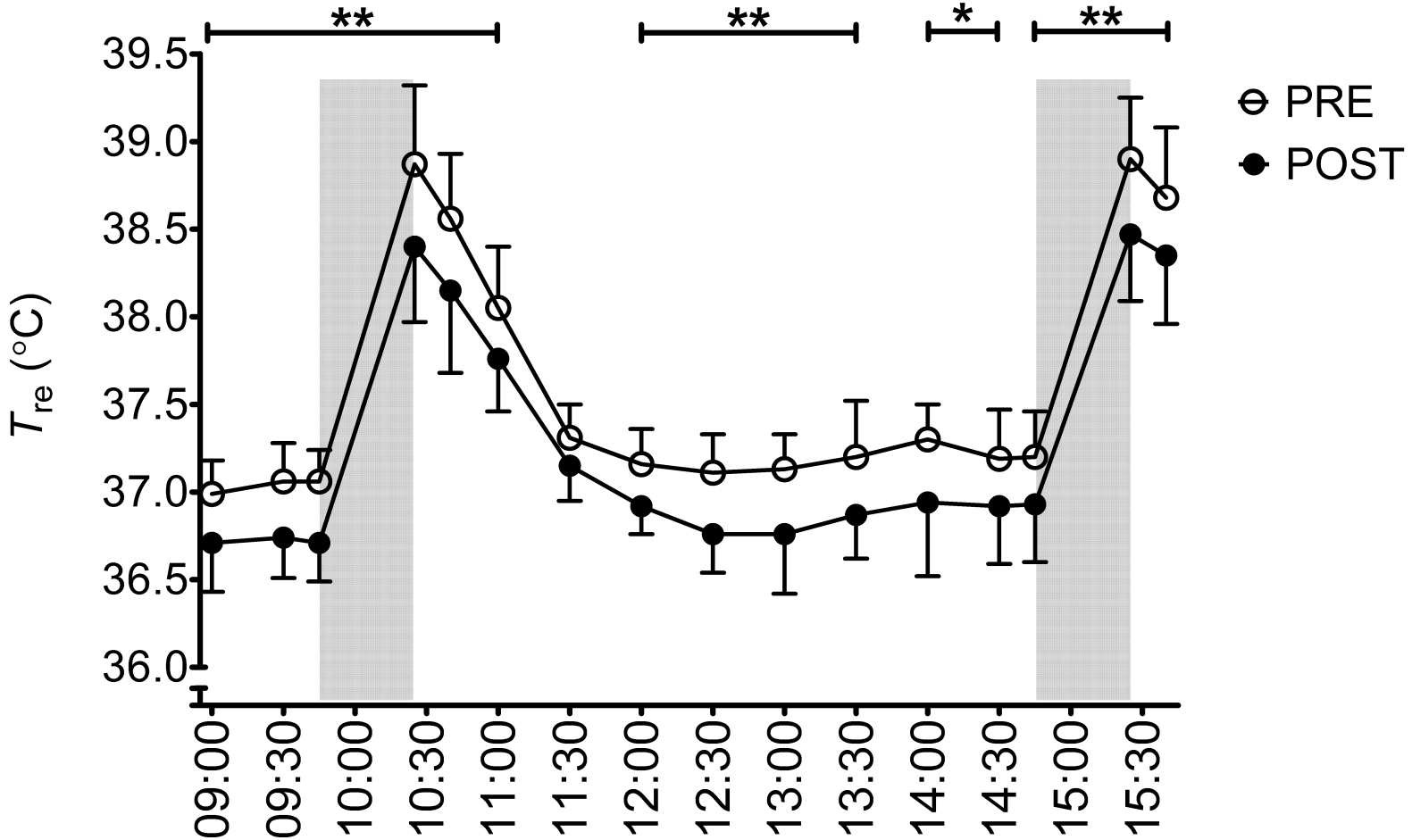

Figure 5.1 Effect of 6-day post-exercise hot water immersion heat acclimation on rectal temperature (T_{re}) responses between 0900-h and 1540-h. Filled grey boxes on x-axis represents period of submaximal treadmill running (40 min, 65 % $\dot{V}O_{2max}$) in the heat (33 °C, 40 % RH). * $P < 0.05$ and ** $P < 0.01$ indicates POST less than PRE. Data displayed as mean ± SD.

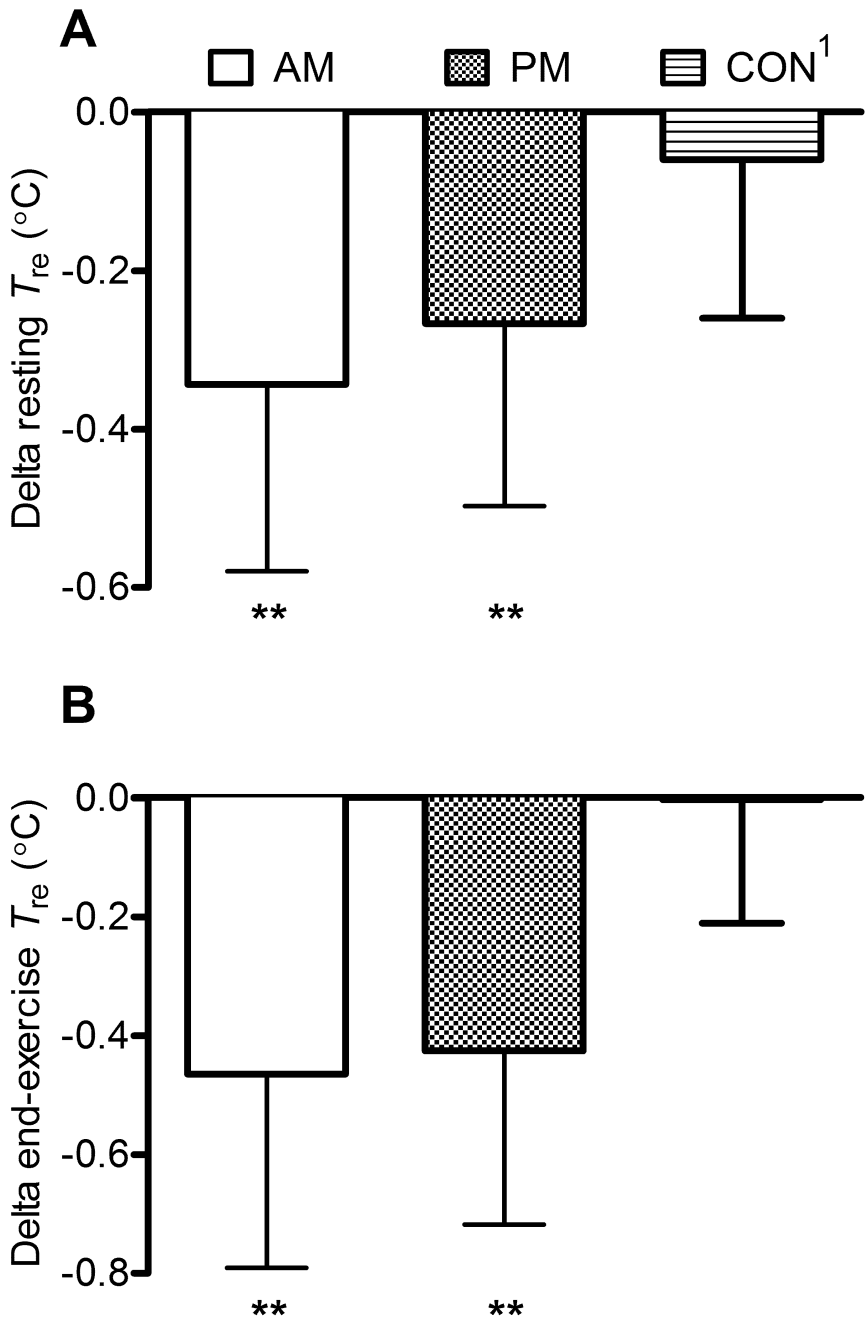

Figure 5.2 Change in resting (A) and end-exercise heat stress (40 min, 65 % $\dot{V}O_{2max}$, 33 °C, 40 % RH; B) rectal temperature (T_{re}) following 6-day post-exercise hot water immersion (40 °C) heat acclimation in the morning (AM) and afternoon (PM).[1] Morning control data (CON) following 6-day post-exercise thermoneutral water (34 °C) immersion intervention shown for comparison only (**Chapter 4**). Data displayed as mean ± SD. * $P < 0.05$ and ** $P < 0.01$ indicates POST less than PRE.

Table 5.2 Physiological and perceptual responses during submaximal treadmill running (40 min, 65 % $\dot{V}O_{2max}$) in the heat (33 °C, 40 % RH) in both the morning (AM) and afternoon (PM) following 6-day post-exercise hot water immersion heat acclimation.

	AM		PM	
	PRE	POST	PRE	POST
T_{re} at sweating onset (°C)	37.03 ± 0.21 #	36.68 ± 0.28 # **	37.23 ± 0.28	36.92 ± 0.32 **
End-exercise T_{sk} (°C)	35.01 ± 0.93	34.11 ± 0.85 *	34.86 ± 1.08	34.17 ± 1.04 *
End-exercise HR (beats·min^{-1})	178 ± 11	164 ± 11 ## **	180 ± 12	167 ± 9 **
End-exercise RPE	15 ± 2	13 ± 1 *	15 ± 3	13 ± 1 *
End-exercise thermal sensation	10 ± 2	9 ± 1 **	11 ± 1	9 ± 1 **
Mean $\dot{V}O_2$ (L·min^{-1})	2.99 ± 0.42	2.84 ± 0.47 *	2.98 ± 0.37	2.87 ± 0.49 *
Mean RER	0.87 ± 0.03	0.86 ± 0.02	0.86 ± 0.04	0.86 ± 0.03
WBSR (L·h^{-1})	1.04 ± 0.41	0.97 ± 0.28	0.92 ± 0.20	0.96 ± 0.25
Hb (g·dL)	14.8 ± 0.6	14.6 ± 0.6	-	-
Hct (%)	45 ± 1	44 ± 2	-	-

T_{re}, rectal temperature; T_{sk}, mean skin temperature; HR, heart rate; RPE, rating of perceived exertion; $\dot{V}O_2$, oxygen consumption; RER, respiratory exchange ratio; WBSR, whole body sweating rate; Haemoglobin, Hb; Haematocrit, Hct. # $P < 0.05$ and ## $P < 0.01$ indicates AM less than PM. * $P < 0.05$ and ** $P < 0.01$ indicates POST less than PRE. Data displayed as Mean ± SD.

5.5 Discussion

The novel findings of the current study confirm and advance those previous by showing that hallmark heat acclimation adaptations by post-exercise hot water immersion are not restricted to the clock-time of daily heat exposures (**Chapter 4**). These data provide clear evidence that post-exercise hot water immersion can be performed in the morning to reduce thermal strain during exercise-heat stress in both the morning and afternoon (end-exercise T_{re} AM -0.47 °C; PM -0.43 °C; Figure 5.2B). The observed reduction in thermal strain during exercise-heat stress in the morning and afternoon was achieved, at least in part, through a reduction in T_{re} at rest in temperate conditions (AM -0.34 °C; PM -0.27 °C; Figure 5.2A). Other hallmark heat acclimation adaptations were also evident during exercise-heat stress in both the morning and afternoon; these included a reduction in T_{re} at sweating onset and a reduction in end-exercise HR, RPE, thermal sensation and T_{sk}. However, in line with short-term exercise-heat acclimation and our previous work (**Chapter 4**; Tyler *et al.*, 2016), six days of post-exercise hot water immersion did not alter WBSR during submaximal exercise in the heat.

Current heat acclimation recommendations, based upon the work of Shido and colleagues (1991b; 1999), state that to acquire the greatest benefit, daily heat exposures should be scheduled at the anticipated clock-time of future exercise-heat stress (**Section 2.4.2**; Cable *et al.*, 2007; Beaudin *et al.*, 2009; Chalmers *et al.*, 2014; Patterson *et al.*, 2014; Taylor, 2014; Periard *et al.*, 2015). However, the present data demonstrate that 6-days of post-exercise hot water immersion heat acclimation does not need to be constrained to the same clock-time of future exercise-heat stress, when performed between 0900 h and 1540 h (Figure 5.1). The magnitude of adaptation appears to be slightly smaller in the afternoon compared with the morning for hallmark adaptations (Figure 5.2, Table 5.2). However, a recent meta-analysis considered a 0.3 °C reduction to be a meaningful change in exercising T_{re} (Tyler *et al.*, 2016); as such, the -0.47°C (AM) and -0.43°C (PM) reduction in end-exercise T_{re} observed in the current study can both be considered meaningful adaptations. Indeed, the currently available evidence from short-term exercise-heat acclimation studies challenges the notion that heat acclimation adaptations are clock-time dependent. For example, comparable reductions in thermal and cardiovascular strain were demonstrated during exercise-heat stress when the clock-time of the daily intervention and the exercise-heat stress was either matched (Gibson *et al.*, 2015) or performed at different times of the day (Garrett *et al.*, 2009); albeit these studies were not designed to assess whether adaptations are clock-time dependent. It is

conceivable that the subtle, clock-time dependent reduction in resting T_{re} shown previously (Shido *et al.*, 1999), may be explained by the mild thermal stimulus for adaptation during daily passive heat exposures (+0.7 °C change in T_{re}; Shido *et al.*, 1999). The large, daily disruption to homeostasis during post-exercise hot water immersion heat acclimation (e.g. +2.1 °C change in T_{re}; **Chapter 4**) and controlled hyperthermia exercise-heat acclimation (e.g. +1.7 °C change in T_{re}; Gibson *et al.*, 2015), provides a greater stimulus for adaptation. This larger stimulus, may account for the reduction in T_{re} at rest and reduction in thermal strain during exercise-heat stress in both the morning and afternoon. Notwithstanding, before any changes can be made to current heat acclimation recommendations, further research is required specifically to assess the purported clock-time dependency of exercise-heat acclimation adaptations.

Heat acclimation recommendations state that to acquire the greatest benefit daily heat exposures should be scheduled at the anticipated clock-time of future exercise-heat stress (**Section 2.4.2**; Cable *et al.*, 2007; Beaudin *et al.*, 2009; Chalmers *et al.*, 2014; Patterson *et al.*, 2014; Taylor, 2014; Periard *et al.*, 2015). The data from the current study shows that post-exercise hot water immersion on six consecutive days in the morning reduces thermal strain during exercise-heat stress in both the morning and afternoon. As such, when the time of day of future exercise-heat stress is unknown (e.g. in military or other occupational settings), post-exercise hot water immersion could be considered as a practical heat acclimation strategy. The post-exercise hot water immersion heat acclimation intervention presents an accessible strategy to alleviate thermal strain during exercise-heat stress that could be incorporated into post-exercise washing routines, reducing the interference with daily training (**Chapter 4**). Future research should determine the extent of adaptation across the daily circadian rhythm of core temperature; specifically, from the mid-point of the nadir phase (≈ 0600 h) to the acrophase (≈ 1800 h; Aschoff, 1983). Appropriately controlled studies, in highly trained males and females, should also determine the effect of afternoon heat acclimation on morning exercise-heat stress and determine whether any improvements translate to an enhanced endurance performance and reduced susceptibility to heat illness. It is important these studies assess exercise performance because adaptations and performance outcomes to exercise training may have temporal specificity (Hill *et al*., 1989; Hill *et al*., 1998; Arnett, 2001). In addition, to improve the practical relevance of these findings, future research should investigate whether adaptations are beneficial across different time zones that

replicate international travel for competition (e.g. 9 h, Greenwich Mean Time to Tokyo, Japan, 2020 Olympics).

Hot water immersion after exercise in temperate conditions in the morning on six consecutive days induced heat acclimation adaptions evident at rest and during morning and afternoon exercise-heat stress. Thus, this novel heat acclimation method is a strategy that could be adopted to reduce heat strain when it is unknown if future exercise-heat stress will occur in the morning or afternoon.

CHAPTER SIX

Post-exercise hot water immersion induces heat acclimation in endurance trained and recreationally active individuals

6.1 Summary

Endurance training initiates partial adaptation to the heat and is suggested to speed up the acquisition of complete adaptation from exercise-heat acclimation. Six days of post-exercise hot water immersion provides heat acclimation adaptations however, it remains unknown if habitual endurance training influences the induced adaptations. Thus, we examined if 6-days of post-exercise hot water immersion provides heat acclimation for eight endurance trained (ET; 9 ± 3 h weekly endurance exercise) and eight recreationally active (RA; 3 ± 1 h weekly endurance exercise) males. Before (PRE) and after (POST) post-exercise hot water immersion, hallmarks of heat acclimation were assessed during a 40 min treadmill run (65 % $\dot{V}O_{2max}$) in the heat (33 °C, 40 % RH). Post-exercise hot water immersion induced a similar extent of heat acclimation in endurance trained and recreationally active participants (P > 0.05), evidenced by reduction at POST in end-exercise T_{re} (endurance trained; -0.36 ± 0.17°C, recreationally active; -0.47 ± 0.37°C, P < 0.001). In addition, resting T_{re}, T_{re} at sweating onset, end-exercise thermal sensation, perceived exertion and mean skin temperature were reduced in both groups (P < 0.05). At POST, end-exercise heart rate was reduced in the recreationally active (-15 ± 7 bpm; P < 0.001) whereas, non-significant reductions were observed in the endurance trained (-4 ± 5 bpm; P = 0.13). Whole body sweat rate, respiratory exchange ratio and plasma volume were not different in either group following heat acclimation (P > 0.05). Heat acclimation by post-exercise hot water immersion attenuates thermal strain during exercise-heat stress in endurance trained and recreationally active individuals, without practical alterations to the intervention protocol.

6.2 Introduction

Exercise in the heat augments physiological strain, attenuates exercise capabilities and increases susceptibility to exertional heat illness (**Section 2.1**; Young *et al.*, 1985; Binkley *et al.*, 2002; Racinais *et al.*, 2015b). To attenuate the detrimental effect of exercise-heat stress and to optimise subsequent adaptations, recommendations suggest that athletes and military personnel prepare by completing 5 to 14-days of conventional exercise-heat acclimation, with T_{re} clamped (≥ 38.5 °C, ≥ 60 min) throughout to maintain a progressive thermal impulse (**Section 2.3**; Taylor, 2014; Periard *et al.*, 2015). The induced adaptations from conventional exercise-heat acclimation include an enhanced sweating response, reductions in cardiovascular stain and body temperatures and an increase in aerobic capacity that, in turn, reduces perceptual strain and improves endurance performance in the heat (**Section 2.2**; Gagge *et al.*, 1967; Sawka *et al*., 1985a; Lorenzo *et al.*, 2010; Taylor, 2014). Habitual endurance exercise, which initiates perfuse sweating and elevates body temperatures, improves parameters of physical fitness and initiates a reduction in thermal strain during exercise-heat stress akin to 'partial' heat acclimation (**Section 2.4.3**; Piwonka *et al.*, 1965; Strydom *et al.*, 1966; Gisolfi and Robinson, 1969). In spite of the thermoregulatory benefits demonstrated by the endurance trained, conventional exercise-heat acclimation protocols, that expose individuals to the heat and increase training load, initiate a larger reduction in thermal strain and further improvements in performance in the heat (Strydom and Williams, 1969; Nadel *et al.*, 1974; Shvartz *et al.*, 1979; Lorenzo *et al*., 2010; Racinais *et al.*, 2015b; James *et al*., 2017).

A high level of endurance fitness is suggested to speed up the acquisition of adaptation from conventional exercise-heat acclimation, with a lower number of constant work-rate (100 min, treadmill walking at 4.8 $km \cdot h^{-1}$) heat acclimation sessions required to 'fully' acclimate to the heat (4 *vs* 8 days) in individuals with a greater oxygen uptake capacity ($\dot{V}O_{2max} \approx 61$ $mL \cdot kg^{-1} \cdot min^{-1}$ *vs* ≈ 38 $mL \cdot kg^{-1} \cdot min^{-1}$; **Section 2.4.3**; Pandolf *et al.*, 1977). Full adaptation in this research was however defined as a plateau in end-exercise T_{re} (± 0.1 °C), and as physiological strain progressively decreases with the application of a non-progressive thermal stress, this attenuation in the rise of T_{re} more likely provides a measure of tolerance or habituation, rather than the optimisation of adaptation (**Section 2.3.1**; Pandolf *et al.*, 1977; Taylor, 2014). The partial heat acclimation adaptations demonstrated by the endurance trained may therefore reduce the number of overloading heat acclimation sessions completed compared to the

untrained when completing the same absolute workload, thus exposing the endurance trained to a smaller thermal and physical training stimulus (Pandolf *et al.*, 1977; Taylor, 2014). Consequently, although meaningful reductions in thermal strain are initiated following constant work-rate heat acclimation in the endurance trained, exposure to a larger thermal and physical training stimulus augments reductions in core temperature (-0.4 *vs* -0.7 °C) and HR (-12 *vs* -22 beats·min^{-1}) in the untrained (**Section 2.4.3**; Shvartz *et al.*, 1977; Tyler et al., 2016). The differences in the magnitude of induced adaptation may however be negated if endurance trained and untrained individuals are exposed to a similar progressive thermal stimulus. For example, controlled hyperthermia heat acclimation, which clamps an elevation in core temperature, may negate the partial heat acclimation advantages acquired through habitual endurance exercise, thus exposing all individuals to a similar relative thermal and physical training stimulus to induce a similar magnitude of adaptation (Fox *et al.*, 1963; Piwonka *et al.*, 1965; Mee *et al.*, 2015; Neal *et al.*, 2016a).

Post-exercise hot water immersion is a novel, practical and effective heat acclimation protocol that exposes individuals to a constant endogenous thermal stimulus (**Chapter 4**; Tyler *et al.*, 2016). The passive hot water immersion may also reduce the physical training stimulus and, although initial tolerance to immersion in hot water may be greater in the endurance trained, all individuals may be exposed to a similar thermal stimulus as the immersion is limited to 40 min and perceptual tolerance increases over the 6-day protocol. Therefore, post-exercise hot water immersion may induce a similar extent of adaptation in endurance and lesser trained individuals. However, date, no study has assessed whether endurance trained and recreationally active individuals establish a similar magnitude of adaptation following post-exercise hot water immersion. In addition, it is unknown whether the stimulus for heat adaptation, namely physical training and/or the thermal impulse, differs between the groups. To this end, we hypothesized that taking a hot bath after submaximal exercise in temperate conditions on six consecutive days would induce similar heat acclimation adaptations in both endurance trained and recreationally males although, the stimulus for adaptation may differ between the groups.

6.3 Methods

Participants: Eight endurance trained (ET: 9 ± 3 h weekly endurance exercise) and eight recreationally active (RA: 3 ± 1 h weekly endurance exercise) males who had not been exposed to hot environmental conditions in the past 3-months and were not regular hot bath/sauna users, participated in the current investigation (Table 6.1).

Table 6.1 Participant characteristics. Data displayed as mean ± SD.

	Endurance Trained	Recreationally Active
n	8	8
Age (years)	25 ± 4	21 ± 2
Mass (kg)	69.0 ± 3.5	71.1 ± 8.5
$\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	68.4 ± 6.1	54.1 ± 5.5$^{\#\#}$
Weekly endurance exercise (h)	9 ± 3	3 ± 1$^{\#\#}$

$^{\#\#}$ $P < 0.01$ recreationally active less than endurance trained.

Study design: To assess the effect of training status on heat acclimation adaptations, participants completed a 40 min submaximal treadmill run in the heat (65 % $\dot{V}O_{2max}$, 33 °C, 40 % RH) before (PRE) and after (POST) heat acclimation as previously described (**Section 4.3**). Heat acclimation involved a daily 40 min submaximal treadmill run in temperate conditions (65 % $\dot{V}O_{2max}$), following by a ≥ 40 min hot water immersion (40 °C) on six consecutive days as described previously (**Section 4.3**).

Preliminary measurements: In temperate conditions (20 °C), a continuous incremental exercise test on a motorised treadmill assessed $\dot{V}O_{2max}$ as previously described (**Section 3.3**). The interpolation of the running speed–$\dot{V}O_2$ relationship determined a running speed that elicited 65 % $\dot{V}O_{2max}$. This speed was verified 30 min later and was used during submaximal exercise in experimental trials and the daily exercise throughout the 6-day heat acclimation intervention.

Experimental trials: Twenty-four hours prior to and on the day of experimental trials, participants were asked to prepare as described (**Sections 4.5 and 5.3**). On the morning of experimental trials, participants arrived at the laboratory fasted and were provided with a standardised breakfast (0.03 $MJ \cdot kg^{-1}$) and a bolus of water equivalent to 7 $mL \cdot kg^{-1}$ of body

mass. Following a 20 min seated rest in temperate conditions (20 °C), a venous blood sample was taken without stasis. This blood sample was assessed for Hb concentration and Hct for the calculation of plasma volume change as described (**Section 3.4.5**). A pre-exercise nude body mass was taken using a digital platform scale following voiding. A urine sample was analysed for USG to confirm euhydration as described (**Section 3.4.7**; Armstrong, 2005). The participant was then instrumented for the exercise protocol. Prior to exercise, dressed in T-shirt, running shorts, socks and shoes, a seated rest was completed in a temperate laboratory (20°C) to establish baseline measures.

Dressed in running shorts, socks and shoes the participant entered the environmental chamber and completed a submaximal treadmill run (40 min, 65 % $\dot{V}O_{2max}$, 1 % gradient) in the heat (33 ºC, 40 % RH). No fluids were consumed during exercise. T_{re}, T_{sk} and HR were monitored continuously as described (**Sections 3.4.1 and 3.4.3**). Local forearm sweat rate, $\dot{V}O_2$, RER, RPE (Borg, 1970) and thermal sensation (Hollies and Goldman, 1977) were measured as described (**Sections 3.4 and 3.5**). On completion of the exercise protocol participants exited the environmental chamber and rested in temperate conditions (20°C) for 15 min. To estimate WBSR participants provided a nude body mass following the seated rest. Participants were then provided with water equivalent to sweat losses and were free to leave the laboratory when $T_{re} \leq 38.5$°C.

Post-exercise hot water immersion intervention: Post-exercise hot water immersion heat acclimation involved a daily 40 min submaximal run (65 % $\dot{V}O_{2max}$, 1 % gradient) on a motorised treadmill in temperate conditions (20 °C), followed by a ≤ 40 min hot water immersion (40 °C) to the neck completed on six consecutive days as described (**Section 4.3**). During the intervention, participants were instructed to reduce their normal endurance exercise volume by that completed during the intervention in the laboratory and to consume their normal diet and fluid intake, including caffeine and alcohol (≤ 3 units per day).

Measurement and instrumentation: T_{re}, T_{re} at sweating onset, T_{sk} (Ramanathan, 1964), local sweating rate, WBSR, $\dot{V}O_2$, RER, RPE (Borg, 1970) and thermal sensation (Hollies and Goldman, 1977) were measured as described (**Chapter 3**). A resting venous blood sample was taken prior to exercise during experimental trails and assessed for Hb concentration (g·dL) and Hct for the measurement of plasma volume as described (**Section 3.4.5**). The

change in plasma volume was estimated by correcting the initial plasma volume at PRE for the percentage change in plasma volume at POST as described (**Section 3.4.5;** Dill and Costill, 1974).

Statistical Analysis: A sample size calculation (G*Power 3.1.2) was performed using mean data from an 8-day exercise-heat acclimation study (Shvartz *et al.,* 1977). A two-way (group × time) repeated measures ANOVA, with alpha level set at 0.05, power of 0.80 and a strong correlation of 0.7, calculated a sample size of eight participants per group to detect a significant difference in end-exercise T_{re} (-0.3°C) between endurance trained and untrained individuals following heat acclimation. Data are presented as mean ± standard deviation (SD) and statistical significance was accepted at $P < 0.05$. All data were checked for normality and sphericity. Two-way repeated measures analysis of variance (ANOVA) with Greenhouse Geisser correction to the degrees of freedom (where necessary) were used to assess for differences between heat acclimation status (PRE to POST and day 1 to day 6 of the intervention) in endurance trained and recreationally active participants. Bonferroni-adjusted pairwise comparisons were used where appropriate to determine where differences occur. Cohen's *d* was used to analyze the magnitude of the effect, with 0.2, 0.5 and 0.8 representing small, medium and large effects (Cohen, 1988). To determine the stimulus for heat acclimation adaptation Pearson's correlations determined the strength of the relationship between factors such as; habitual weekly endurance exercise; $\dot{V}O_{2max}$; and the thermal stimulus (AUC) experienced from heat acclimation, on the reduction of thermal strain (end-exercise T_{re}) and cardiovascular strain (end-exercise HR) in the endurance trained and recreationally active.

6.4 Results

6.4.1 Post-exercise hot water immersion intervention

All participants completed a 40 min treadmill run at 65 % $\dot{V}O_{2max}$ followed by a hot water immersion (≤ 40 min) on six consecutive days. During the intervention, weekly endurance exercise volume was unchanged in ET (8 ± 4 h) and increased in RA (4 ± 1; $P < 0.001$). Heat acclimation was demonstrated (time main effect) through a reduction in end-exercise HR ($P = 0.04$, $d = 0.51$) and an increase in WBSR ($P = 0.02$, $d = 0.68$) from day 1 to day 6 (Table 6.2). Partial heat acclimatisation was demonstrated in ET with a greater WBSR during the intervention ($P = 0.03$, $d = 1.31$). A group × time interaction signified that hot water immersion time increased from day 1 to day 6 in ET ($P = 0.03$, $d = 1.31$) and RA ($P < 0.001$, $d = 3.79$, Table 6.2) and that the duration of immersion was longer on day 1 in ET ($P = 0.01$, $d = 1.04$, Table 6.2). As such, total hot water immersion time from the 6-day protocol tended to be longer in ET ($P = 0.08$, $d = 0.97$). A constant endogenous stimulus for adaptation was demonstrated in both groups with daily end-immersion T_{re} and AUC similar on day 1 and day 6. Although, a group main effect demonstrated that end-immersion T_{re} was higher in ET ($P = 0.04$, $d = 0.80$). Additionally, there was a trend for daily AUC to be higher in ET on day 1 and day 6 ($P = 0.06$, $d = 0.92$) however, the total stimulus for adaptation (AUC) was not different between the groups.

6.4.2 Experimental trials

Following post-exercise hot water immersion, the reduction in thermal strain during exercise-heat stress was not influenced by training status, evidenced by no group × time interaction and no main effect for group in end-exercise T_{re}. As such, the PRE to POST reduction in end-exercise T_{re} ($P < 0.001$), which was observed in all participants ($n = 16$), was similar in ET (-0.36 ± 0.17 °C, $d = 0.68$) and RA (-0.47 ± 0.37 °C, $d = 1.52$, Figure 6.1). Interestingly, the reduction in end-exercise T_{re} was very strongly negatively correlated with the size of the thermal stimulus in ET (total AUC, $r = -0.71$, $P = 0.05$). This relationship was not observed in RA ($r = 0.12$, $P = 0.78$, Figure 6.3). There was also no group × time interaction or group main effect for measures of; resting T_{re}; T_{re} at sweating onset; end-exercise T_{sk}; RPE; thermal sensation and mean RER (Table 6.3). There was also no group × time interaction in WBSR or mean $\dot{V}O_2$ although, these measures were higher in ET than RA ($P < 0.05$, Table 6.3). Main effects of time were observed at POST, with reductions in; resting T_{re} ($P = 0.003$, $d = 0.60$); T_{re}

Table 6.2. The influence of submaximal running at 65 % $\dot{V}O_{2max}$ for 40 min in 20 °C and post-exercise hot water immersion in 40 °C on thermoregulatory variables, heart rate, and immersion time in endurance trained (ET) and recreationally active (RA) participants.

	ET		RA	
	Day 1	Day 6	Day 1	Day 6
Submaximal exercise				
End-exercise T_{re} (°C)	38.37 ± 0.48	38.27 ± 0.43	38.34 ± 0.32	38.22 ± 0.23
End-exercise HR (beats·min^{-1}) *	147 ± 13	144 ± 10	150 ± 9	144 ± 9
Hot water immersion				
End-immersion T_{re} (°C) $^{\#}$	39.44 ± 0.44	39.36 ± 0.31	39.15 ± 0.18	39.21 ± 0.20
Immersion time (min) ** $^{\#}$	35 ± 8	40 ± 0 †	28 ± 5	40 ± 1 ††
n completing 40 min immersion	5 of 8	8 of 8	0 of 8	7 of 8
Submaximal exercise and hot water immersion				
WBSR (L·h^{-1}) * $^{\#}$	1.08 ± 0.34	1.25 ± 0.26	0.72 ± 0.17	0.95 ± 0.18
AUC (°C·min^{-1})	33 ± 24	29 ± 15	18 ± 7	20 ± 7

T_{re}; rectal temperature, AUC; area under the curve. * $P < 0.05$ and ** $P < 0.01$, main effect of time. $^{\#}$ $P < 0.05$, main effect of group. † $P < 0.05$ and †† $P < 0.01$, *post hoc* time effects. Data displayed as mean ± SD.

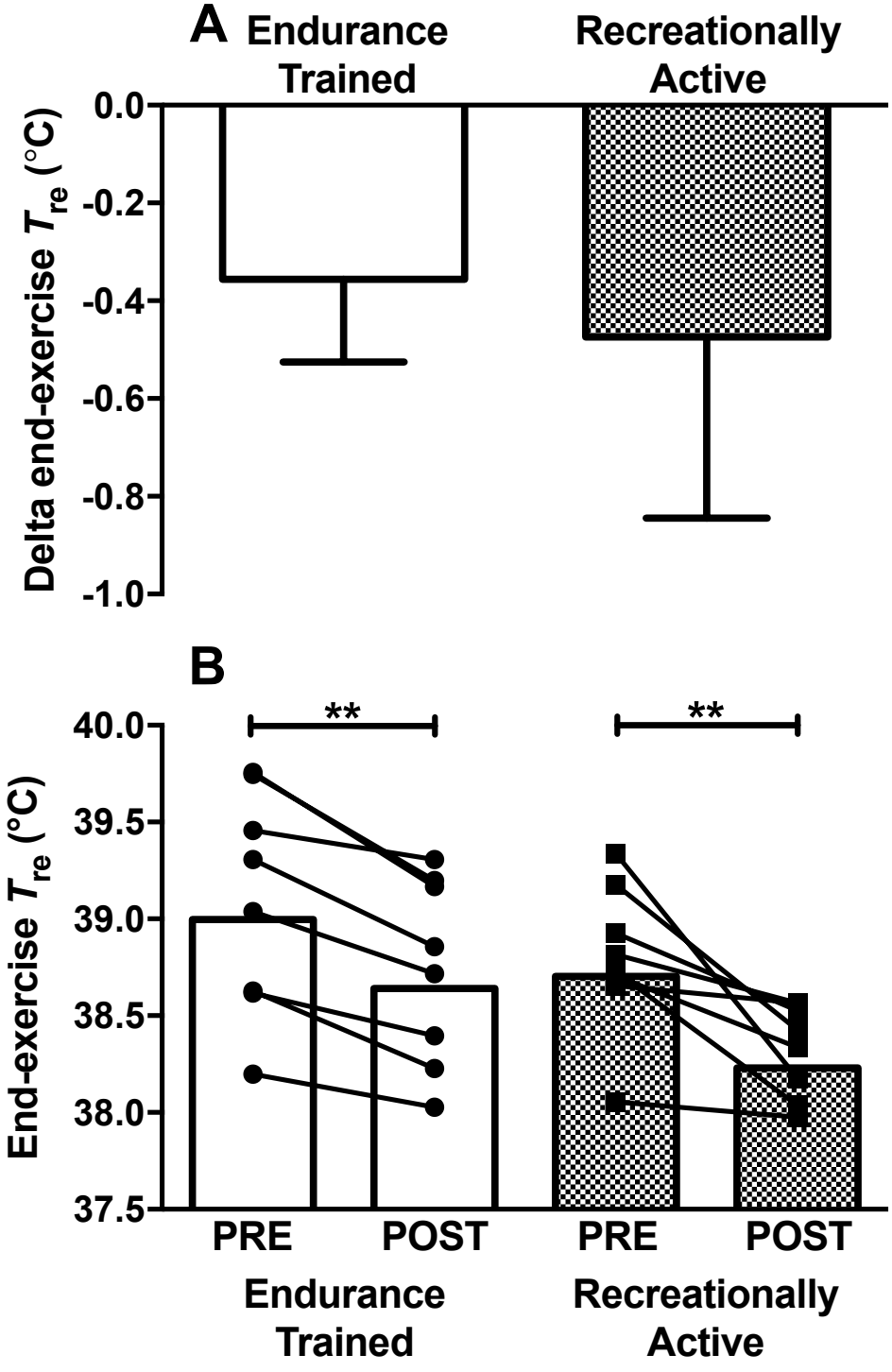

Figure 6.1. Effect of 6-day post-exercise hot water immersion heat acclimation on end-exercise rectal core temperature (T_{re}) following a 40 min submaximal treadmill run at 65 % $\dot{V}O_{2max}$ in the heat (33 °C, 40 % RH) in endurance trained and recreationally active individuals. Bars represent mean ± SD of the PRE to POST change (A) and mean at PRE and POST (B). Lines between bars represent individual participant responses. ** $P < 0.01$, main effect of time.

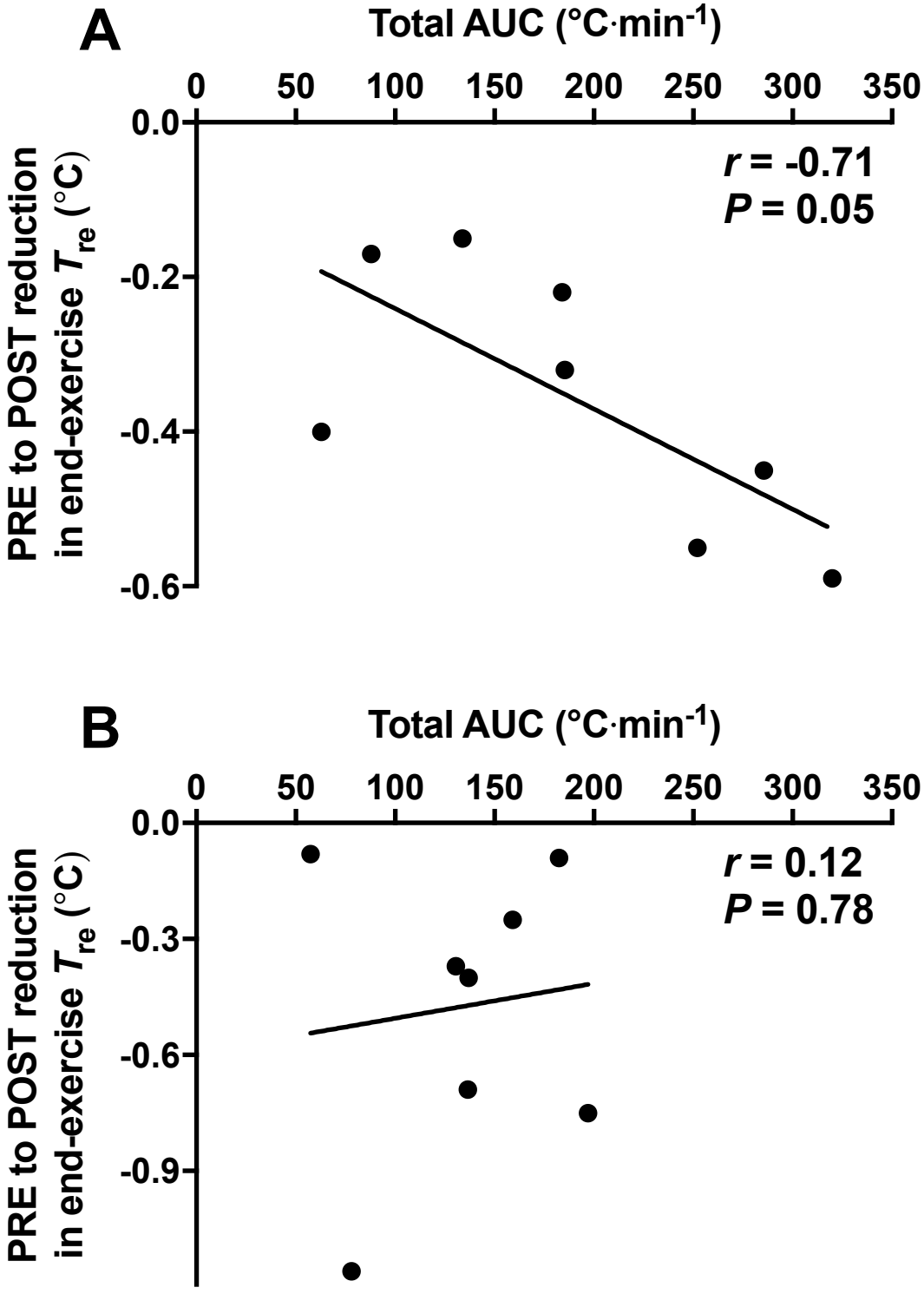

Figure 6.2. The relationship between the thermal stimulus (total AUC), from post exercise hot water immersion heat acclimation, and the reduction in end-exercise rectal core temperature (T_{re}) at POST following 40 min submaximal treadmill running at 65 % $\dot{V}O_{2max}$ in the heat (33 °C, 40 % RH) in endurance trained (A) and recreationally active (B) individuals.

Table 6.3. Effect of 6-day post-exercise hot water immersion heat acclimation on 40 min submaximal treadmill running at 65 % $\dot{V}O_{2max}$ in the heat (33 °C, 40 % RH) in endurance trained (ET) and recreationally active (RA) participants.

	ET	RA
Resting T_{re} (°C) **	-0.17 ± 0.25	-0.23 ± 0.17
T_{re} at sweating onset (°C) **	-0.22 ± 0.24	-0.23 ± 0.29
End-exercise T_{sk} (°C) **	-0.67 ± 0.38	-0.75 ± 0.70
End-exercise HR (beats·min^{-1}) **	-4 ± 5	-15 ± 7 ††
WBSR (L·h^{-1}) $^{\#\#}$	0.13 ± 0.02	-0.03 ± 0.25
End-exercise RPE *	-1 ± 1	-2 ± 3
End-exercise thermal sensation **	-1 ± 1	-1 ± 1
Mean $\dot{V}O_2$ (L·min^{-1}) ** $^{\#\#}$	-0.07 ± 0.11	-0.12 ± 0.15
Mean RER	-0.02 ± 0.04	0.00 ± 0.05
Plasma volume (%)	4 ± 8	3 ± 7

T_{re}, rectal temperature; T_{sk}, mean skin temperature; HR, heart rate; WBSR, whole body sweat rate, RPE; rating of perceived exertion, $\dot{V}O_2$; volume of oxygen uptake, RER; respiratory exchange ratio. Data displayed as mean ± SD of the PRE to POST change. * $P < 0.05$ and ** $P < 0.01$, main effect of time. $^{\#\#}$ $P < 0.05$, main effect of group. †† $P < 0.01$, post hoc time effects. Data displayed as mean ± SD.

at sweating onset (P = 0.005, d = 0.56); end-exercise T_{sk} (P < 0.001, d = 0.75); RPE (P = 0.01, d = 0.66); thermal sensation (P = 0.002, d = 1.03); and mean $\dot{V}O_2$ (P = 0.01, d = 0.20; Table 6.3). There was no PRE to POST change in WBSR, mean RER or plasma volume (Table 6.3). The PRE to POST reductions in end-exercise HR were however influenced by training status, demonstrated by a significant group × time interaction (P = 0.003, Table 6.3). Post-hoc analysis demonstrated that end-exercise HR was non-significantly reduced in five of the eight participants in ET (P = 0.13, d = 0.22), whereas at POST, end-exercise HR was reduced in all (n = 8) RA individuals (P < 0.001, d = 1.42, Table 6.3). Moreover, large correlations suggested that a greater reduction in end-exercise HR was observed in participants who routinely completed less weekly habitual endurance exercise (r = 0.68, P = 0.003) and had a lower $\dot{V}O_{2max}$ (r = 0.57, P = 0.02, Figure 6.3).

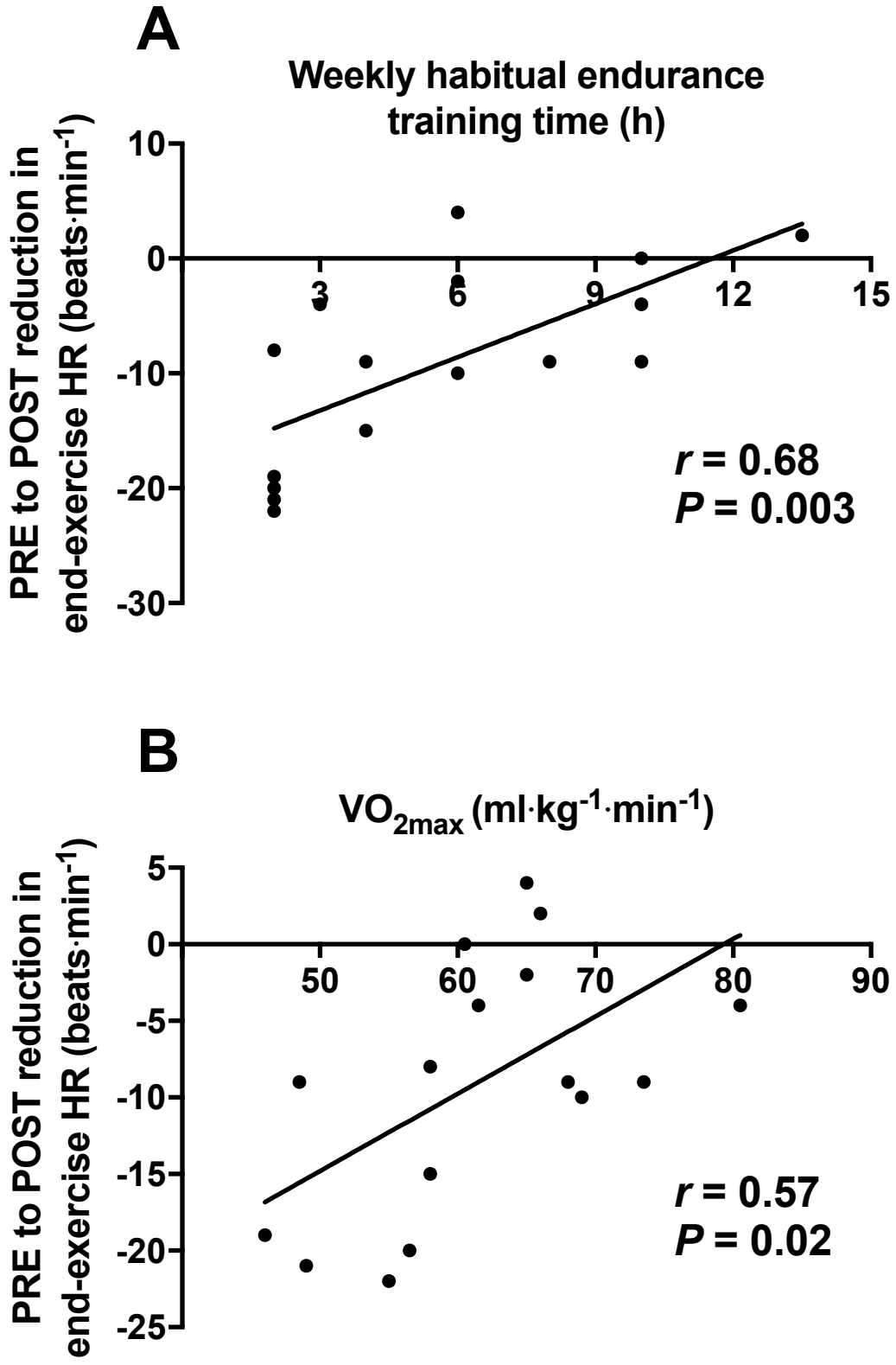

Figure 6.3. The relationship between the reduction at POST in end-exercise heart rate (HR, beats·min^{-1}), following 40 min submaximal treadmill running at 65 % $\dot{V}O_{2max}$ in the heat (33 °C, 40 % RH), and weekly habitual endurance exercise training hours (A) and $\dot{V}O_{2max}$ (B).

6.5 Discussion

The novel finding of the current study demonstrates that post-exercise hot water immersion attenuates thermal strain during exercise-heat stress in both ET and RA, evidenced through a meaningful reduction in end-exercise T_{re}. This practical 6-day post-exercise hot water immersion intervention also provides other hallmark heat acclimation adaptations in both ET and RA, including a decrease in resting T_{re}, and reductions in T_{re} at sweating onset, T_{sk}, mean $\dot{V}O_2$, RPE and thermal sensation during submaximal exercise in the heat. Post-exercise hot water immersion also initiates a reduction in HR during exercise heat stress in RA, whereas, the reduction in exercising HR in ET did not reach statistical significance. The present data also demonstrates the partial acclimitisation status of ET. For example, thermal tolerance, demonstrated by longer immersion times and tolerance to a higher end-immersion Tre, was greater on day one of the 6-day post-exercise hot water immersion.

Current recommendations state that habitual endurance exercise initiates partial heat acclimation adaptations and alters the temporal patterning of adaptation from conventional exercise-heat acclimation (Piwonka *et al*., 1965; Strydom *et al*., 1966; Gisolfi *et al*., 1969; Pandolf *et al*., 1977). Our data demonstrates that individuals experienced a similar meaningful attenuation in thermal strain, which is likely initiated by the large daily elevations in core (T_{re} ≈ 39. 3°C) and peripheral body temperatures, where skin temperature equilibrates with water temperature (≈ 40 °C) during immersions (**Chapter 4**). In addition, the increase in subjective tolerance to immersion in hot water, or the completion of the daily post-exercise hot water immersion protocol, initiates a similar daily elevation in T_{re} and AUC throughout the 6-day intervention, demonstrating a progressive thermal stimulus in ET and RA (**Chapters 4 and 5**; Fox *et al.*, 1963; Taylor, 2014). The greater tolerance to immersion in hot water demonstrated in ET also exposed these individuals to a larger thermal stimulus (total mean AUC, ≈ 40%). Even so, these individuals experienced a smaller (≈ 25 %), albeit meaningful, attenuation in end-exercise T_{re} (-0.36 °C) compared to RA (-0.47 °C). This association between the stimulus and adaptation is similar to that demonstrated in strength or endurance trained individuals who, to acquire a similar magnitude of training adaptation as untrained individuals, require exposure to a larger stimulus (Wenger and Bell, 1986; Ahtiainen *et al.*, 2003). The strong relationship between the thermal stimulus and the attenuation in thermal strain (r = -0.71, P = 0.05) following post-exercise hot water immersion in ET, suggests that increasing the thermal stimulus may initiate a similar

magnitude of adaptation as seen in RA, but this requires confirmation. This negative relationship between the thermal stimulus and the reduction in end-exercise T_{re} ($r = 0.12$, $P = 0.78$) was not observed in RA therefore, exposure to an additional stimulus during post-exercise hot water immersion, such as an increase in endurance exercise volume, may provide additional adaptations that augment the attenuation in thermal strain. For example, it may be speculated that 6-days of post-exercise hot water immersion may increase $\dot{V}O_{2max}$ and/or improve exercise economy to reduce relative exercise intensity and metabolic heat production during exercise-heat stress, further attenuating thermal strain and initiating the observed reduction in HR, this however, was not measured in the current research (Jones and Carter, 2000; Lorenzo *et al.*, 2010; Taylor, 2014; James *et al.*, 2017).

Data from the current study demonstrates that post-exercise hot water immersion initiates a reduction in thermal strain during exercise-heat stress in both ET and RA. A meaningful, albeit smaller reduction was observed in ET in spite of these individuals experiencing a larger thermal stimulus, provided through a greater perceptual tolerance to immersion in hot water. The post-exercise hot water immersion intervention therefore presents a cost effective, accessible, practical strategy that attenuates thermal strain during exercise-heat stress without relocation to a hot climate, access to a heat chamber, manipulation of external work rate to maintain the thermal stimulus or alterations in the protocol to acclimate both endurance trained and recreationally active individuals. Future research is required to establish the stimulus required to initiate the same extent of adaptation in ET as RA and to assess whether habituation to the daily post-exercise hot water immersion protocol, demonstrated through a plateau in physiological variables, occurs after a larger number of exposures (Pandolf *et al.*, 1977). Furthermore, future research should determine whether adaptations following post-exercise hot water immersion translate to an improved exercise capacity and reduced susceptibility to heat illness, whilst establishing the timeframe of adaptation retention in endurance trained and recreationally active males and females.

In conclusion, six consecutive daily post-exercise hot water immersion sessions reduce thermal strain during exercise-heat stress similarly in both endurance trained and recreationally active males, with the only notable adaptation difference between the groups observed in end-exercise HR. In addition, subjective tolerance to immersion in hot water, or completion of the 40 min protocol, exposes both endurance trained and recreationally active

individuals to a thermal stimulus that initiates heat acclimation adaptations, without any practical alterations to the novel intervention protocol.

CHAPTER SEVEN

Heat acclimation by post-exercise hot water immersion is retained for two weeks

7.1 Summary

Post-exercise hot water immersion induces heat acclimation adaptations that reduce thermal strain during exercise-heat stress. The timeframe for the retention of heat acclimation adaptations following 6-day post-exercise hot water immersion is however currently unknown. Thus, we examined the induction and the temporal patterning of heat acclimation decay from post-exercise hot water immersion. Thirteen physically active, non-heat acclimatised males completed a 6-day heat acclimation intervention consisting of a daily submaximal treadmill run (40 min, 65 % $\dot{V}O_{2max}$), followed by a hot water immersion (≤ 40 min, 40 °C). Before (PRE) heat acclimation, participants completed a submaximal treadmill run (40 min, 65 % $\dot{V}O_{2max}$) in the heat (33 °C, 40 % RH). Heat acclimation adaptations were then assessed during exercise-heat stress tests repeated following (POST) post-exercise hot water immersion and seven (WK 1) and fourteen (WK 2) days following POST. Post-exercise hot water immersion induced heat acclimation, demonstrated at POST through a reduction in resting T_{re} (-0.32 ± 0.22 °C, $P < 0.001$) and end-exercise T_{re} (-0.42 ± 0.30 °C, $P < 0.001$). T_{re} and T_{es} at sweating onset, end-exercise T_{sk}, HR, RPE and thermal sensation were also lower at POST ($P < 0.05$). At POST, plasma volume tended to be increased ($P = 0.08$) whereas, sweating sensitivity was unchanged from PRE ($P > 0.05$). The induction of hallmarks of heat acclimation were retained at WK 2. For example, resting T_{re} (-0.36 ± 0.25 °C) and end exercise T_{re} (-0.36 ± 0.37 °C) were lower than PRE ($P < 0.05$), and similar to POST at WK 2 ($P > 0.05$). Other hallmarks of heat acclimation were also retained at WK 2, including reductions in T_{re} at sweating onset, end-exercise T_{sk}, HR, RPE and thermal sensation ($P < 0.05$). Heat acclimation adaptations including; reductions in resting T_{re} and end-exercise T_{re}, HR, RPE and thermal sensation are retained for two weeks following 6-day post-exercise hot water immersion.

7.2 Introduction

To reduce thermal strain, improve exercise capabilities/performance in the heat and reduce susceptibility to EHI, athletes and military personnel are advised to complete heat acclimation protocols prior to movement to the heat (**Section 2.2**; Binkley *et al.*, 2002; Lorenzo *et al.*, 2010; Racinais *et al.*, 2015b). Recommendations suggest that 5 to 14-days of submaximal exercise in the heat is required to initiate hallmark adaptations of heat acclimation (**Section 2.3**; Periard *et al.*, 2015). Taking a hot bath following exercise in temperate conditions on six consecutive days, has been demonstrated to also initiate hallmark heat acclimation adaptations such as, a reduction in resting and exercise-heat stress core temperature (**Chapters 4, 5 and 6**). However, subtle adaptation differences may exist between post-exercise hot water immersion and exercise-heat acclimation (**Chapters 4, 5 and 6**). For example, exercise-heat acclimation initiates a large expansion in plasma volume (+9-15 %; Senay et al., 1976; Nielsen *et al.*, 1997; Gibson *et al.*, 2015b), whereas, using the relative changes in Hb and Hct, a smaller expansion has been demonstrated following post-exercise hot water immersion (+3 %; **Chapters 4, 5 and 6**; Dill and Costill, 1974). Additionally, the effect of post-exercise hot water immersion on the sensitivity of the sweating response is yet to be defined. Therefore, using appropriate measurement techniques, examination of blood compartment and sweating sensitivity changes is required to provide a more comprehensive assessment of the induced adaptations and the mechanisms for the reduction in thermal strain during exercise-heat stress, following post-exercise hot water immersion.

Information regarding the induction of heat acclimation is extensive, with a great deal of research conducted examining the induced adaptations and the methods used to initiate them (**Sections 2.2 and 2.3**; Taylor, 2014). However, the induced adaptations are transient and adaptations will be lost without regular exposure to the heat (**Section 2.5**; Pandolf, 1998; Taylor, 2014). Observations regarding the decay of heat acclimation report that those adaptations that occur first, such as an expansion in plasma volume and a reduction in exercising HR, exhibit the most rapid decay (Williams *et al.*, 1967; Pandolf *et al.*, 1977; Armstrong and Maresh, 1991; Flouris *et al.*, 2014). Additionally, the decay of heat acclimation is traditionally thought to be more rapid than its acquisition, with 1-day of adaptation lost every 2-days without a heat exposure (Givoni and Goldman, 1973). This timeframe of heat acclimation decay has however been questioned, with suggestions that

adaptations are present 18 to 26-days following exercise-heat acclimation (Pandolf *et al.*, 1977; Weller *et al.*, 2007; Daanen *et al.*, 2011). This ambiguity within the research may however be due to inconsistencies between experimental designs, a lack of control of habitual exercise during decay protocols and/or differences in the physical characteristics of participants (Pandolf *et al*., 1977; Pandolf, 1998). It is however worth noting that the number of heat acclimation exposures, the size of the thermal stimulus and/or the magnitude of initiated adaptation may also effect the retention timeframe of heat acclimation (**Section 2.5**). For example, following STHA a -0.27 °C reduction in exercising T_{re} is retained for 7-days and not apparent after 14-days (Garrett *et al.*, 2009). Whereas, heat acclimation adaptations are observed for up to 26-days following MTHA, which produces a more profound reduction in exercising core temperature (≈ -0.50 °C) and a reduction in core temperature at rest (Weller *et al.*, 2007; Daanen *et al.*, 2011; Poirier *et al.*, 2015). In spite of these advantages of MTHA, short-term exercise-based protocols remain the preferred preparatory method for the heat, as these programmes reduce training disruptions (Garrett *et al.*, 2011). Nevertheless, the rapid decay of adaptation from STHA dictates that these protocols should be completed in the days before moving to the heat, which may compromise an athletes taper (Pandolf, 1998).

The practical, post-exercise hot water immersion protocol has been demonstrated to initiate a preferential magnitude of adaptation compared to exercise-based STHA (exercise T_{re}; ≈ -0.4 °C, resting T_{re}; ≈ -0.3 °C; **Chapters 4, 5 and 6**; Tyler *et al.*, 2016). The timeframe of adaptation retention from post-exercise hot water immersion is yet to be established however, as the extent of induced adaptation is similar to that following 10 to 14-days of exercise-heat acclimation, the retention timeframe may be longer than that following STHA and similar to MTHA. Therefore, it may not be necessary to complete the 6-day post-exercise hot water immersion intervention during an athlete's taper to gain the greatest heat acclimation benefit. The primary aim of the current study was to establish the temporal patterning of heat acclimation adaptation decay from post-exercise hot water immersion. Due to the similar, although unconfirmed, extent of initiated adaptation following MTHA (Weller *et al.,* 2007) and post-exercise hot water immersion, we hypothesised that hallmarks of heat acclimation would demonstrate minimal (end-exercise T_{re}; 15 %, end-exercise HR; 30 %), or no (resting T_{re}) adaptation decay 2-weeks following 6-day post-exercise hot water immersion. Secondarily, we aimed to provide a more extensive appraisal of the induced adaptations and the mechanisms that may initiate the reduction in thermal strain during exercise-heat stress

following post-exercise hot water immersion. This includes, using appropriate measurement techniques, assessments of the impact of the intervention on blood compartments and sweating sensitivity.

7.3 Methods

Participants: Thirteen physically active, non-heat acclimatise males (mean ± SD, age: 23 ± 3 years; body mass: 73.5 ± 7.0 kg; $\dot{V}O_{2max}$: 58.2 ± 9.1 $mL \cdot kg^{-1} \cdot min^{-1}$) who completed ≥ 2 h of endurance exercise per week were recruited to participate in the study.

Study design: Participants completed 6-days of post-exercise hot water immersion heat acclimation as described (Figure 7.1; **Section 4.3**). Prior to (PRE), following (POST), 7-days (WK 1) and 14-days (WK 2) following POST, heat acclimation adaptations were assessed during submaximal treadmill exercise in the heat as described (**Section 4.3**).

Preliminary measurements and familiarisation: Following the assessment of $\dot{V}O_{2max}$ and verification of a running speed that elicited 65 % $\dot{V}O_{2max}$, as described (**Section 3.3**), all participants were familiarised with procedures used in the study. Due to the lack of availability of equipment, a subsample of participants were also familiarised to the breathing procedure used in the optimised CO rebreathing protocol, without the inhalation of CO, for the future assessment of total Hb mass (n = 9; **Section 3.4.6**; Schmidt and Prommer, 2005).

Experimental trials: Twenty-four hours prior to and on the day of experimental trials, participants were asked to prepare as described (**Section 5.3**). This included, sleeping between 2200 h and 0700 h to ensure a similar circadian pattern prior to each experimental trial, confirmed by Actigraph and the completion of a food diary to confirm no alcohol, diuretics, or caffeine, were consumed. Participants were also asked to replicate this prior to all other subsequent experimental trials (POST, WK 1 and WK 2).

On the day of each experimental trial, participants arrived at the laboratory fasted, at 0730 h. A standardised breakfast and bolus of water was provided, and at 0800 h, participants completed a 20 min seated rest. A venous blood sample was taken without stasis and a subsample (n = 9) of participants completed the optimised CO rebreathing protocol as described (**Section 3.4.6**). To confirm euhydration, urine samples were collected and analysed for USG as described (**Section 3.4.7**; Armstrong, 2005). A pre-exercise nude body mass was taken and the participant was instrumented for the exercise protocol as described (**Section 3.4**). To establish baseline measures, at 0915 h, a 30 min seated rest in a temperate laboratory (20 °C) dressed in T-shirt, running shorts, socks and shoes was completed.

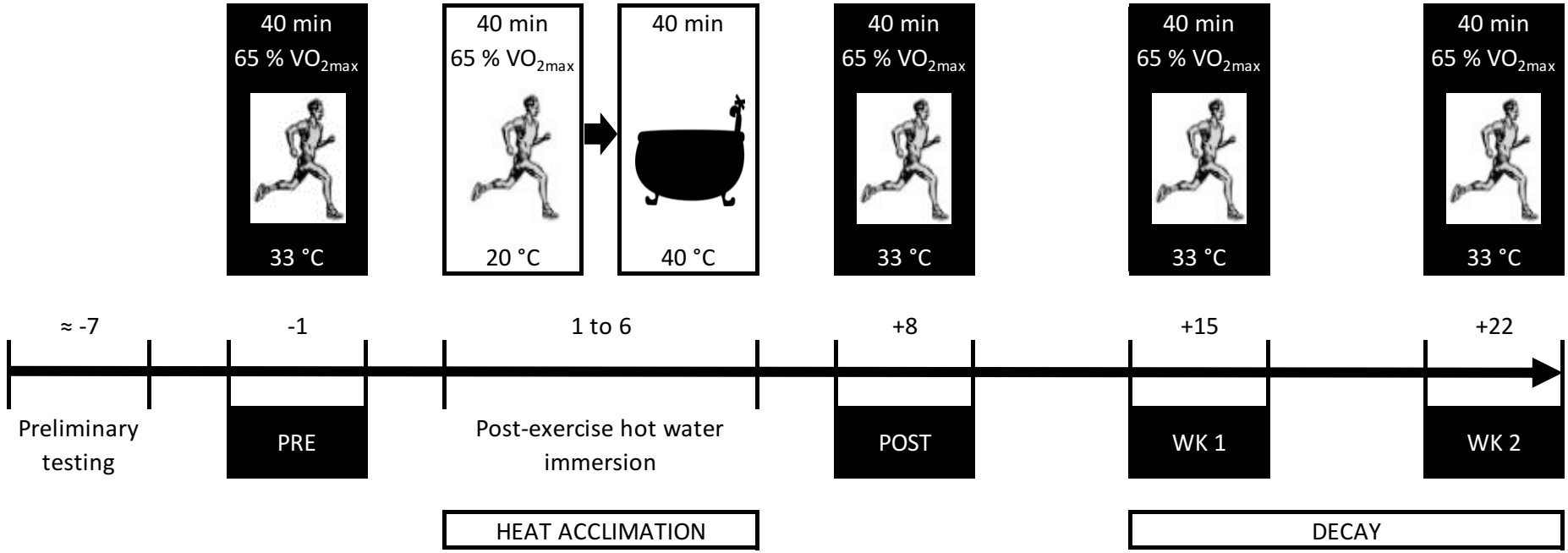

Figure 7.1 Experimental schematic.

Submaximal exercise: At 0945 h, wearing shorts, socks and trainers, participants entered the environmental chamber and completed a 40 min submaximal treadmill run (65 % $\dot{V}O_{2max}$, 1 % gradient) in the heat (33 ºC, 40 % RH). T_{re}, T_{es}, T_{sk}, local forearm sweating rate, HR, RPE (Borg, 1970), thermal sensation (Hollies and Goldman, 1977), $\dot{V}O_2$ and RER were examined as described (**Sections 3.4 and 3.5**). Following the completion of exercise, a nude body mass was taken and WBSR was assessed as described (**Section 3.4.2**). Participants were then provided with water equivalent to sweat losses and were free to leave the laboratory when T_{re} ≤ 38.5°C.

Post-exercise hot water immersion: Post-exercise hot water immersion heat acclimation was completed on six consecutive days with sessions commencing between 0630 h and 0900 h as described (**Section 4.3**). During the 6-day intervention participants were asked to consume their normal diet and fluid intake, including caffeine and alcohol (≤ 3 units per day) and were instructed to wear an activity tracker (Fitbit Flex, San Francisco, USA) on their non-dominant arm for the duration of the protocol to monitor physical activity.

Heat acclimation decay: Following POST, participants began a 14-day heat acclimation decay protocol. To assess for the decay of heat acclimation adaptations participants completed experimental trials (submaximal treadmill run in the heat) on day +8 (WK 1) and day +15 (WK 2; Figure 7.1) as described (**Section 4.3**). During the heat acclimation decay protocol participants were instructed to maintain physical activity levels, consume their normal diet and fluid intake, including caffeine and alcohol (≤ 3 units per day) and wear the activity tracker for the duration of the protocol.

Measurement and instrumentation: T_{re}, T_{es} and T_{sk}, calculated using a four-site skin temperature equation (Ramanathan, 1964), local forearm and WBSR were measured as outlined (**Section 3.4**). Resting whole blood samples were taken without stasis, prior to exercise during experimental trials (PRE, POST, WK 1 and WK 2) and used for the immediate determination of Hb and Hct (**Section 3.4.5**). Additionally, total Hb mass, blood volume and plasma volume were also assessed (n = 9) using the optimised CO rebreathing technique as described (**Section 3.4.6**; Schmidt and Prommer, 2005). The change in blood and plasma volume (at POST, WK1 and WK 2) were estimated by correcting the initial plasma volume (at PRE) for the percentage change in plasma volume.

Statistical analysis: A one-tailed *t*-test sample size calculation (G*Power 3.1.2) with alpha level set at 0.05 and power set at 0.8 was performed using mean data taken from the decay phase of a 5-day heat acclimation study (Garrett *et al.*, 2009). To detect a meaningful heat acclimation induced difference in end exercise T_{re} (0.3 °C) 7-days following the cessation of heat exposures, a sample size of 9 participants was calculated. To ensure adequate power and allowing for dropout, 13 participants were recruited. Data is presented as mean ± SD and statistical significance was accepted at $P < 0.05$. All data were checked for normality and sphericity and to assess for differences between the four experimental trials (PRE, POST, WK 1 and WK 2) data was analysed using either *t*-tests or repeated measures ANOVA's, with Greenhouse Geisser correction to the degrees of freedom (where necessary). When a main effect was observed, results were followed up using Tukey's HSD paired *t*-test post hoc tests.

7.4 Results

7.4.1 Post-exercise hot water immersion intervention

Thirteen participants completed six consecutive days of submaximal treadmill exercise (65 % $\dot{V}O_{2max}$) in temperate conditions followed by a hot water immersion (≤ 40 min). Hot water immersion time progressively increased from day 1 (33 ± 7 min) to day 6 (40 ± 0; P = 0.002, d = 2.17) which, in part, maintained a constant stimulus throughout the intervention. This constant endogenous adaptation stimulus is demonstrated through no observable differences in end-immersion T_{re} or daily AUC between day 1 (end-immersion T_{re}, 39.34 ± 0.30 °C; AUC, 27 ± 16 °C·min^{-1}) and day 6 (end-immersion T_{re}, 39.24 ± 0.30 °C; AUC, 25 ± 13 °C·min^{-1}; P > 0.05). Evidence of heat acclimation was observed from day 5 of the intervention through an increase in WBSR (day 1, 0.97 ± 0.29 L·h^{-1}; day 5, 1.06 ± 0.26 L·h^{-1}; P < 0.05).

7.4.2 Heat acclimation induced adaptations

There were no differences in environmental temperature (33.0 ± 0.2 °C), RH (39.4 ± 3.4 %), USG (1.02 ± 0.01), sleep duration (7 ± 1 h), or sleep efficiency (86 ± 9 %) between PRE and POST experimental trials (P > 0.05). The induction of heat acclimation following 6-days post-exercise hot water immersion was evidenced by a reduction from PRE to POST in resting T_{re} (P < 0.001, d = 1.31), resting T_{es} (n = 8; P < 0.001; d = 1.30), end-exercise T_{re} (P < 0.001, d = 0.97) and end-exercise T_{es} (n = 8; P = 0.01; d = 1.05; Table 7.1). Other hallmark adaptations of heat acclimation were evident, including; a reduction at POST in T_{re} (P < 0.001; d = 1.09; Table 7.1) and T_{es} (n = 8; P = 0.001; d = 1.02; Table 7.1; Figure 7.2) at sweating onset and reductions in mean $\dot{V}O_2$ (P = 0.003; d = 0.33) and end-exercise HR (P < 0.001; d = 0.99), T_{sk} (P < 0.001; d = 1.03), RPE (P = 0.007; d = 0.87) and thermal sensation (P = 0.01; d = 0.86; Table 7.1). At POST, WBSR was unchanged (P > 0.05; Table 7.1) and reported as a function of T_{es}, there was no PRE to POST difference in sweating sensitivity (Figure 7.2 A) or the drive (ΔT_{es}) for sweating onset (Figure 7.2 B). Mean RER responses during exercise-heat stress were also unchanged PRE to POST (P > 0.05; Table 7.1). The optimised CO rebreathing technique did not detect any significant PRE to POST changes in total Hb mass, blood volume or plasma volume (+6 %; P > 0.05; Table 7.1).

Table 7.1 The change in physiological and perceptual variables following 6-day post-exercise hot water immersion heat acclimation at rest in temperate conditions (20 °C), and during submaximal treadmill running (65 % $\dot{V}O_{2max}$) in the heat (33 °C, 40 % RH).

	PRE-POST change
Resting T_{re} (°C)	-0.32 ± 0.22 **
Resting T_{es} (°C)	-0.41 ± 0.15 **
End-exercise T_{re} (°C)	-0.42 ± 0.30 **
End-exercise T_{es} (°C)	-0.61 ± 0.51 *
T_{re} at sweating onset (°C)	-0.31 ±0.23 **
T_{es} at sweating onset (°C)	-0.34 ± 0.17 **
End-exercise T_{sk} (°C)	-0.81 ± 0.71 **
End-exercise HR (beats·min^{-1})	-14 ± 6 **
WBSR (L·h^{-1})	-0.1 ± 0.3
End-exercise RPE	-2 ± 2 **
End-exercise thermal sensation	-1 ± 1 *
Mean $\dot{V}O_2$ (L·min^{-1})	-0.1 ± 0.1 **
Mean RER	-0.01 ± 0.03
Total Hb mass (g)	26 ± 45 (n = 9)
Blood volume (mL)	236 ± 447 (n = 9)
Plasma volume (mL)	213 ± 334 (n = 9)

T_{re}, rectal core temperature; T_{es}, oesophageal core temperature; T_{sk}, mean skin temperature; HR, heart rate; WBSR, whole body sweat rate; RPE, rating of perceived exertion; $\dot{V}O_2$, volume of oxygen consumption; RER, Respiratory exchange ratio; Hb, haemoglobin. Data displayed as mean ± SD of the change from PRE (n = 13). * $P < 0.05$, ** $P < 0.01$, POST less than PRE.

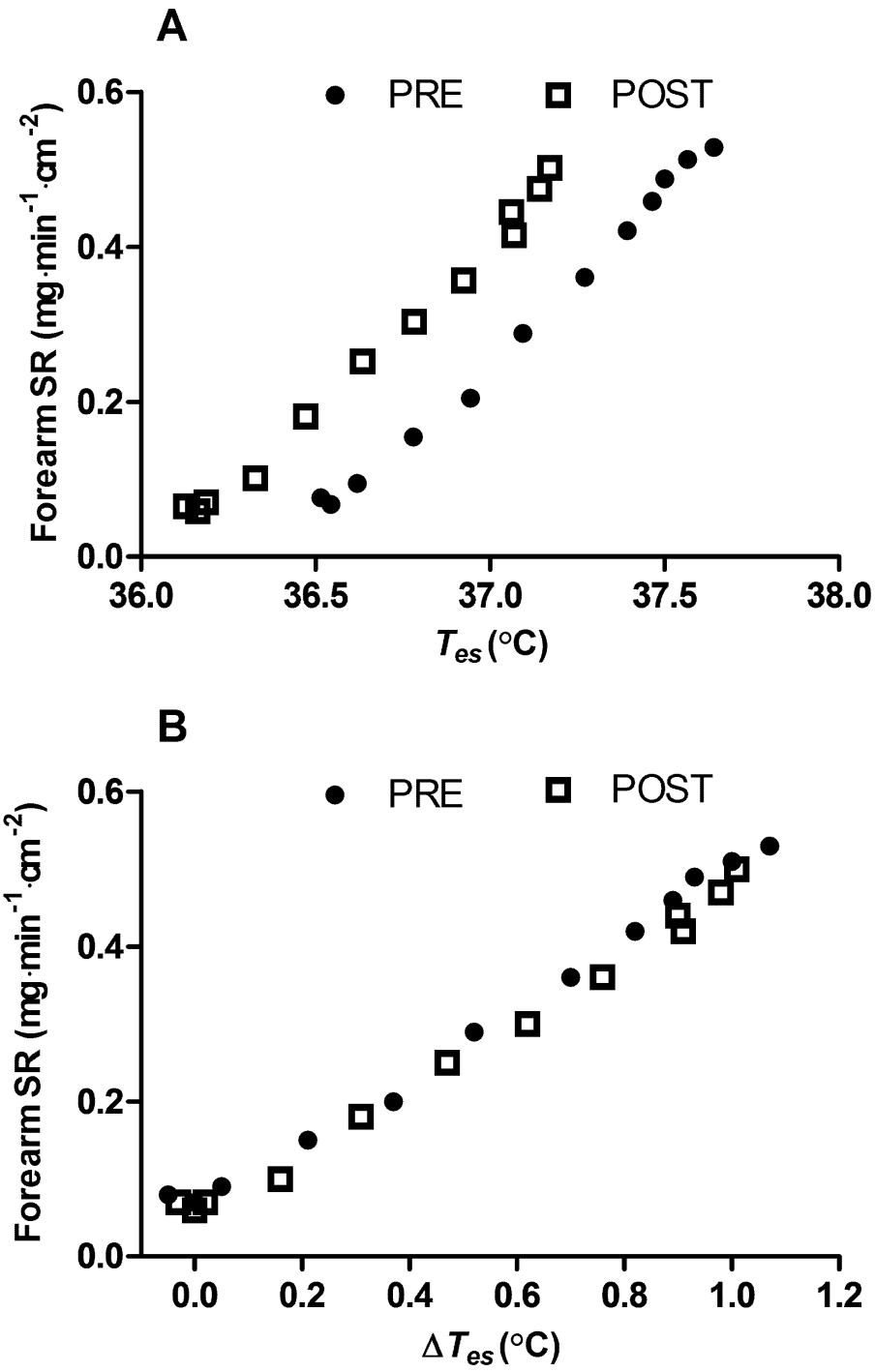

Figure 7.2 Mean local forearm sweating rate reported as a function of oesophageal core temperature (T_{es}; A) and as a function of the change in oesophageal core temperature (ΔT_{es}; B) during the first 12 min (1 min average) of submaximal treadmill running (65 % $\dot{V}O_{2max}$) in the heat (33 °C, 40 % RH). Data demonstrates a reduction in T_{es} at sweating onset (A), no difference in ΔT_{es} to initiate sweating (B) and no difference in sweating sensitivity (A and B) following 6-day post-exercise hot water immersion heat acclimation (n = 8).

7.4.3 Decay of heat acclimation

Nine of thirteen participants completed the 14-day heat acclimation decay protocol. Four participants were excluded from this stage of the protocol due to non-compliance to the experimental procedures. During the decay protocol activity levels from POST to WK 1 (8 ± 4 h per week) and WK 1 to WK 2 (6 ± 2 h per week) were lower compared with activity between PRE and POST (10 ± 4 h per week). Sleep duration or sleep quality the night before all experimental trials was not different ($P > 0.05$).

Adaptations following 6-day post-exercise hot water immersion were retained two weeks after the cessation of heat acclimation with a similar attenuation from POST ($P > 0.05$) in hallmarks of heat acclimation at WK 1 and WK 2. For example, the magnitude of attenuation in resting T_{re} and end-exercise T_{re} at WK 2 was similar to that observed at POST ($P > 0.05$, Figure 7.3). As such, from PRE to WK 2, a reduction in resting T_{re} (-0.36 ± 0.25 °C, $P = 0.01$, $d = 1.19$) and end-exercise T_{re} (-0.36 ± 0.37 °C, $P = 0.005$, $d = 0.98$) was observed. Other hallmarks of heat acclimation were also evident two weeks after the cessation of post-exercise hot water immersion. This retention of heat acclimation is demonstrated through the similar ($P > 0.05$) PRE to POST and PRE to WK 2 attenuations in T_{re} at sweating onset (-0.26 ± 0.24 °C, $P = 0.05$; $d = 0.83$) and end-exercise HR (-14 ± 10 beats·min^{-1}, $P < 0.001$; $d = 0.93$), T_{sk} (-0.77 ± 0.70 °C, $P = 0.002$; $d = 1.34$), RPE (-1 ± 2, $P = 0.03$; $d = 0.87$) and thermal sensation (-1 ± 1, $P = 0.002$; $d = 1.36$, Figure 7.4). However, the reduction in mean $\dot{V}O_2$ observed at POST was not evident at WK 1 or WK 2 ($P > 0.05$) and the modest PRE to POST expansion in plasma volume (+6 %) was not observed at WK 1 (+2 %) or WK 2 (+2 %; $P > 0.05$). WBSR and mean RER responses during exercise-heat stress were unchanged from PRE at WK 1 or WK 2 ($P > 0.05$).

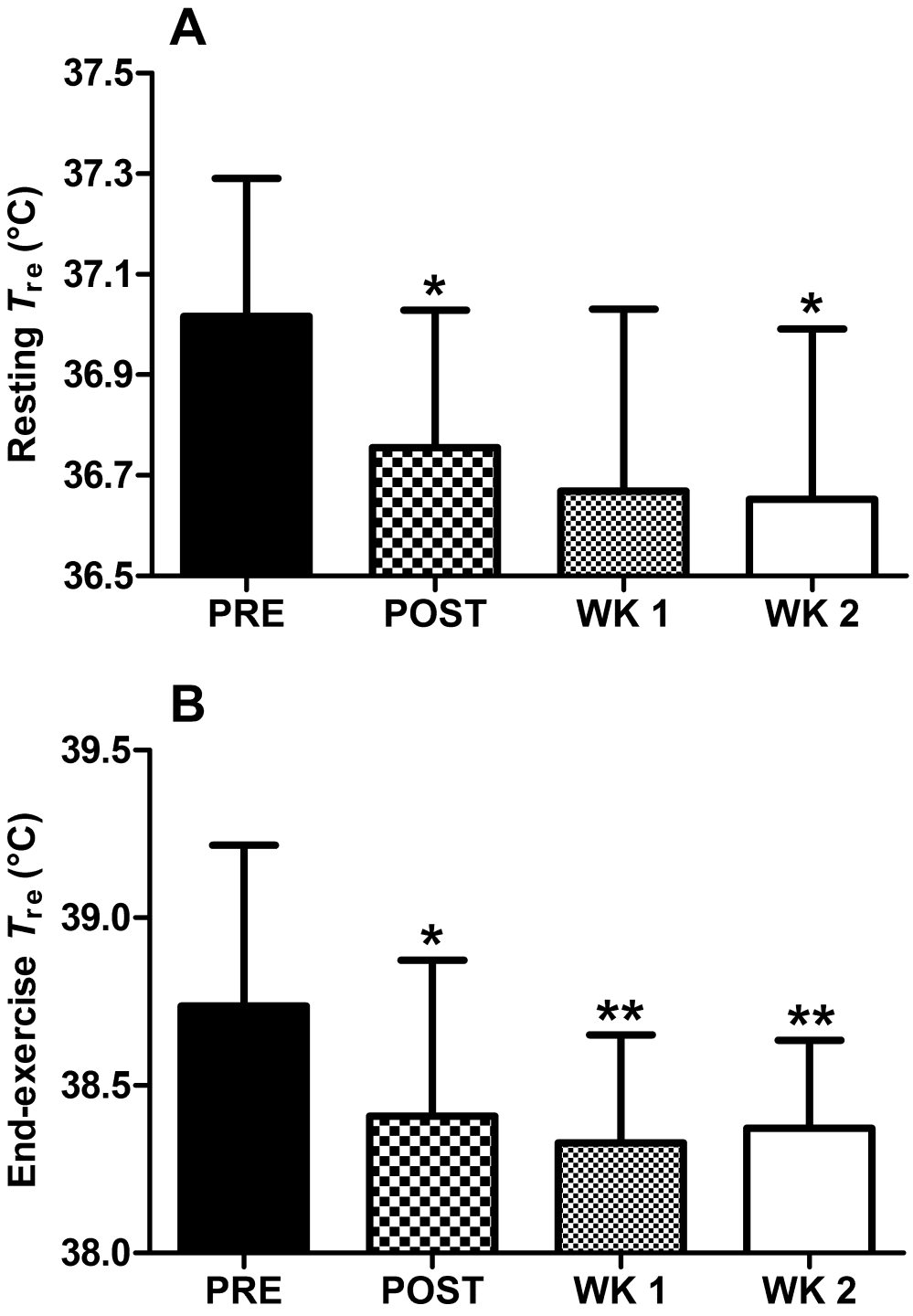

Figure 7.3 Influence of a 6-day post-exercise hot water immersion heat acclimation intervention on resting rectal core temperature (T_{re}, A) and end-exercise T_{re} (B) following 40 min treadmill running at 65 % $\dot{V}O_{2max}$ in the heat (33 °C, 40 % RH). Bars show mean ± SD responses before (PRE), after (POST), 7-days (WK 1) and 14-days (WK 2) following heat acclimation (n = 9). * P < 0.05, ** P < 0.01, POST, WK1 and WK 2 less than PRE.

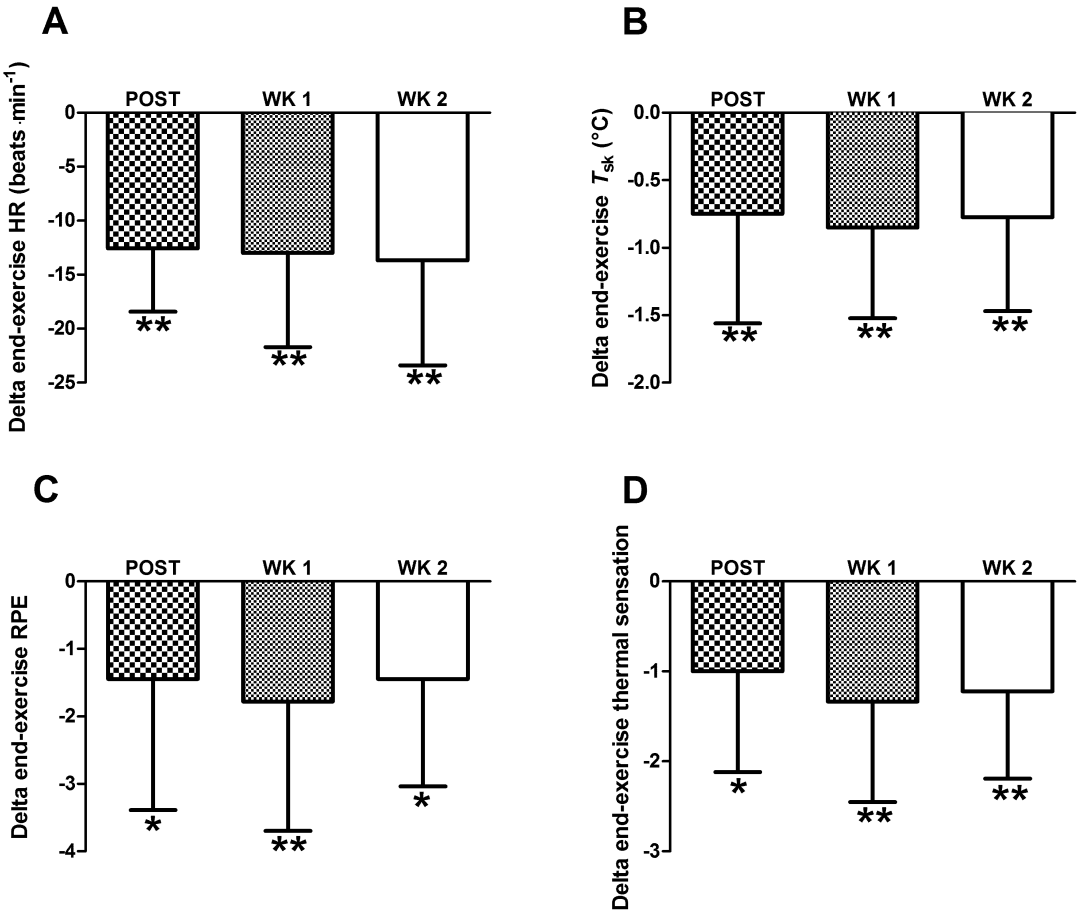

Figure 7.4 Influence of a 6-day post-exercise hot water immersion intervention on end-exercise heart rate (HR; A), end-exercise mean skin temperature (T_{sk}; B) end-exercise rating of perceived exertion (RPE; C) and end-exercise thermal sensation (D) assessed following 40 min treadmill running at 65 % $\dot{V}O_{2max}$ in the heat (33 °C, 40 % RH). Bars show mean ± SD of the change from PRE at POST and 7-days (WK 1) and 14-days (WK 2) following heat acclimation (n = 9). * $P < 0.05$, ** $P < 0.01$, POST, WK1 and WK 2 less than PRE.

7.5 Discussion

The present research is the first to examine the temporal patterning of adaptation decay following 6-day post-exercise hot water immersion heat acclimation. Contrary to our hypothesis, the novel findings from this research demonstrate that the attenuation in thermal strain during exercise-heat stress is retained for 14-days, with no loss in the extent of induced adaptation. This finding is supported through the similar reductions in T_{re} at rest and following exercise-heat stress at POST and at WK 2 (Figure 7.2). Additionally, the extent of adaptation in other hallmarks of heat acclimation demonstrated at POST, such as reduction in T_{re} at sweating onset, end-exercise HR, T_{sk}, RPE and thermal sensation, were similar at WK 1 and WK 2. Additional novel findings from the current research support our previous findings that post-exercise hot water immersion initiates a moderate and variable expansion in plasma volume (Table 7.1; **Chapters 4, 5 and 6**) and demonstrates that the intervention also does not alter the sensitivity of the sweating response during exercise heat stress when reported as a function of T_{es} (Figure 7.2). The appropriate measurement techniques for the assessment of plasma volume, using from the optimised CO rebreathing technique, and the sensitivity of the sweating response, using T_{es} and local sweating rate, provides confidence for these findings.

The current findings reaffirm our previous research that suggests 6-days of submaximal exercise in temperate conditions, followed by a hot water immersion, initiates hallmark heat acclimation adaptations including reductions in; resting (T_{re}, -0.32 °C; T_{es}, -0.32 °C) and end-exercise core temperature (T_{re}, -0.42 °C; T_{es}, -0.61 °C; Table 7.1; **Chapters 4, 5 and 6**). However, the present research also suggests that other hallmarks of heat acclimation, such as a large expansion in plasma volume and an increase in sweating sensitivity may not be initiated from post-exercise hot water immersion (**Chapters 4, 5, 6 and 7;** Senay *et al.*, 1976; Nielsen *et al.*, 1997; Gibson *et al.*, 2015b). Exposure to the passive thermal stimulus and/or the semi-recumbent body position during immersion in hot water may provide an explanation for why these adaptations were not observed. For example, the prone body position and/or hydrostatic squeeze when immersed in hot water may maintain vascular volumes, with interstitial fluid entering the vascular space during the initial stages of immersion, to reduce fluid regulatory stress and attenuate the expansion in plasma volume (Boning *et al.*, 1988; Bradford *et al.*, 2015; Nagashima *et al.*, 1999). In addition, the passive heat exposures may not initiate an improvement in sweating sensitivity due to the lack of a substantial endurance training stimulus and the initiation of associated adaptations, which may be observed

following repeated days of exercise-heat stress; this hypothesis however requires investigation (Nadel *et al*., 1974; Henane *et al*., 1977; Sawka *et al*., 1885a; Lorenzo *et al*., 2010; James *et al*., 2017).

The novel findings from this investigation supports previous research in suggesting that once acclimated to the heat, the rate of adaptation decay is much slower than its acquisition (**Section 2.5**; Pandolf *et al.*, 1977; Weller *et al.*, 2007; Taylor, 2014), with adaptations from 6-day post-exercise hot water immersion retained for at least 14-days. This extent of heat acclimation retention may compare positively with that demonstrated following short-term exercise-heat acclimation, where the loss of adaptation is rapid (< 14-days; Garrett *et al.*, 2009), and may align with the retention timeframe observed following longer duration exercise based protocols (14-26-days; Pandolf *et al.*, 1977; Weller *et al.*, 2007; Daanen *et al*., 2011). This comparison is yet to be made however, if correct, the potential favourable retention of adaptation following both MTHA and post-exercise hot water immersion, compared to STHA, may be induced through exposure to a larger cumulative thermal stimulus, which may initiate acclimatory homeostasis and adaptations that occur later on the adaptation continuum, such as a reduction in resting core temperature (Horowitz, 2016; Daanen *et al*., 2017). Exposure to a larger thermal stimulus may be initiated either by the completion of a greater number of exercise-heat exposures (MTHA), or through larger daily elevations in core temperature (T_{re}; Δ2.1 °C) and a maintained increase in skin temperatures, where skin equilibrates with water temperature (40 °C), during post-exercise hot water immersion (**Chapter 4**). Exposure to the dual endogenous and exogenous thermal stimulus, which is thought to initiate a more complete adaptation (Fox *et al*., 1964; Regan *et al*., 1996), during immersion in hot water may therefore optimise the speed of adaptation uptake and prolong the retention of heat acclimation. Additionally, the present data, and information presented by Daanen and colleagues (2011), may also indicate that the benefits from heat acclimation could be augmented following a period of recovery in cool conditions (Daanen et al., 2017). This concept of supercompensation (Budgett, 1998; Smith, 2003; Kellmann, 2010), which may represent the delay in physiological remodelling following exposure to the heat, warrants further investigation (Horowitz, 2016).

Heat acclimation recommendations suggest that short-term exercise based protocols provide heat acclimation adaptations and cause fewer disturbances to training than protocols

involving > 7 exposures (Garrett *et al*., 2011; Taylor, 2014; Periard *et al*., 2015). However, the decay of adaptations from these programmes is rapid (< 2 weeks) and as such, to acquire the greatest benefit, athletes and military personnel are required to complete these protocols in the week prior to moving to the heat, compromising pre-competition taper (Garrett *et al.*, 2009). Findings reported in the current research however suggest that post-exercise hot water immersion compares positively to short-term exercise-based heat acclimation in terms of both the initial extent of adaptation and its retention, although this requires confirmation (Garrett *et al.*, 2009; Tyler *et al.*, 2016). Therefore, this cost-effective, practical heat acclimation strategy has the potential to be incorporated alongside regular training, as part of post-exercise washing routines, a number of weeks before movement to the heat, potentially reducing disruptions to taper or pre-deployment training (**Chapters 4, 5 and 6**). We recognise that the current research does not fully illustrate the timeframe of decay from post-exercise hot water immersion or directly compare the retention of heat acclimation with STHA or MTHA. However, previous exercise-based MTHA research that initiates a similar attenuation in resting and exercise core temperature as post-exercise hot water immersion, suggests that the heat acclimation adaptations may be retained for up to a month (Weller *et al.*, 2007). Future research is required to establish this and to elucidate the responsible mechanisms for the retention of adaptation following post-exercise hot water immersion. Additionally, investigations are required to establish if the retention of physiological and perceptual adaptations translates to a retention in performance improvements in endurance trained males and females.

The novel findings from the current research suggest that once heat acclimation adaptations are attained from 6-days of post-exercise hot water immersion, physically active males retain the benefits for at least 14-days. The post-exercise hot water immersion intervention offers a simple and practical heat acclimation strategy that, unlike short-term exercise based heat acclimation protocols, may be integrated into regular training a number of weeks prior to relocation to the heat therefore, reducing the negative impact of acquiring heat acclimation adaptations on taper or pre-deployment training.

CHAPTER EIGHT

General discussion

8.1 Summary of main findings

The aim of this thesis was to investigate the efficacy of a post-exercise hot water immersion heat acclimation intervention and to question current practical heat acclimation recommendations. All chapters explore the phenotypic adaptations induced from 6-days of post-exercise hot water immersion and its effect on exercise capabilities in the heat (**Chapters 4, 5, 6 and 7**). **Chapter 4** also demonstrates that post-exercise hot water immersion improves endurance exercise performance in the heat but does not demonstrate an ergogenic effect on 5 km TT performance in 18 °C (Figure 4.4), even though thermal strain was reduced during submaximal exercise in temperate conditions (Figures 4.2 and 4.3). **Chapter 5** questions current recommendations and reports that similar heat acclimation benefits are observed when post-exercise hot water immersion is completed in the morning and exercise-heat stress occurs in the morning or afternoon (Table 5.2, Figures 5.1 and 5.2). Post-exercise hot water immersion also induces meaningful heat acclimation adaptations in both endurance trained and recreationally active individuals, without any practical alterations to the experimental protocol (Table 6.3, Figure 6.1; **Chapter 6**). Finally, **Chapter 7** reports that heat acclimation adaptations are retained for at least 14-days following 6-days of post-exercise hot water immersion (Figures 7.3 and 7.4). Together, these studies demonstrate that post-exercise hot water immersion induces a larger magnitude of heat acclimation adaptation compared to exercise based protocols of a similar number of exposures, while overcoming many of the practical restrictions associated with acclimating to the heat.

8.1.1 The induction of heat acclimation adaptations

Findings from **Chapters 4, 5, 6 and 7** demonstrate that the novel post-exercise hot water immersion intervention induces heat acclimation adaptations including; a reduced resting T_{re} in temperate conditions and attenuations in T_{re} at sweating onset and T_{re}, T_{sk}, HR and perceptual strain (RPE and thermal strain) during exercise-heat stress (Tables 5.2 and 6.3, Figures 4.2, 4.3, 5.1, 5.3, 6.1, 7.3 and 7.4). Additionally, the magnitude of attenuation in thermal strain is large (resting T_{re}, -0.3 °C; exercise-heat stress T_{re}, -0.4 °C), and compares positively to that demonstrated following STHA, with the level of adaptation aligning closely

with that of MTHA, although this claim requires conformation (Nielsen *et al.*, 1997; Garrett *et al.*, 2009; Tyler *et al.*, 2016). This large magnitude of adaptation during exercise-heat stress following post-exercise hot water immersion is, in part, initiated through the meaningful reduction in T_{re} at rest, which may (Gibson *et al.*, 2015a; Neal *et al.*, 2016a; Neal *et al.,* 2016b; Rendell *et al.,* 2017) or may not (Garrett *et al.,* 2009; Garrett *et al.,* 2012; Garrett *et al.,* 2014) be observed following conventional exercise based STHA. The reduction in resting T_{re} acts to 'pre-cool' individuals, reducing T_{re} at sweating onset and initiating the reduction in T_{re} during exercise (**Chapters 4, 5, 6 and 7**). The reduction resting T_{re} is likely initiated through an increase in skin blood flow for a given T_{re}, which distributes body temperature throughout the body and away from the core (Taylor, 2014). The incorporation of a control group into **Chapter 4** adds further support to efficacy of this intervention by demonstrating that daily submaximal exercise in temperate conditions and immersion in thermoneutral water on six consecutive days does not initiate improvements in thermoregulatory and cardiovascular variables during submaximal exercise in both temperate and hot environments.

Post-exercise hot water immersion induces commonly observed heat acclimation adaptations however, the data within this thesis suggests that subtle differences in adaptation may exist with those reported following exercise-based protocols. For example, conventional exercise-heat acclimation interventions initiate a large +9-15 % expansion in plasma volume (Senay *et al.*, 1976; Nielsen *et al.*, 1997; Gibson *et al.*, 2015b). Whereas **Chapters 4, 5 and 6**, using the relative changes in Hb and Hct (Dill and Costill, 1974) and **Chapter 7**, using optimised CO rebreathing technique, demonstrate that post-exercise hot water immersion initiates a smaller non-significant expansion in plasma volume (+3-6 %). Explanations for this apparent attenuation in plasma volume expansion may include hydrostatic pressure during immersions limiting net fluid regulatory stress through the movement of interstitial fluid into the vascular space during the initial stages of immersion (Boning *et al.*, 1988; Bradford *et al.*, 2015; Akerman *et al.*, 2016). In addition, much like observations made during bed rest, the semi-recumbent body position during the hot water immersion may decrease hydrostatic forces, limiting the rise in plasma albumin content which, for example, occurs following upright exercise and facilitates the expansion in plasma volume (Convertino *et al.*, 1980; Nagashima *et al.*, 1999; Nagashima *et al.*, 2000; Bradford *et al.*, 2015). Data presented in **Chapter 7** also suggests that post-exercise hot water immersion does not improve the sensitivity of the

sweating response (Table 7. 1, Figure 7.2). However, as introduced in **Sections 2.2.2 and 7.5**, the cause of the improvement in sweating sensitivity following exercise-heat acclimation may be associated with peripheral adaptations that occur alongside improvements in physical fitness and $\dot{V}O_{2max}$ following exercise-heat acclimation (Nadel *et al.*, 1974; Roberts *et al.*, 1977; Sawka *et al.*, 1985; Lorenzo *et al.*, 2010; Lorenzo and Minson, 2010; James *et al.*, 2017). It is currently unknown if post-exercise hot water immersion initiates improvements in physical fitness however, as training load is theoretically lower during post-exercise hot water immersion than during conventional exercise-heat acclimation, it may be hypothesised that improvements in aerobic fitness and the associated sweating adaptations may not be initiated.

Data from this thesis also suggests that heat acclimation adaptations, or a portion of the acquired benefits, may be gained in fewer than six post-exercise hot water immersion exposures. Markers of heat acclimation including, a reduction in HR during submaximal exercise in thermoneutral conditions and an increase in WBSR, from post-exercise hot water immersion heat acclimation sessions, were observed from day 4 of the intervention (Tables 4.1, 5.1 and 6.2; **Chapters 4, 5, 6 and 7**). However, due to the experimental model of investigations and the constant forcing function applied during the 6-day intervention, future is required to ascertain a timeframe, or the thermal stimulus required, to initiate all thermoregulatory and cardiovascular adaptations from post-exercise hot water immersion heat acclimation.

The heat acclimation adaptations initiated from 6-days of post-exercise hot water immersion improves self-paced 5 km treadmill TT performance in the heat, with performance restored, to the level achieved in thermoneutral conditions (Figure 4.4; **Chapter 4**). This finding is in agreement with the small, but growing body of evidence regarding the effect of heat acclimation on not only endurance exercise capabilities but, on self-paced endurance performance in the heat (Nielsen *et al.*, 1997; Lorenzo *et al.*, 2010; Garrett *et al.*, 2012; Racinais *et al.*, 2015b). In view of the reported reductions in thermoregulatory, cardiovascular and perceptual strain during submaximal exercise in temperate conditions (Figures 4.4.2 and 4.3), it was anticipated, but not observed, that post-exercise hot water immersion would initiate an improvement in 5 km TT performance in 18 °C (**Chapter 4**). However, the improved physiological and perceptual responses during submaximal exercise

in temperate conditions (Figures 4.4.2 and 4.3), tentatively suggest that the post-exercise hot water immersion intervention may initiate improvements in performance in this environment. Reasoning for why this was not observed in **Chapter 4** could be that the performance test was not sufficiently thermally demanding to demonstrate the ergogenic performance potential of post-exercise hot water immersion (**Chapter 4**).

8.1.2 Factors that affect the induction of heat acclimation adaptations

The number of heat acclimation exposures completed currently categorises interventions into short, medium and long-term programmes. STHA induces favourable cardiovascular and thermoregulatory adaptations that reduce thermal strain during exercise-heat stress (Armstrong and Maresh, 1991). The extent of induced adaptation increases with the number of heat exposures, with complete adaptation thought to occur following exercise based MTHA (Pandolf, 1998). The extent of adaptation from 6-days of post-exercise hot water immersion however, compares positively to controlled hyperthermia heat acclimation of a similar number of exposures (Garrett *et al.*, 2009), with the attenuation in thermal strain comparable to that seen following MTHA (Nielsen *et al*., 1997). Research investigating this comparison is required to confirm this, however, upon comparison of the size of the thermal impulse during these protocols this claim may be substantiated. For example, short-term controlled hyperthermia heat acclimation aims to maintain mild hyperthermia on ≈ 5 days (ΔT_{re}; 1.7 °C; Gibson *et al.*, 2015a) whereas, 8-13 submaximal bouts of exercise in the heat to exhaustion and 6-days of post-exercise hot water immersion, induce similarly large elevations in core temperature (ΔT_{re}; 2.1-2.3 °C; Table 4. 1 and 5.1; **Chapters 4, 5, 6 and 7**; Nielsen *et al.*, 1997). Therefore, when the time to acclimate is limited, post-exercise hot water immersion provides a practical and economic alternative to exercise based protocols, which ultimately may induce a larger magnitude of adaptation than exercise based protocols in fewer heat exposures.

To acquire the greatest benefits, heat acclimation recommendations also state that the clock-time of exposure sessions should be matched with the anticipated clock-time of future exercise-heat stress (Shido *et al.*, 1999; Cable *et al.*, 2007; Beaudin *et al.*, 2009; Chalmers *et al.*, 2014; Patterson *et al.*, 2014; Taylor, 2014; Periard *et al.*, 2015). However, **Chapter 5** demonstrates that post-exercise hot water immersion completed in the morning induces hallmark heat acclimation adaptations at rest and during exercise-heat stress similarly in both

the morning and afternoon (Figures 5.1 and 5.2, Table 5.2). Support for this finding is also evident from exercise-heat acclimation research. For example, when the clock-time of heat acclimation sessions and subsequent exercise-heat stress were matched or performed at a different clock-time, similar attenuations in thermal strain during exercise-heat stress are reported (Garrett *et al.*, 2009; Gibson *et al.*, 2015a). Explanations for these differences with the research of Shido and colleagues (1999), whose work the recommendations are based upon, are apparent when comparing the thermal impulse and the magnitude of adaptation. Although Shido *et al.* (1999) report a statistically significant reduction in core temperature at rest, this change was subtle (-0.09 to -0.19 °C) and initiated through a mild thermal stimulus (daily ΔT_{re}; ≈0.75 °C). The data presented within **Chapter 5**, although limited through the lack of a crossover group acclimating in the afternoon and an exercising control group, suggests that when exposed to a larger thermal stimulus, the clock-time heat acclimation adaptations may not be apparent.

Research also suggests that an individual's training status influences the temporal patterning of adaptation from exercise-heat acclimation (Pandolf *et al*., 1977; Shvartz *et al*., 1977. **Chapter 6** demonstrates that both endurance trained and recreationally active individuals acquire a meaningful reduction in thermal strain during exercise-heat stress following post-exercise hot water immersion (Figure 6.1, Table 6.3). **Chapter 6** also indicates that post-exercise hot water immersion exposes both endurance trained and recreationally active individuals to a progressive thermal stimulus demonstrated through the similar daily AUC and rise in T_{re} throughout the 6-day intervention (Table 6.2). Additional findings also demonstrate that the recreationally active acquire a greater benefit from post-exercise hot water immersion and that the endurance trained require exposure to a larger thermal stimulus, provided by a greater perceptual tolerance to immersion in hot water, to experience a meaningful reduction in thermal strain during exercise-heat stress. Of interest, the extent of adaptation in the endurance trained very strongly negatively correlates with the size of the thermal stimulus (r = -0.71). As this relationship is not evident in the recreationally active (r = 0.12), heat acclimation is conceivably induced in these individuals through the combined exposure to the thermal stress, and an increase in habitual endurance exercise during the intervention which may be improved aerobic fitness, although this requires investigation.

8.1.3 The decay of heat acclimation adaptation

The aim of **Chapter 7** was to outline the decay of heat acclimation from post-exercise hot water immersion. However, a similar attenuation in thermal strain during exercise-heat stress was observed 48 h and 16-days following the final post-exercise hot water immersion exposure, demonstrating retention of heat acclimation for at least 2-weeks (Figures 7.3 and 7.4). This data suggests that the benefits of post-exercise hot water immersion are retained for longer than those following short-term exercise heat acclimation (Garrett *et al.*, 2009), where the benefits are lost after 7-days, and closely align with the magnitude of heat acclimation retention following MTHA (Weller *et al.*, 2007; Daanen *et al.*, 2011). For example, Weller *et al.* (2007) report minimal losses of adaptation following a 26-day decay protocol and Daanen *et al.* (2011) report a slight amplification after 18-days without exposure to the heat. The present data also indicates that a small, non-significant improvement in heat acclimation benefits may be observed seven and fourteen days following the completion of post-exercise hot water immersion, which may demonstrate a delay in physiological remodelling following heat acclimation (Figures 7.3 and 7.4; Horowitz, 2016). An explanation for these differences in adaptation retention between short and medium-term heat acclimation is likely due to differences in the size of the thermal stimulus and the extent of induced adaptation (Daanen *et al.*, 2017). For example, compared to STHA, MTHA and post-exercise hot water immersion may augment reductions in cardiovascular and thermoregulatory strain during exercise-heat stress, and reduces core temperature at rest, although this comparison is yet to be made (Tyler *et al.*, 2016). The comparable reductions in thermal strain between MTHA and post-exercise hot water immersion, and the reduction in resting core temperature, may therefore indicate a similar retention timeframe between these two protocols (**Chapter 7**; Weller *et al.*, 2007; Daanen *et al.*, 2011).

8.2 Limitations

The novel findings outlined within this thesis provide evidence for the efficacy of heat acclimation by post-exercise hot water immersion. **Chapters 4, 5 and 6** provide a clear proof of concept for the intervention however, these findings are not without limitation. For example, within **Chapter 4**, between group (HWI vs. CON) comparisons were not made and, due to the lower participant numbers within CON, this group may be under powered. Although heat acclimation research commonly does not include work-matched control comparisons, the lack of group comparisons and possible lack of power, may limit the

efficacy of the post-exercise hot water immersion intervention. In addition, the mechanisms behind the initiated adaptations are not fully investigated. **Chapter 7** does, in part, address these issues and provides a greater examination of the induced adaptations including the evaluation of blood compartment changes (optimised CO rebreathing technique) and a greater assessment of sweating responses (T_{es} measurement). However, further elucidation of the mechanisms involved to initiate adaptation from immersion in hot water including the purported cellular adaptations associated with heat adaptation are required. A favourable retention of heat acclimation following post-exercise hot water immersion is also outlined within **Chapter 7** however, the mechanisms involved are not outlined, a full demonstration of adaptation decay is not made and the effect of decay on endurance performance is not addressed. This thesis also does not outline the speed of adaptation induction nor does it directly compare post-exercise hot water immersion with conventional exercise based protocols. Additionally, as within most areas of sports science, the influence of the intervention on physiological, perceptual and performance adaptations within an elite athlete or female population is not assessed.

8.3 Perspectives

Current heat acclimation recommendations state that to acquire heat acclimation adaptations individuals should exercise in the heat for 1-2 h on 10 to 14 consecutive days (Gill and Sleivert, 2001; Racinais *et al.*, 2015a; Tyler *et al.*, 2016). STHA protocols however provide a portion of adaptation in a shorter timeframe, causing fewer disturbances to training (Pandolf, 1998; Garrett *et al.*, 2011). Despite this reduced time commitment, exercise based STHA protocols still suffer in terms of cost and practicality. One may also speculate that the completion of daily fatiguing exercise in the heat may actually attenuate athletic performance and lead to non-functional overreaching if training load and recovery is not correctly balanced (Meeusen *et al.*, 2010; Meeusen *et al.*, 2013). It is perhaps for these physiological and psychological reasons and practical restraints, that prior to 2015 IAAF World Athletics Championships only 15 % of athletes attempted to adapt to the heat and only two long distance athletes trained in an artificially hot environment prior to the championships (Periard *et al.*, 2017).

In contrast, post-exercise hot water immersion overcomes many of these practical limitations as the protocol could be easily incorporated into regular training as part of post-exercise

washing routines and access to a hot environment is not required. Additionally, unlike the described 'gold-standard' exercise based controlled hyperthermia technique (**Section 2.3.1**), precise measurement and control of core body temperature, and a practitioner who dictates exercise intensity is not required to maintain the thermal stimulus. A further benefit of post-exercise hot water immersion is that the intervention could also be easily manipulated to suit a number of different scenarios. For example, when the aim is to habituate large numbers of military personnel to a standard thermal stress, the duration and/or frequency of exposures could be reduced. Alternatively, for an athlete wishing to maximise the benefits, the adaptation stimulus could be amplified with a progressive increase in immersion duration or by allowing immersions to be limited through volitional discomfort. The findings presented within this thesis also suggest that the completion of post-exercise hot water immersion may reduce the impact of acquiring heat acclimation. This reduction in training disturbance/load compared to exercise based protocols is yet to be shown however, as the 6-day post-exercise hot water immersion protocol may provide a comparable magnitude of adaptation as MTHA (T_{re} reduction; -0.3 °C at rest, -0.4 °C during exercise-heat stress) in fewer exposures, the time required to acclimate, and the number of exhaustive, dehydrating heat acclimation sessions may be reduced (Nielsen *et al.*, 1997; Aoyagi *et al.*, 1998; Patterson *et al.*, 2004). Therefore, although post-exercise hot water immersion may increase physiological strain experienced, the completion of the intervention may not substantially increase training load, compared with exercise based protocol, and accordingly may reduce the likelihood of the occurrence of overreaching and the associated decrement in performance, although this is yet to be established (Meeusen *et al.*, 2010; Meeusen *et al.*, 2013), compared to exercise based protocols. In addition, immersion in hot water may also have therapeutic benefits which, for example, may improve sleep quality (Horne and Reid, 1985).

Experimental chapters (**Chapters 4, 5, 6 and 7**) presented within this thesis demonstrate the effectiveness of the post-exercise hot water immersion intervention in inducing heat acclimation adaptations in physically active males. These chapters also provide suggestions to acquire the greatest benefits and improve the integration of post-exercise hot water immersion heat acclimation into training/taper. For example, **Chapter 5** conflicts with current views and reports that time of day heat acclimation adaptations are not apparent when post-exercise hot water immersion is completed in the morning and exercise-heat stress occurs in the afternoon (Shido *et al.*, 1999; Taylor, 2014; Periard *et al.*, 2015). As such, this

commonly reported recommendation may require examination. **Chapter 6** demonstrates that endurance trained individuals acquire heat acclimation from post-exercise hot water immersion without any alterations to the experimental protocol and **Chapter 7** proposes that adaptations from the 6-day intervention are retained for longer than adaptations following exercise based STHA (Garrett *et al.*, 2009). In combination, these findings suggest that it may not be necessary to align the clock-time of heat exposures with the anticipated clock-time of subsequent exercise-heat stress and that the intervention could be completed well before an athlete enters their taper, therefore reducing disturbances in training and sleeping patterns especially if international travel between time zones is required.

8.4 Future directions

The findings from this research not only highlight the benefits of heat acclimation by post-exercise hot water immersion, but also question traditional heat acclimation recommendations. Firstly, contrary to the popular consensus that passive protocols initiate a smaller magnitude of adaptation (Taylor and Cotter, 2006), the reported data suggests that post-exercise hot water immersion initiates a similar reduction in thermal strain as longer duration exercise based protocols; however, confirmation of this is required. Future research should also outline the effect of the intervention on aerobic fitness (e.g. $\dot{V}O_{2max}$), blood flow distribution and cellular thermal tolerance while also investigating the mechanisms that initiate the induced adaptations. These include, examining the effect of the large elevation of skin temperatures during immersion in hot water on the reduction in thermal strain and the reduction in resting core temperature that acts to pre-cool individuals (Regan *et al.*, 1996; Tansey and Johnson, 2015; Tan *et al.*, 2016).

Experimental chapters within this thesis assess the induction and decay of heat acclimation adaptation following six post-exercise hot water immersion exposures. To optimise the protocol so that it may be adapted to suit various different scenarios, future research should investigate the efficacy of a shorter (≤ 4 exposures) and longer (≥ 10 exposures) duration post-exercise hot water immersion protocol on the induced adaptations. The timeframe of full adaptation decay from these interventions of different length, as well as from the 6-day intervention is also required. Outlining these timeframes would provide information for individuals wishing to acclimate when the time is limited (< 6-days) and for those planning for the integration of protocols into training/taper. The benefits of hot water immersion are

also likely greater when core temperature is elevated following exercise in temperate conditions. Future research should however verify this and establish if the thermal stimulus can be elevated with an increase in exercise intensity and/or duration prior to immersion or through an increase in water temperature or immersion duration. Findings from **Chapter 4** also suggest that post-exercise hot water immersion heat acclimation does not improve self-paced performance in temperate conditions. This finding adds to the work completed in this area however, as suggested, a performance test that is sufficiently thermally demanding may demonstrate the ergogenic potential of post-exercise hot water immersion (Lorenzo *et al.*, 2010; Minson and Cotter, 2016; Nybo and Lundby, 2016). Future research is also required to assess the potential that post-exercise hot water immersion heat acclimation may increase muscular strength (Kodesh and Horowitz, 2010; Goto *et al.*, 2011), improve sleep quality (Horne and Reid , 1985), improve cardiovascular (Brunt *et al.*, 2016) and metabolic health (Faulkner *et al.*, 2017) and/or improve immune status (Walsh *et al.*, 2011).

Statements from within this thesis have also suggested that post-exercise hot water immersion would better integrate into regular training and cause less disruption to training/taper than conventional exercise-heat acclimation. However, the safe, real-world application of this protocol, with or without a measure of core temperature, is yet to be established. Additionally, endurance athlete's perceptions of the protocol have not been investigated. These individuals for example, may either prefer to prepare for competition in the heat by training in a natural hot environment or to alleviate thermal strain on the day of competition through precooling or fluid consumption strategies (Periard *et al.*, 2017). To further assist with the integration of post-exercise hot water immersion heat acclimation into training and to verify theories outlined within **Chapter 7**, future research should also investigate the time course for the induction of adaptations and establish if a recovery phase following these exhaustive programmes provides an improvement in the observed adaptation.

An area that this thesis does not address is the common assumption that becoming acclimated to the heat reduces susceptibility to EHI. Historical research suggests that the heat acclimation strategies employed in South African gold mines reduced mortality numbers within the mines (Dreosti, 1935) and theoretically, reducing thermal strain during exercise-heat stress, either through an improvement in physical fitness or through the acquisition of heat acclimation adaptations, should reduce EHI symptoms (Armstrong *et al.*, 2007).

However, there is no empirical evidence regarding the aetiology of this condition. Various other factors such as a genetic predisposition (Smith *et al.*, 2016) immune dysfunction (Shephard and Shek, 1999), malignant hyperthermia susceptibility (Hopkins, 2007), and previous EHI occurrence (Keren *et al.*, 1981) may all be underlying factors of this condition and require investigation.

8.5 Conclusions

The findings from this thesis include:

1. A hot water immersion immediately after exercise in temperate conditions on six consecutive days reduces thermal strain and improves exercise capabilities during submaximal exercise in both temperate and hot environments.

2. The novel 6-day post-exercise hot water immersion intervention improves self-paced endurance exercise performance in the heat.

3. An ergogenic benefit of post-exercise hot water immersion heat acclimation is not demonstrated during 5 km treadmill TT performance in temperate conditions.

4. Adaptations from post-exercise hot water immersion, completed in the morning, are observed in the morning and afternoon. Therefore, post-exercise hot water immersion exposures may not need to be aligned with the clock-time of future exercise-heat stress to acquire the greatest benefit.

5. Post-exercise hot water immersion induces meaningful heat acclimation adaptations in endurance trained and recreationally active males. However, the recreationally active acquire a larger benefit.

6. Two weeks following post-exercise hot water immersion there is no observable decay in the induced heat acclimation adaptations.

7. The extent of adaptation from the post-exercise hot water immersion intervention may compare positively to the recognised gold-standard short-term controlled hyperthermia heat acclimation technique. With the extent of adaptation more aligned

to medium-term exercise based protocols, although research is required to confirm this.

8. A large portion of the reduction in thermal strain during submaximal exercise seems likely caused by the meaningful reduction in resting core temperature.

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APPENDIX A

Example Informed Consent

Bangor University
HEALTH AND EXERCISE SCIENCES

1	Title of project		
2	Name and e-mail addresses of all researchers	Michael Zurawlew Prof. Neil Walsh Dr Jessica Mee	pep409@bangor.ac.uk n.walsh@bangor.ac.uk j.mee@bangor.ac.uk

Please tick boxes

1 I confirm that I have read and understand the Information Sheet dated ……. for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2 (i) Patients:
I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason, without my medical care or legal rights being affected. ☐

(ii) Students:
I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason. If I do decide to withdraw I understand that it will have no influence on the marks I receive, the outcome of my period of study, or my standing with my supervisor or with other staff members of the School. ☐

(iii) General members of the public:
I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason. ☐

3 I understand that I may register any complaint I might have about this experiment with Professor Tim Woodman, Head of School of Sport, Health and Exercise Sciences, and that I will be offered the opportunity of providing feedback on the experiment using the standard report forms. ☐

4 I agree to take part in the above study. ☐

Name of Participant …….

Signature ……. Date ……..

Name of Person taking consent…….

Signature …….

APPENDIX B

Example Medical Questionnaire

Bangor University
SCHOOL OF SPORT, HEALTH AND EXERCISE SCIENCES

Name of participant ……..……..

Age ……

Are you in good health? ☐ YES ☐ NO

If no, please explain

How would you describe your present level of activity?
Tick intensity level and indicate approximate duration.

Vigorous		Moderate		Low intensity	

Duration (min)…….

How often?

< Once per month		2-3 times per week	
Once per month		4-5 times per week	
Once 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 from allergies? ☐ YES ☐ NO

If yes, please give particulars:

Do you suffer, or have you ever suffered from:

	YES	NO		YES	NO
Asthma			Epilepsy		
Diabetes			High blood pressure		
Bronchitis					

Are you currently taking medication? ☐ YES ☐ NO

If yes, please give particulars:

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 laboratory experiment? ☐ YES ☐ NO

PLEASE READ THE FOLLOWING CAREFULLY

Persons will be considered unfit to do the experimental exercise task if they:

- have a fever, cough or cold, or suffer from fainting spells or dizziness;
- have suspended training due to a joint or muscle injury;
- have a known history of medical disorders, i.e. high blood pressure, heart or lung disease;
- have had hyper/hypothermia, heat exhaustion, or any other heat or cold disorder;
- have anaphylactic shock symptoms to needles, probes or other medical-type equipment;
- have chronic or acute symptoms of gastrointestinal bacterial infections (e.g. Dysentery, Salmonella);
- have a history of infectious diseases (e.g. HIV, Hepatitis B); and if appropriate to the study design, have a known history of rectal bleeding, anal fissures, haemorrhoids, or any other condition of the rectum.

PLEASE COMPLETE AND SIGN THE DECLARATION BELOW

DECLARATION

I agree that I have none of the above conditions and I hereby volunteer to be a participant in experiments/investigations during the period of ……20……

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 explained to my satisfaction the purpose of the experiment and possible risks involved.

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

Furthermore, if I am a student, I am aware that taking part or not taking part in this experiment, will neither be detrimental to, or further, my position as a student.

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 *(participant)* …….. Date ……..

Print name ……..

Signature *(experimenter)* ……. Date ……..

Print name ……..…...

APPENDIX C

Measurement reliability of total haemoglobin mass using the optimised carbon monoxide method

C.1 Purpose

The optimised CO rebreathing technique (Schmidt and Prommer, 2005) is an accurate (Gore *et al.*, 2005), harmless dilution technique to determine total Hb mass. Total Hb mass is measured through the changes of CO bound to Hb (% COHb) after inhaling a defined volume of this gas. CO is used as a marker due to its strong affinity to Hb. Erythrocyte, blood and plasma volumes can also be calculated from total Hb mass with measurements of Hb concentration and Hct. To acquire accurate and reproducible results the examination instructions need to be carefully followed. The typical error which indicates the accuracy of the method is reported from qualified laboratories to be between 1.1 % and 2 % (Schmidt and Prommer, 2005; Gore *et al.*, 2006; Gibson *et al.*, 2015). The typical error also aligns with traditional CO rebreathing techniques (≈ 2 %; Gore *et al.*, 1997; Ashenden *et al.*, 1999) where the O_2-CO gas bolus is rebreathed between 10-15 min. It is advised that before the protocol is used in research investigations that the experimenter's reliability is within this range. Therefore, the aim of the study was to establish the experimenter's typical error for the measurement of total Hb mass using the optimised CO rebreathing technique.

C.2 Methods

Experimental procedures: Nine participants completed three experimental trials (T1, T2 and T3) with ≥ 24 h between measurements. During each trial, the same optimised CO rebreathing procedure was completed (**Section 3.4.6**). In brief, following a 20 min seated rest to stabilise blood volumes, earlobe capillary blood samples (65 μL) were collected and assessed for % COHb. Participants were then instructed to exhale into a CO gas meter, to provide baseline end-tidal CO concentration in the lungs. Participants then rebreathed within a closed system a bolus (0.7 - 1.0 $mL \cdot kg^{-1}$ body mass) of CO (99.9 %) and medical grade oxygen (O_2; 3 L; 99.5%) that was mixed on the first inhalation, for 2 min. During rebreathing a CO meter was held close to the spirometer to check for leaks. After 2 min, participants exhaled to residual volume and were disconnected from the spirometer, this expired air sample was collected and the CO content was measured. End-tidal CO concentration in the

lungs was again assessed and earlobe capillary samples were then taken at 6 and 8 min following the commencement of the rebreathing protocol and analysed for % COHb. The pre to post change in % COHb and the volume of CO distributed in the body was used to calculate total Hb mass.

Data analysis: Data in results are displayed as mean ± SD. Total Hb mass was calculated using SpiCO calculation software (Version 2.0; Blood tec, Bayruth, Germany). Pearson's correlations were performed to determine the relationship in measured total Hb mass between T1 and T2 and between T1 and T3. To indicate the accuracy of the method reliability was quantified by the calculation of typical error of measurement (Hopkins, 2000) using Microsoft Excel (Microsoft, Washington, USA). Typical error (TE) is the standard deviation of the difference scores of *n* series of measurements divided by *n*.

C.3 Results

Strong relationships with total Hb mass (Figure C.1) were found when comparing T1 *vs* T2 (r^2 = 0.97) and T1 *vs* T3 (r^2 = 0.96) with a mean difference in total Hb mass of 0.20 ± 2.79 % (T1 *vs* T2) and -0.67 ± 2.83 % (T1 *vs* T3). Calculated typical error was 1.98 % (± 17 g) between T1 *vs* T2 and 2.00 % (± 17 g) between T1 *vs* T3. When comparisons between the three tests were combined typical error decreased to 1.96 % (± 16 g).

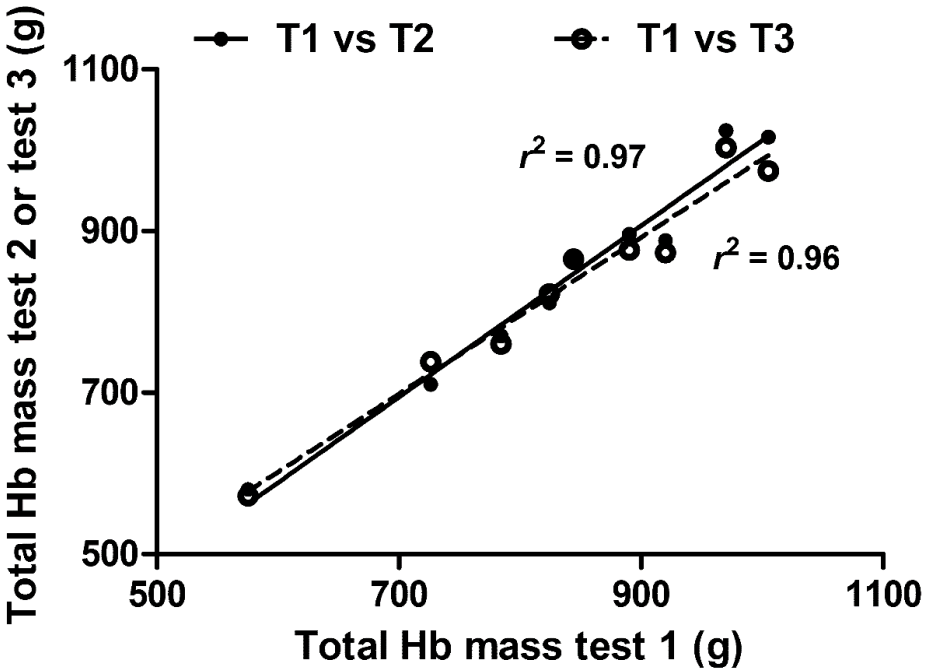

Figure C.1 Correlation of the measurement of total Hb mass between T1 *vs* T2 and T1 *vs* T3 using the optimised CO rebreathing technique.

C.4 Conclusions

The aim of the investigation was to establish the experimenter's reliability for the measurement of total Hb mass using the optimised CO rebreathing technique. The small mean differences between trials demonstrate strong linear relationships and the variability between trials is aligned with the typical error of 1.1 % to 2.0 % commonly reported for the optimised CO rebreathing technique (Schmidt and Prommer, 2005; Gore *et al.*, 2006; Gibson *et al.*, 2015). These findings suggest that experimenter error is low and within the bounds for reliable measurements of total Hb mass.