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Novel insights into heat acclimation by post-exercise hot water immersion

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Novel insights into heat acclimation by post-exercise hot water immersion

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Supervisors: Prof. Neil P. Walsh, Dr Jessica A. Mee, & Dr Samuel J. Oliver

Summary

Hot environmental conditions can reduce exercise performance, work productivity, and increase the risk of exertional heat illness. In preparation for competing or working in the heat, athletes, military personnel, and occupational workers who reside in temperate conditions are advised to heat acclimate to alleviate thermal strain and improve physical capabilities. Conventional exercise heat acclimation protocols can be costly, impractical, and ineffective over short durations. Six days of post-exercise hot water immersion presents an alternative heat acclimation strategy that provides thermal adaptations and improvements in endurance performance. However, direct comparisons between short- (≤7 exposures) and medium-term (8–14 exposures) post-exercise hot water immersion and exercise heat acclimation are yet to be made. Further, previous findings are limited to the effects of 6 days of post-exercise hot water immersion, hence, the temporal patterning of adaptation is unknown. Finally, the mechanism(s) for the achieved thermal benefits after heat acclimation requires elucidation. Previous literature suggests that the reduction in core temperature after heat acclimation may be associated with changes in circulating thyroid hormones; however, research is required to confirm this. Therefore, the aims of this thesis were to compare thermal adaptations after short- and medium-term post-exercise hot water immersion with exercise heat acclimation and thermoneutral exercise, establish whether shortening the 6-day post-exercise hot water immersion intervention provides meaningful adaptations and whether extending it achieves a more complete state of heat acclimation, and to investigate the relationship between thermal adaptations and changes in plasma thyroid hormone concentrations after heat acclimation. We demonstrated that compared with conventional short-term exercise heat acclimation, 6 days of post-exercise hot water immersion elicited larger thermal adaptations, observable after 3 days. Extending the 6-day post-exercise hot water immersion intervention to 12 days provided no further thermal benefits or improvements in endurance capacity. Medium-term post-exercise hot water immersion conferred a more complete heat acclimation than exercise heat acclimation and thermoneutral exercise. Short- and medium-term heat acclimation by post-exercise hot water immersion and exercise heat acclimation was partly associated with changes in thyroid hormone concentrations. However, temporal and intervention disconnects between thermal adaptations and thyroid hormones changes indicate that changes in thyroid hormones are not the mechanism for short- and medium-term heat acclimation adaptation.

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Declaration of Originality

Yr wyf drwy hyn yn datgan mai canlyniad fy ymchwil fy hun yw'r thesis hwn, ac eithrio lle nodir yn wahanol. Caiff ffynonellau eraill eu cydnabod gan droednodiadau yn rhoi cyfeiriadau eglur. Nid yw sylwedd y gwaith hwn wedi cael ei dderbyn o'r blaen ar gyfer unrhyw radd, ac nid yw'n cael ei gyflwyno ar yr un pryd mewn ymgeisiaeth am unrhyw radd oni bai ei fod, fel y cytunwyd gan y Brifysgol, am gymwysterau deuol cymeradwy.

I hereby declare that this thesis is the results of my own investigations, except where otherwise stated. All other sources are acknowledged by bibliographic references. This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree unless, as agreed by the University, for approved dual awards.

Publications

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

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CHAPTER SEVEN

Thesis Format

This thesis contains a general introduction (**Chapter 1**), which provides a brief background and outlines the aims of the thesis. The literature review (**Chapter 2**) then provides a more in-depth overview of previous literature and identifies the gaps in current knowledge. The general methods section (**Chapter 3**) provides details of the procedures and measures that have been used in two or more of the subsequent experimental chapters. Three experimental chapters form the focus of the thesis. **Chapter 4** is a peer-reviewed article and has been included as published in the *Journal of Science and Medicine in Sport*, except for some minor amendments in the interest of consistency through the thesis. This chapter compares adaptations after 3 and 6 days of post-exercise hot water immersion and exercise heat acclimation. The second experimental chapter investigates the relationship between the reduction in resting core temperature after heat acclimation and changes in circulating thyroid hormone concentrations (**Chapter 5**). The third experimental chapter expands upon the previous work, comparing adaptations after 12 days of post-exercise hot water immersion and exercise heat acclimation, and investigates the effect of medium-term heat acclimation on circulating thyroid hormones concentrations (**Chapter 6**). Finally, the general discussion (**Chapter 7**) provides a summary and critical analysis of the main findings with consideration of the recognised limitations and suggests potential areas for future research. Lists of abbreviations, figures and tables are included before **Chapter 1**. **Bold type** is used to refer to chapters or sections within this thesis.

List of Figures

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List of Abbreviations

Δ	change
%ΔPV	percentage change in plasma volume
°C	degrees Celsius
α	alpha
ß	beta
µm	micrometre
ANCOVA	analysis of covariance
ANOVA	analysis of variance
AUC	area under the curve
BAT	brown adipose tissue
BDNF	brain-derived neurotrophic factor
Ca^{2+}	calcium ion
CI	confidence interval
cm	centimetre
CO	carbon monoxide
COHb	carboxyhaemoglobin
d	Cohen's *d* effect size
dL	decilitre
EDTA	edthylenediaminetetraacetic acid
EHA	exercise heat acclimation
EHI	exertional heat illness
F/F	fluorescence response
g	gram
h	hour
HR	heart rate
HR_{max}	maximal heart rate
Hsp	heat shock protein
HWI	post-exercise hot water immersion
IAAF	International Association of Athletics Federations
I-FABP	intestinal fatty-acid binding protein
IL	interleukin
JSP	Joint Service Publication
kh	kilogram
km	kilometre

L	litre
LPS	lipopolysaccharide
LTHA	long-term heat acclimation
m	metre
METs	metabolic equivalents
min	minute
mL	millilitre
n	number
ng	nanogram
nmol	nanomole
O_2	oxygen
pmol	picomoles
PV	plasma volume
r	Pearson correlation coefficient
RER	respiratory exchange ratio
RH	relative humidity
RPE	rating of perceived exertion
s	second
SD	standard deviation
SEM	standard error of the mean
SPSS	Statistical Package for the Social Sciences
STHA	short-term heat acclimation
T3	triiodothyronine
T4	thyroxine
T_{amb}	ambient temperature
T_{core}	core body temperature
TNE	thermoneutral exercise
TNF-α	tumour necrosis factor alpha
T_{re}	rectal core temperature
TS	thermal sensation
T_{sk}	mean skin temperature
TTE	time to exhaustion
UK	United Kingdom of Great Britain and Northern Ireland
USA	United States of America
USG	urine specific gravity
$\dot{V}O_2$	oxygen uptake
$\dot{V}O_{2max}$	maximal oxygen uptake

$\dot{V}O_{2peak}$	peak oxygen uptake
W	watt
WBSR	whole-body sweat rate
W_{max}	maximal work capacity

CHAPTER ONE

General Introduction

In 1924, the high occupational workloads and rock temperatures approaching 60°C resulted in the first recorded death from exertional heat stroke in the Deep City Mine, Johannesburg (Schneider, 2016). Following several more heat-related deaths, the Manager of the Rand Mines Ltd. and their chief medical officer, Dr A. J. Orenstein hired Dr Aldo Dreosti to conduct experiments to prepare recruits for working in the heat. In 1927, Dr Dreosti began the first investigation into the on-going heat-related deaths, the findings of which resulted in the number of acclimation days being modified to consider the regional origin of the native workers (Dreosti, 1935, 1949). Despite this new acclimation protocol, heat-related deaths continued to occur, leading to further revisions to heat acclimation guidelines.

Almost a century has passed, and exertional heat illness remains a problem in athletic and occupational settings. Several major sporting events take place in conditions with substantial environmental heat stress, such as the 2019 International Association of Athletics Federations (IAAF) World Athletics Championships in Doha and the Tokyo 2020 Olympics. Similarly, military training and field operations involve personnel being deployed to hot climates (Schickele, 1947; Cook, 1955). Athletes and military personnel who endure prolonged exercise in these conditions are at risk of homeostatic perturbations that can impair exercise performance (Galloway & Maughan, 1997; Maughan *et al.*, 2012) and result in exertional heat illness (Casa *et al.*, 2015). For example, of the 307 surveyed athletes competing in the 2015 IAAF World Championships in Beijing, 48% had previously experienced exertional heat illness symptoms and 8.5% had been diagnosed with exertional heat illness (Périard *et al.*, 2017). The incidence rate of exertional heat illness in the military is also high and has not changed over the past 5 years (Joint Service Publication (JSP) 539, 2019). Specifically, there were 333 UK Armed Forces personnel who were identified as having a heat illness during the period October 2015–September 2016.

Before engaging in exercise in hot environments, consensus recommendations state that athletes and military personnel should complete a period of heat acclimation to alleviate heat strain and improve exercise capacity in the heat (Armstrong *et al.*, 2007; Casa *et al.*, 2015; Racinais *et al.*, 2015a; Tyler *et al.*, 2016). To induce heat acclimation adaptations, current recommendations are to complete ≥ 15 daily exercise-heat stress exposures, which initiate profuse sweating and maintain an elevated body temperature for ≥ 60 min (Périard *et al.*, 2015; Saunders *et al.*, 2019). However, adherence to these recommendations is impractical as they require access to an environmental chamber or travel to a hot climate to complete the intervention (Ashworth *et al.*, 2020), and interfere with pre-competition taper and pre-deployment training guidelines (Mujika & Padilla, 2003; Bosquet *et al.*, 2007; Saunders *et al.*, 2019). Consequently, athlete and military engagement with conventional exercise heat acclimation is poor (Périard *et al.*, 2017; Parsons *et al.*, 2019).

Taking a hot bath immediately after exercise in temperate conditions provides an alternative and more accessible heat acclimation strategy compared with conventional exercise-based approaches. The results from recent research into post-exercise hot water immersion are promising, demonstrating both performance enhancing and thermal adaptations (Zurawlew *et al.*, 2016; Zurawlew *et al.*, 2018b). However, current findings are limited to six post-exercise hot water immersion exposures and have not been directly compared with conventional exercise heat acclimation. Furthermore, the underlying mechanism(s) for post-exercise hot water immersion adaptation is unknown. Therefore, the primary aim of this thesis was to compare the temporal patterning of post-exercise hot water immersion adaptation with conventional exercise heat acclimation. This thesis also aimed to investigate the mechanism for post-exercise hot water immersion and exercise heat acclimation adaptation.

CHAPTER TWO

Literature Review

The focus of this review is heat acclimation in adult humans, with specific regard to methodology, adaptation, maladaptation, and mechanisms. To appreciate these processes, a brief background in thermoregulation and the acute response to heat stress is provided. This review aims to provide an in-depth overview of previous literature and highlight the gaps in current knowledge, which may identify and guide future directions for heat acclimation research.

2.1 Thermoregulation

Normal human core body temperature (T_{core}) is around 37.0°C and controlled within a narrow range (36.7–37.5°C; Sund-Levander *et al*., 2002). There are normal fluctuations that occur throughout the day (circadian rhythm; Waterhouse *et al*., 2005), throughout a month (menstrual cycle; Cagnacci *et al*., 1996), and throughout a lifetime (aging; Lu *et al*., 2010). Abnormal T_{core} deviations outwith this value places the individual at risk of hyperthermia ($T_{core} \geq 38.5$°C) or hypothermia ($T_{core} < 36.0$°C; Armstrong *et al*., 1996). Despite the need to maintain T_{core} within such a narrow range, humans can survive in extreme conditions, from ultra-marathon running in the Sahara Desert to ice diving in the Arctic Circle.

The ability to regulate T_{core} is a fundamental homeostatic process that is critical to survival. Thermoregulation is comprised of numerous mechanisms that are centrally governed by the hypothalamus. Table 2.1 shows physiological and behavioural responses to a change in ambient or internal temperature (Tansey & Johnson, 2015). Classically, it was believed that temperature control involved the integration of central and peripheral thermal signals coordinated in the preoptic area of the hypothalamus and the comparison of this integrated signal with a "set-point" signal (Hammel *et al.*, 1963). If an error between the input temperatures and the set-point occurs, then this would trigger appropriate heat gain or heat loss mechanisms. However, in the last two decades technological advancements have allowed a greater understanding of how T_{core} is controlled (Tansey & Johnson, 2015). The current consensus is that central and peripheral temperatures influence individual effector circuits independently. Thermoreceptor neurons are activated when the appropriate temperature

threshold for that neuron is reached, and action potentials ascend, via synaptic relays, to the preoptic area. These signals, along with thermoreceptive signals arising within the preoptic area, act on several effector outputs, and the influence of central and peripheral signals varies between different effectors. Hence, the concept of the "set-point" has been updated with a more nuanced model, and the term "balance point" has been proposed as an alternative (Romanovsky, 2007, 2018).

Table 2.1. Physiological and behavioural responses to a change in ambient or internal temperature.

Core temperature stimulus	Effector	Response
Increase	Endocrine tissue	Decreased metabolic rate
	Skin blood vessels	Vasodilation
	Sweat glands	Sweating
	Behaviour	Reduced activity, stretched body position, and loss of appetite
Decrease	Skeletal muscle	Shivering and thermogenesis
	Brown adipose tissue	Non-shivering thermogenesis
	Endocrine tissue	Increased metabolic rate
	Skin blood vessels	Vasoconstriction
	Arrector pili muscles	Piloerection and air trapping
	Behaviour	Increased activity, huddled body position, and increased appetite

Since the identification of the preoptic area as an important region for central temperature detection more than 80 years ago (Teague & Ranson, 1936; Magoun *et al.*, 1938), the specific neurological pathways that regulate thermoregulation have largely remained elusive and are only now beginning to emerge. Several recent ground-breaking studies, exploiting innovative mice models, have started to elucidate the complex neural circuitry that regulates T_{core} (Song *et al.*, 2016; Tan *et al.*, 2016; Zhao *et al.*, 2017). Song *et al.* (2016) used calcium imaging to demonstrate that transient receptor potential M2 ion channels function as ionotropic warm receptors in hypothalamic neurons that limit the fever response and may detect increased temperature to prevent overheating. These findings provide direct *in vivo* evidence that the action potential firing rate of warm-sensitive neurons in the preoptic area underlies T_{core}

homeostasis. Additionally, recent work by Tan and colleagues (2016) confirmed that activation of warm-sensitive neurons induce a decrease in T_{core}. The authors also demonstrated that these warm-sensitive neurons were rapidly activated by environmental warmth, indicating that they are regulated by thermal signals from the periphery (Figure 2.1).

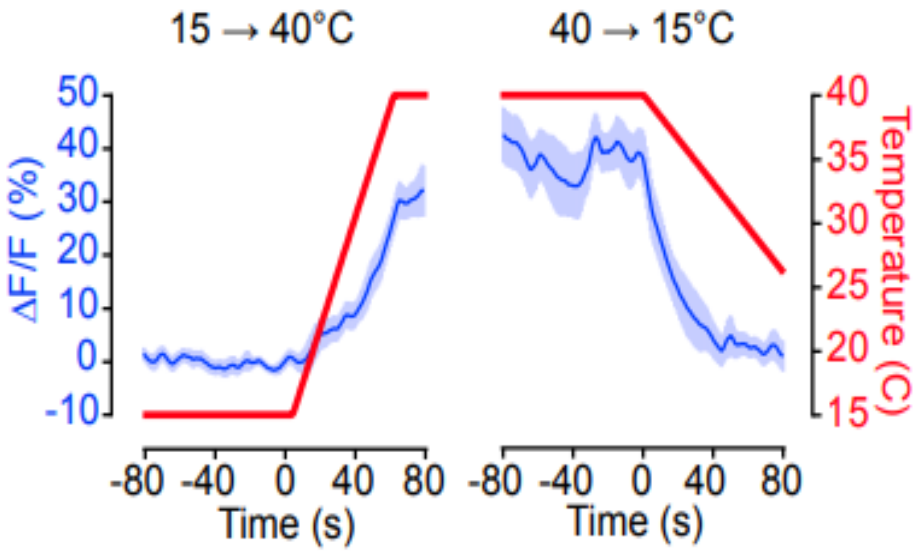

Figure 2.1. Mean ventromedial preoptic area neuron responses (recorded using fiber photometry) during rapid temperature transitions. Fiber photometry is a calcium imaging technique used to monitor fluorescent signals emitted by green fluorescent protein-bound Ca^{2+} via an implanted fiber optic cannula. Blue line = fluorescence response (F/F; %), red line = temperature (°C). Taken from Tan *et al.* (2016).

More recently, a study by Zhao *et al.* (2017) verified connections within the hypothalamic neural network that coordinate changes in T_{core} in either direction. Interestingly, both Tan *et al.* (2016) and Zhao *et al.* (2017) independently discovered that brain-derived neurotrophic factor (BDNF) is transcriptionally activated after a heat challenge. BDNF is one of the four mammalian neurotrophic factors that control synaptic function and synaptic plasticity (Huang & Reichardt, 2001). Taken together, these findings suggest that long-term heat exposure may affect nerve growth and cause remodelling. Such plasticity in the thermoregulatory centre may partly explain the physiological adaptations associated with repeated exposure to a hot environment (Siemens & Kamm, 2018). These recent discoveries have opened a new line of

inquiry into mammalian thermoregulation, which may promote the design of ergogenic paradigms geared towards promoting these adaptations.

2.2 Heat stress

2.2.1 Exercise-heat-stress

Exercise in hot and hot-humid conditions poses one of the greatest challenges to homeostasis of the human body. As the ambient temperature and humidity increases, thermoregulatory heat-loss mechanisms become less effective; hence, a greater proportion of the heat produced from working muscles is stored in the body and T_{core} rises. Work efficiency and exercise performance are impaired in hot environments. Galloway and Maughan (1997) acutely depicted this phenomenon in a study comparing the effects of different ambient temperatures on cycling performance in recreationally active males. The authors observed that endurance capacity was markedly reduced when cycling in the heat (31°C) compared to temperate (21°C), and cool (11°C) conditions (Figure 2.2A). Similarly, Maughan *et al.* (2012) demonstrated that time to exhaustion in a warm environment is progressively impaired as the relative humidity (RH) increases (Figure 2.2B). The effect of heat stress on endurance performance has also been observed during self-paced time trials in well-trained cyclists (Tatterson *et al.*, 2000). The authors observed a reduction in mean power output during exercise in the heat compared with exercise in thermoneutral conditions (Figure 2.2C).

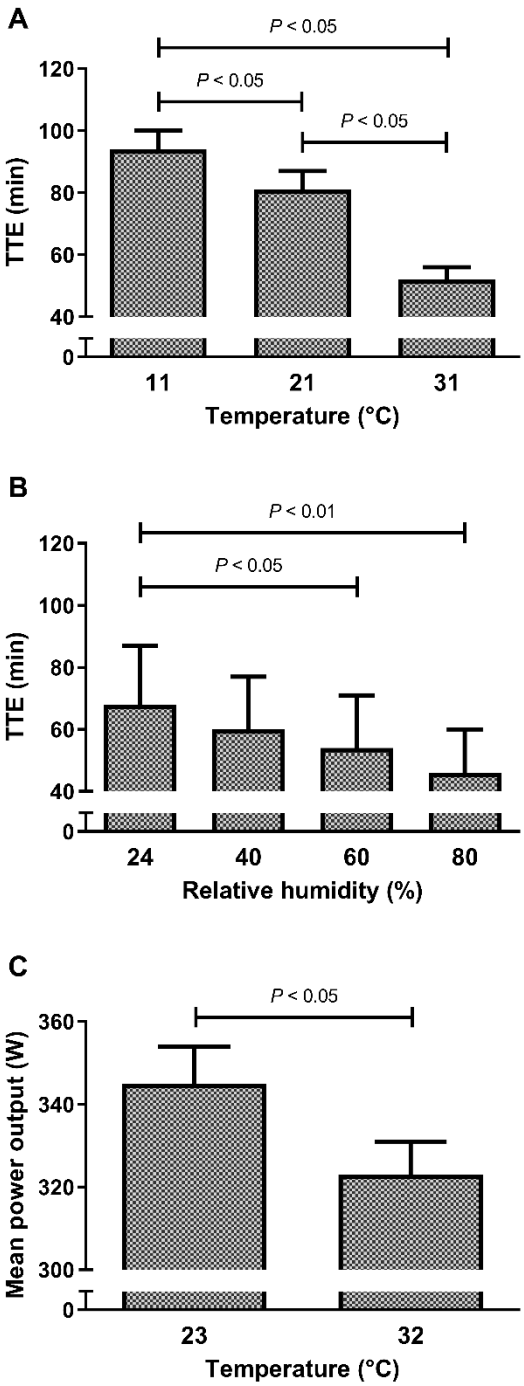

Figure 2.2. The influence of temperature and humidity on exercise performance.

(A) Time to exhaustion (TTE) under four different ambient temperature conditions at 70% RH. Bars represent mean ± SEM. (Galloway & Maughan, 1997)

(B) TTE under three different ambient humidity conditions at 30°C. Bars represent mean ± SD. (Maughan *et al.*, 2012)

(C) Mean power output during 30-min time-trial in thermoneutral and hot conditions. Bars represent mean ± SEM. (Tatterson *et al.*, 2000)

Fatigue during prolonged exercise in the heat is rarely caused by a single physiological parameter. Instead, it is more likely that fatigue is influenced by a complex interplay between the cardiovascular, respiratory, central nervous, and peripheral (muscular) systems (Nybo *et al.*, 2014). Also, it is considered that different factors might limit maximal exercise than submaximal intensity aerobic performance in the heat. At maximal exercise intensities, the reduced endurance capacity is likely owing to cardiovascular mechanisms related to oxygen delivery (Rowell *et al.*, 1966; Nybo & Nielsen, 2001a; Gonzalez-Alonso & Calbet, 2003). Whereas, for submaximal intensities it is more complex, involving several integrated parameters, such as oxygen delivery, T_{core} and perceived strain (Cheuvront *et al.*, 2010; Nybo, 2012; Sawka *et al.*, 2012).

During exercise-heat-stress there is a competing demand for blood flow between the working muscles to enable oxygen supply and to the skin to facilitate heat loss to the surrounding environment (Gonzalez-Alonso *et al.*, 2008). This increased demand for blood flow to the periphery results in a reduced central blood volume (Rowell *et al.*, 1966). Consequently, diastolic filling of the heart is impaired, which lowers end-diastolic volume and stroke volume. As a result, an elevated heart rate is required to maintain cardiac output. At rest and during light/moderate exercise in the heat, elevations in heart rate may fully compensate for any reduction in stroke volume; indeed, heart rate is usually higher to meet the increased demand (Rowell *et al.*, 1966). Conversely, during exercise at high intensities in the heat, cardiac output declines as heart rate reaches maximum, leading to an earlier onset of fatigue. In a study by Gonzalez-Alonso and Calbet (2003), eight trained males cycled at 80% peak power output until exhaustion in both hot and temperate conditions. The authors observed that heat stress accelerated the decline in cardiac output and mean arterial pressure, which resulted in a reduced $\dot{V}O_{2max}$ and exercise time to exhaustion. These findings are consistent with those of previous research completed in endurance-trained cyclists (Nybo *et al.*, 2001). These decrements in performance during exercise in the heat are typically observed alongside increases in mean skin temperature (T_{sk}) and sweat rate compared to exercise at the same relative intensity in thermoneutral conditions.

In addition to the physiological consequences of exercise-heat-stress, there are also concurrent increases in perceptual measures, such as rating of perceived exertion (Tatterson *et al.*, 2000; Nybo & Nielsen, 2001b) and thermal comfort/sensation (Périard *et al.*, 2014). An increase in perceptual strain can have a compounding, and potentially independent, detrimental influence on exercise performance. Marcora *et al.* (2009) demonstrated that mentally fatigued

participants with increased ratings of perceived exertion at isotime reached exhaustion sooner than those in the control condition, despite similar physiological responses. These findings support Brehm's theory of motivation, which postulates that participants decide to disengage when a task is perceived to be either too difficult or effort demands exceed the upper limit of what they are willing to do (Wright, 2008). Furthermore, increased thermal sensation during match-play tennis in the heat was associated with an increase in time between points and a reduction in effective playing percentage (Périard *et al.*, 2014). The authors proposed that these adjustments represent a behavioural strategy adopted to reduce or offset the sensation of environmental conditions being perceived as difficult.

Exercise in hot conditions can also have deleterious effects at a cellular level. As previously discussed, during prolonged exercise-heat-stress blood is redistributed to the working muscles and peripheral circulation. Consequently, blood is drawn away from the gastrointestinal tract resulting in splanchnic hypoperfusion and ischaemia (Dokladny *et al.*, 2016). If gut hypoperfusion is prolonged it can lead to increased intestinal permeability (Figure 2.3), and in turn provoke endotoxin leakage (Smetanka *et al.*, 1999; Jeukendrup *et al.*, 2000; Lambert *et al.*, 2002). Endotoxins are highly toxic lipopolysaccharides (LPS) of the outer cell wall of Gram-negative bacteria. Endotoxin leakage is a hallmark of pathological conditions including exertional heat stroke (Bouchama *et al.*, 1991). On entering the blood stream, LPS trigger an immune response involving the production of pro-inflammatory cytokines, such as tumour necrosis factor-α (TNF- α), interleukin-6 (IL-6) and interleukin-1β (IL-1β; Peake *et al*., 2008; Selkirk *et al*., 2008). These pyrogenic cytokines promote an additional increase in temperature, initiating a vicious cycle of events that may lead to possible organ damage (Lambert, 2009).

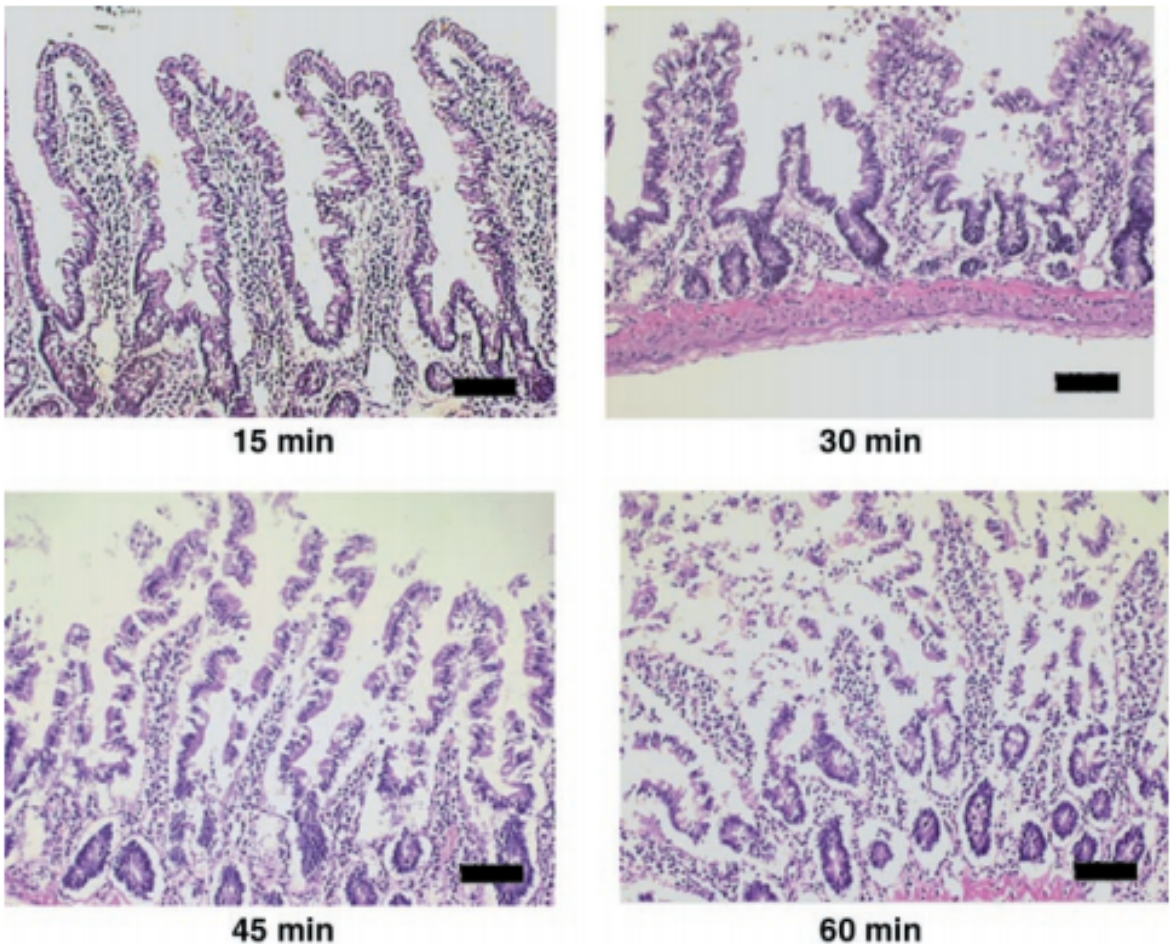

Figure 2.3. Representative light micrographs of haematoxylin and eosin-stained rat everted small intestinal sac tissue over a 60-min time course at 41.5–42°C. Note generally normal-appearing villi at 15 min and progressive deterioration of the structural integrity at subsequent timepoints. Bars represent 100 μm. Taken from Lambert *et al.* (2002).

Exercise-heat-stress is also a potent stimulus for the release of heat shock protein 72 (Hsp72) into the extracellular matrix (Marshall *et al.*, 2006; Périard *et al.*, 2012), and intracellular changes in total protein expression and/or gene transcription in monocytes and systemic tissue (McClung *et al.*, 2008; Selkirk *et al.*, 2009; Magalhaes *et al.*, 2010b; Amorim *et al.*, 2011). Hsp72 is highly responsive to cellular stress including that associated with exercise and is thought to be primarily responsible for the protective effects of the heat shock response (Henstridge *et al.*, 2016). Lee *et al.* (2017) found that extracellular Hsp72 was increased (baseline, 0.32 ± 0.38 ng·L^{-1} vs post-exercise, 0.77 ± 0.58 ng·mL^{-1}) after a 90-min treadmill run at a speed equivalent to 65% $\dot{V}O_{2max}$ (1% gradient) in the heat (32°C and 47% RH). Research investigating intracellular changes in Hsp72 expression in endurance-trained and

untrained individuals showed that after exercise-heat-stress, Hsp72 expression in circulating monocytes was elevated (Selkirk *et al.*, 2009). Interestingly, a greater induction of intracellular Hsp72 was observed in trained compared to untrained participants, which was accompanied by an attenuated inflammatory response and reduced apoptosis at exhaustion and following *in vitro* heat shock. These results imply that Hsp72 provides cellular protection against heat exposure and that this is enhanced in endurance-trained individuals.

2.2.2 Exertional heat illness

In extreme cases of exercise-heat-stress, where heat production from working muscles is not dissipated effectively, T_{core} can rise to dangerous levels, thereby putting the individual at risk of exertional heat illness (Armstrong *et al.*, 2007). Exertional heat illness refers to a spectrum of heat illnesses that occur during activity in conditions of environmental heat stress often characterised by high ambient temperatures and humidity levels (Nichols, 2014). The severity of exertional heat illness ranges from exercise-associated muscle cramps and heat exhaustion to heat syncope and the potentially fatal, exertional heat stroke (Casa *et al.*, 2015).

Athletes and military personnel are required to work in hot or hot/humid climates making them particularly susceptible to thermal injury (Armstrong *et al.*, 2007; Stacey *et al.*, 2015). Of the 307 athletes surveyed by Périard *et al.* (2017), 48% had previously experienced exertional heat illness symptoms and 8.5% had been diagnosed with exertional heat illness. Similarly, 2017 defence statistics show that the incidence of exertional heat illness and heat stroke in the military was 1.7 per 1,000 personnel and had not changed in the past 5 years ([Health] Report to Heat Illness Working Group, 2017). Furthermore, evidence suggests that these values may be subject to significant underreporting bias (Stacey *et al.*, 2016), therefore the true number of military heat illness casualties may be much higher.

The risk of heat illness can be exacerbated by numerous factors, which include, but are not limited to, elevated ambient temperature, high humidity, work intensity, physical fitness, hydration status, and lack of heat acclimation (Moore *et al.*, 2016; Nelson *et al.*, 2018). Other potential risk factors, which are less well supported include disruption of sleep, and the circulating inflammatory response (Lim & Mackinnon, 2006; Moore *et al.*, 2013). Causes of inflammation that could contribute to the development of exertional heat illness include muscle damage (Fortes *et al.*, 2013) illness/disease (Lim & Mackinnon, 2006), infection (Wijerathne *et al.*, 2016), and vaccination (Kuhlman *et al.*, 2018).

Despite being well-trained, highly motivated athletes and military personnel are particularly at risk of exertional heat illness as they are more likely to underestimate their thermophysiological state or ignore normal thermoregulatory behavioural cues during competitive events in the heat. This issue was recently revealed in an elegant study, whereby participants competed in a 20-km cycling time trial in the heat against an avatar (Corbett *et al.*, 2018). The crux of the protocol was that participants believed they were racing against another participant, but the competitor's avatar replicated their own preliminary time trial in cool conditions. The authors observed that this head-to-head competition resulted in increased external work rate, metabolic heat production, and improved performance compared to when the participants completed the time trial in the heat alone. Moreover, despite increases in thermophysiological strain, perceptual responses were unchanged with head-to-head competition. These findings demonstrate that competition is a risk factor of exertional heat illness and suggest athletes should be screened for psychological factors that might make them more susceptible to the effects of head-to-head competition.

Within the military population, recruits appear to be at increased risk of exertional heat illness (Nelson *et al.*, 2018). In a study investigating the predictors of heat illness in United States Army soldiers enlisting between January 2011 and December 2014 (n = 238,168), the authors observed 2,612 incident cases of mild heat illness and 732 incident cases of severe heat illness (Nelson *et al.*, 2018). The majority of mild (71%) and severe cases (60%) of heat illness occurred during the first 6 months of service, peaking at month 2 (Figure 2.4). It is likely that during the first months of service, recruits are unaccustomed to the thermal challenges associated with the intense physicality of military training. This lack of prior exposure to exercise-heat-stress may provide some explanation as to why new enlists are at greater risk of exertional heat illness. In addition, new recruits are more likely to be unaccustomed to the high physical demands of military training, hence, experience greater muscle damage (Hill *et al.*, 2012). Exercise-induced muscle damage has been shown to increase heat strain during subsequent exercise-heat-stress (Fortes *et al.*, 2013). It has also been demonstrated that becoming accustomed to exercise-induced muscle damage provides protection against this increase in heat strain (Dolci *et al.*, 2015). It is likely, therefore, that as training continues the impact of muscle damage on heat strain (and associated exertional heat illness) is reduced. In addition, these findings may be a consequence of heat intolerant enlists withdrawing from military training after 2 months, possibly owing to the challenges of training or incidence of

heat illness. Evidently, there is a pressing need for focused prevention methods at the initiation of military duty.

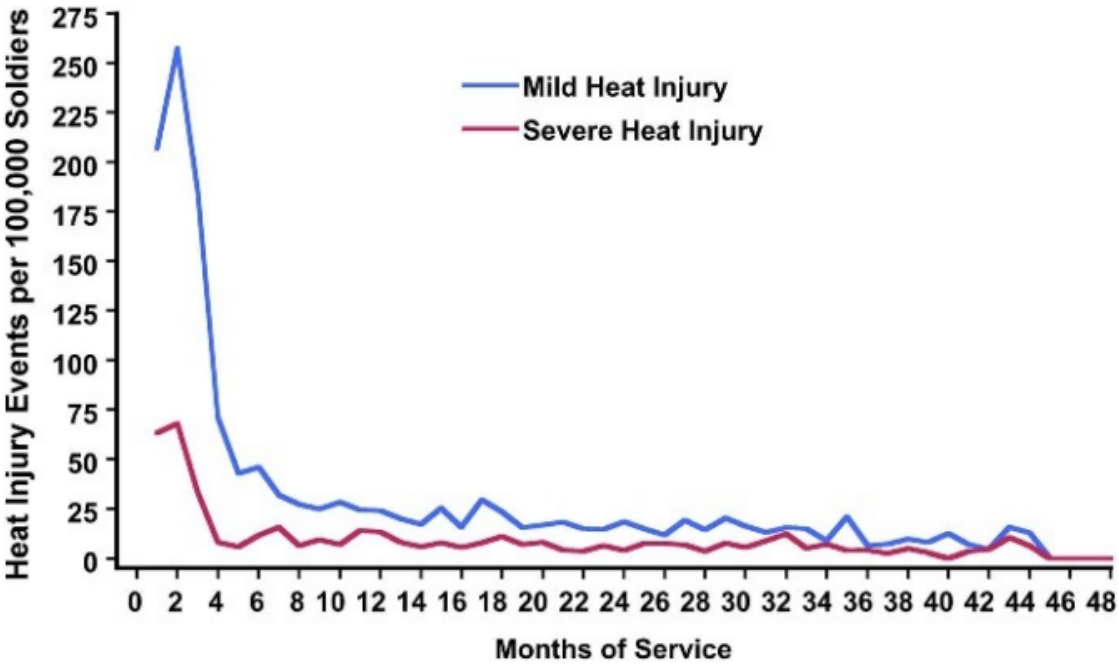

Figure 2.4. Incidence of mild (blue line) and severe (red line) heat illness cases per 100,000 newly enlisted United States Army soldiers in each service month. Taken from Nelson *et al.* (2018) .

2.3 Heat acclimation

2.3.1 Conventional heat acclimation methodology

Before exercise-heat-stress, consensus recommendations state that athletes and military personnel should complete a period of heat acclimation to alleviate heat strain and improve exercise capacity in the heat (Armstrong *et al.*, 2007; Casa *et al.*, 2015; Racinais *et al.*, 2015a; Tyler *et al.*, 2016). Heat acclimation is the manifestation of physiological and cellular adaptations that coalesce to reduce the deleterious effects of heat stress, thereby enhancing thermotolerance to heat exposure. During heat acclimation, adaptations develop after repeated heat exposures to artificial/laboratory settings that are sufficiently stressful to elicit profuse

sweating and elevate T_{core} and T_{sk}. Similarly, heat acclimatisation designates exposure to natural environments that elicit equivalent responses.

The current literature comprises a plethora of methods for attaining heat acclimation adaptations that can be broadly categorised as active or passive strategies, or a combination of both. Active heat acclimation strategies involve exercise in a hot environment, whereby elevated T_{core} is achieved via heat production from working muscles. Conversely, T_{core} is increased during passive heat acclimation methods through non-active heat absorption from the surrounding environment (Minett *et al.*, 2016). Several classic studies have demonstrated how simply resting in a hot environment (e.g., environmental chamber) can result in beneficial thermoregulatory and physiological adaptations in trained (Fox *et al.*, 1964), young and elderly populations (Armstrong & Kenney, 1993).

Traditionally, active heat acclimation interventions involved exercising at a fixed intensity throughout each session (Nielsen *et al.*, 1993; Febbraio *et al.*, 1994; Nielsen *et al.*, 1997; Lorenzo *et al.*, 2010; Lorenzo & Minson, 2010; Poirier *et al.*, 2015). Fixed intensity protocols are relatively simple to implement on a large scale as they only require a measure of external work and limited invasive monitoring. Controlled-hyperthermia (also known as isothermic) heat acclimation is an alternative method that involves targeting and maintaining a specific T_{core} (typically 38.5°C) through a combination of active and passive heat acclimation (Fox *et al.*, 1963; Patterson *et al.*, 2004a, b; Garrett *et al.*, 2009; Garrett *et al.*, 2012; Gibson *et al.*, 2015b; James *et al.*, 2017a). Controlled hyperthermia requires monitoring T_{core} continuously and adjusting exercise intensity to ensure the target temperature is maintained. Consequently, these protocols require alteration in administration throughout each session; hence, they are more complex to conduct, especially with larger groups of individuals.

Despite the practical disadvantages, controlled hyperthermia protocols may result in greater adaptation over a given period than the conventional fixed intensity method as the endogenous thermal stimulus is consistently targeted throughout, whereas during fixed intensity protocols, relative intensity and thermal strain are likely to progressively diminish (habituation) as ongoing adaptation is attained (Taylor, 2000, 2014). However, when directly compared, no differences were observed between methods (Gibson *et al.*, 2015a; Gibson *et al.*, 2015b). An explanation for these findings has not yet been elucidated but may result from the different protocols inducing distinct autonomic responses that result in comparable physiological changes (Périard *et al.*, 2016). For example, a study in rats revealed that both exercise heat

acclimation and exercise training alone improved cardiac efficiency during exercise in the heat; however this was achieved via different pathways (Moran *et al.*, 1996). The authors observed that heat acclimation attenuated the rise in blood pressure during exercise-heat-stress, whereas exercise alone resulted in a more pronounced reduction in heart rate.

Evidently, heat acclimation may be achieved through a variety of means so long as it results in sufficient physiological and thermal stress (Tyler *et al.*, 2016). However, conventional heat acclimation interventions are costly and impractical for athletes and military personnel residing in cooler climates as their completion requires access to an environmental chamber or travel to a hot climate to complete the intervention. Furthermore, the high physical demands of daily exercise with additional heat stress can disrupt training. These practical disadvantages were, at least partly, responsible for only 15% of athletes using conventional heat acclimation before competing in the 2015 IAAF World Championships in which hot/humid conditions were expected (Périard *et al.*, 2017). This is despite it being suggested that heat acclimation is the most important intervention one can adopt to reduce physiological strain and optimise performance in the heat (Armstrong *et al.*, 2007; Casa *et al.*, 2015).

2.3.2 Heat acclimation adaptations

Heat acclimation adaptations include, but are not limited to, an earlier onset of sweating, an increase in whole-body sweating rate (Lorenzo & Minson, 2010), a reduction in cardiovascular strain (Frank *et al.*, 2001) and improved thermal comfort (Gonzalez & Gagge, 1976). Collectively these adaptations result in improved endurance performance in both temperate (Lorenzo *et al.*, 2010) and hot conditions (James *et al.*, 2017a).

Often reported as a hallmark adaptation, a reduction in T_{core} during exercise-heat-stress has been demonstrated in numerous heat acclimation studies (Table 2.2). Reduced exercising T_{core} is partly responsible for the improvements in endurance capacity after heat acclimation owing to delaying the attainment of a critical T_{core} (Nielsen *et al.*, 1993). The reduction in exercising T_{core} after heat acclimation can largely be attributed to a reduction in resting T_{re} (Brazaitis & Skurvydas, 2010; Zurawlew *et al.*, 2016; Charlot *et al.*, 2017) and, to a lesser extent, a reduction in the rate of heat storage (James *et al.*, 2017a). While improved heat loss mechanisms account for the reduced rate of heat storage, the mechanism for the reduction in resting T_{core} is unknown.

Lower resting T_{core} is typically accompanied by a reduction in the T_{core} threshold for the onset of sweating (Gonzalez *et al.*, 1974; Cotter *et al.*, 1997; Zurawlew *et al.*, 2016). This earlier

onset of sweating allows for greater sweat production and promotes further evaporative cooling, which in turn slows the rate of heat storage. The increase in whole-body sweat rate with repeated heat exposure is also achieved through an increase in the number of activated sweat glands and augmented sweat gland output (Poirier *et al.*, 2016). These parameters are susceptible to a high degree of inter-individual variability and regional differences, which may explain the lack of improvement in sweat rates after heat acclimation reported in some studies (Pandolf *et al.*, 1977; Hessemer *et al.*, 1986). In addition, heat acclimation confers reductions in sweat sodium concentration (Allan & Wilson, 1971; Nielsen *et al.*, 1997; Mee *et al.*, 2018). The mechanism for sodium conservation is currently unclear but may involve an increased sodium ion reuptake within the re-absorptive duct of the sweat gland owing to an increase in aldosterone (Allan & Wilson, 1971; Nielsen *et al.*, 1997). However, this view is contested by studies that have shown no relationship between aldosterone and sweat concentration (Kirby & Convertino, 1986). Despite the obvious benefits of improved evaporative heat loss, an increased sweat rate could potentially have underlying disadvantages. If sweat loss exceeds fluid intake, hypohydration will develop, and thus may exacerbate thermophysiological strain and impair endurance performance in the heat (Adams *et al.*, 2017). This decrement in performance is caused by a combination of physiological mechanisms, including increased cardiovascular strain and elevated T_{sk} (Sawka *et al.*, 2015).

Heat acclimation also reduces T_{sk} during exercise in the heat (Weller *et al.*, 2007; Daanen *et al.*, 2011), which likely represents improvements in evaporative cooling (Gonzalez *et al.*, 1974) and skin blood flow (Roberts *et al.*, 1977). Evidence suggests that lowering T_{sk} can improve performance owing to the interaction between T_{sk} and thermal sensation (Faulkner *et al.*, 2015). For example, ten endurance-trained male cyclists pre-cooled using garments frozen overnight or saturated in cool water (14 ± 1°C) and significantly improved their time trial performance by 6% and 3%, respectively. The authors suggested that these improvements were driven by the reduction in T_{sk} after cooling. Furthermore, reductions in T_{sk} and the associated thermal perceptions have been shown to influence the selection of exercise intensity independent of T_{core} (Schlader *et al.*, 2011).

Concurrent with improved thermoregulatory responses are cardiovascular adaptations to heat acclimation (Périard *et al.*, 2016). Plasma volume expansion is purported to be one of the first of these adaptations to develop (Nielsen *et al.*, 1997; Scoon *et al.*, 2007; Goto *et al.*, 2010). This increase in plasma volume (and parallel increase in blood volume) results in reductions in heart rate at a given submaximal intensity during heat stress owing to elevations in stroke

volume (Lorenzo & Minson, 2010). Together these factors improve cardiac output during exercise-heat-stress, and, in turn, enhance endurance performance (Lorenzo *et al.*, 2010; Garrett *et al.*, 2012).

Repeated heat exposure also confers a degree of cellular protection from future thermal challenges (Horowitz & Robinson, 2007; Horowitz, 2016). This acquired cellular thermotolerance is likely mediated through changes in heat shock proteins (Moseley, 1997), particularly increases in the inducible and thermosensitive Hsp72 (McClung *et al.*, 2008). Heat shock proteins have been shown to protect against cell death via proteasomal maintenance (Beckham *et al.*, 2008).

2.3.2.1. Potential mechanisms for the reduction in resting core temperature

The underlying mechanism for the reduction in resting core temperature after heat acclimation remains elusive but may involve a reduction in metabolic heat production via reduced thyroid hormone secretion (Buguet *et al.*, 1988), a decrease in the thermoregulatory balance point (Romanovsky, 2007), or hypothalamic neural network remodelling (Tan *et al.*, 2016; Zhao *et al.*, 2017).

Release of thyroid stimulating hormone by the anterior pituitary gland stimulates the release of two protein-iodine-bound hormones. When unbound, free thyroid hormones are metabolically active and stimulate glucose uptake, gluconeogenesis, lipolysis, and thermogenesis (Iwen *et al.*, 2018). Reductions in thyroid hormones have been demonstrated after prolonged heat exposure in rats (Horowitz *et al.*, 1986; Mirit *et al.*, 2000). Moreover, rats with lower circulating thyroid hormones have been shown to have a lower core temperature at rest and during heat stress (Yang & Gordon, 1997; Maloyan & Horowitz, 2002). However, no study to date in humans has investigated the effect of heat acclimation on thyroid hormone concentrations or thyroid hormone influences on heat acclimation thermoregulatory adaptations e.g., reducing resting core temperature.

Table 2.2. A summary of investigations that adopted short-term exercise heat acclimation or post-exercise hot water immersion heat acclimation interventions.

	Reference	Heat acclimation frequency and duration	Method of heat acclimation	T_{amb} (°C)	RH (%)	Δ Resting T_{core} (°C)	Δ Mean/end-exercise T_{core} (°C)	Δ WBSR (%)	Δ Mean/end exercise T_{sk} (°C)	Δ Mean/end exercise HR (beats·min^{-1})	Δ Plasma volume (%)	Δ RPE	Δ TS
Exercise heat acclimation	Aoyagi *et al.* (1995)	6 × 60 min	Walking/running (45–55% VO_{2max}); fixed intensity	40	30	−0.20	−0.20/−0.20	6	−0.20/−0.20	−8/−8	–	–	–
	Barberio *et al.* (2015)	5 × 25 min	Cycling (78% VO_{2max}) until T_{core} increased by 2°C	40	40	−0.30	–/−0.30	–	–	–/−12	8	0.0	–
	Brade *et al.* (2013)	5 × 48 min	Intermittent cycling (3 min at 244 W, 1 min rest); fixed intensity	35	60	−0.10	–/−0.30	–	–/−0.2	–/−13	–	−1.0	0
	Buono *et al.* (1998)	7 × 100 min	Walking (4.8 km·h^{-1}) or cycling (75 W); fixed intensity	35	75	−0.30	–/−0.60	–	–/–	–/−14	–	–	–
	Casadio *et al.* (2016)	5 × 45–60 min	Cycling (60–70% VO_{2max}); controlled hyperthermia (38.5°C)	35	60	–	−0.46/–	12 *	–/–	−14/–	8	−1.8	−0.6
	Charlot *et al.* (2017)	5 × 32–56 min	Running (50% VO2max); 3–5 × 8 min	40	12	−0.31	–/−0.38	−19	–/–	–/−20	–	−2.3	−2.6
	Febbraio *et al.* (1994)	7 × 90 min	Cycling (50% VO_{2max}); fixed intensity	40	20	−0.40	–/−0.50	–	–/–	−11/–	–	–	–
	Fujii *et al.* (2012)	6 × 120 min	Cycling (50% VO_{2max}); 4 × 20 min with 10 min rest; fixed intensity	37	50	−0.20	–/−0.60	–	−0.15/−0.10	−10/−14	7	0	–
	Garrett *et al.* (2009)	5 × 90 min	Cycling; controlled hyperthermia (38.5°C)	40	60	−0.10	–/−0.30	–	–/–	–/−13	4	–	–
	Garrett *et al.* (2012)	5 × 90 min	Cycling; controlled hyperthermia (38.5°C)	40	60	−0.10	–/−0.30	–	–/–	–/−14	5	0.0	0.0
	Garrett *et al.* (2019)	5 × 90 min	Cycling; controlled hyperthermia (38.5°C)	40	60	0.0	–/−0.20	–	–/−0.50	–/−8	−7	−2.0	−1.0
	Gibson *et al.* (2015b)	5 × 90 min	Cycling (50% VO_{2peak}); fixed intensity	40	39	−0.29	−0.19/−0.22	30	−0.23/−0.77	−9/−1	–	1	−0.3
		5 × 67 min	Cycling (65% VO_{2peak}); controlled hyperthermia (38.5°C)	40	39	−0.10	−0.19/−0.32	21	−0.42/−0.66	−12/−13	–	−1.0	−0.2
		5 × 52 min	Cycling (65% VO_{2peak}); controlled hyperthermia (38.5°C)	40	39	−0.21	−0.15/−0.25	53	−0.67/−0.71	−11/−9	–	−1.0	0.0

Table 2.2. continued

	Reference	Heat acclimation frequency and duration	Method of heat acclimation	T_{amb} (°C)	RH (%)	Δ Resting T_{core} (°C)	Δ Mean/end-exercise T_{core} (°C)	Δ WBSR (%)	Δ Mean/end exercise T_{sk} (°C)	Δ Mean/end exercise HR (beats·min^{-1})	Δ Plasma volume (%)	Δ RPE	Δ TS
Exercise heat acclimation	Goto *et al.* (2010)	5 × 30 min	Cycling (70% VO_{2peak}), fixed intensity	30	50	−0.10 *	–/−0.20 *	8	–/−0.5	–/−5 *	4	–	–
	James *et al.* (2017a)	5 × 90 min	Cycling (2.7 W·kg^{-1}); controlled hyperthermia (38.5°C)	37	59	−0.15	−0.21/–	3	−0.32/–	−3/–	6	0	−0.2
	Kirby *et al.* (2019)	4 × 90 min	Cycling; controlled hyperthermia (38.5°C)	40	30	0.0	0.0/0.0	–	−0.50/−0.50	–/−3	–	–	0.0
	Kuennen *et al.* (2011)	7 × 100 min	Running (6.8 km·h^{-1}); 2 × 50 min with 10 min rest; fixed intensity	47	20		–/−0.45 *	11	–/−0.8 *	−20 *	16	–	–
	Mee *et al.* (2018)	5 × 90 min	Cycling (65% VO_{2max}); controlled hyperthermia (38.5°C)	40	40	−0.07	–/−0.05	58	–/0.03	–/−3	1	−1.0	−0.0
	Moss *et al.* (2020)	5 × 60 min	Cycling; controlled hyperthermia (38.5°C)	40	50	−0.39	−0.37/−0.39	13	−0.53/−0.85	−11/−8	–	−2.0	−0.6
	Neal *et al.* (2016a)	5 × 90 min	Cycling (RPE 15); controlled hyperthermia (38.5°C)	40	50	−0.24	−0.18/–	7	−0.11/–	−7/–	1	-1	−2
	Racinais *et al.* (2015b)	5 × 240 min	Cycling (unspecified)	34	18	–	–/0.00	0	–/–	−3/–	–	–	–
	Ravanelli *et al.* (2019)	6 × 90 min	Walking (70% HR_{max}); fixed intensity	38	65	−0.17	–/−0.25	6 *	–/−0.36	–/–	–	–	–
	Reeve *et al.* (2019)	5 × 30 min	Cycling (6 min at 50 % W_{max} then 12 × 1 min at 100 W_{max}); fixed intensity	35	50	−0.17	0.74/−0.13	−18	0.32/−0.10	−2/6	−1	−0.5	−0.5
	Willmott *et al.* (2016)	4 × 45 min	Cycling (50% VO_{2max}); fixed intensity	35	60	–	−0.15/−0.15	12	–/–	−7/−3	–	−2.0	−0.5
		2 × 2 × 45 min	Cycling (50% VO_{2max}); fixed intensity	35	60	–	−0.11/−0.07	18	–/–	−10/−8	–	−1.0	−0.1
	Willmott *et al.* (2017)	4 × 60 min	Cycling (2 W·kg^{-1}) or running (6–10 km·h^{-1}); controlled hyperthermia (38.5°C)	45	30	−0.02	−0.09/−0.10	14	–/–	−4/−7	4	−2.0	−0.6

Table 2.2. continued

	Reference	Heat acclimation frequency and duration	Method of heat acclimation	T_{amb} (°C)	RH (%)	Δ Resting T_{core} (°C)	Δ Mean/end-exercise T_{core} (°C)	Δ WBSR (%)	Δ Mean/end exercise T_{sk} (°C)	Δ Mean/end exercise HR (beats·min^{-1})	Δ Plasma volume (%)	Δ RPE	Δ TS
Post-exercise hot water immersion	Zurawlew *et al.* (2016)	6 × ≤40 min	Post-exercise hot water immersion	40	–	−0.27	–/−0.36	0	–/−0.79	–/−6	3	−1.3	−0.8
	Zurawlew *et al.* (2018a)	6 × ≤40 min	Post-exercise hot water immersion	40	–	−0.34	–/−0.47	−7	–/−0.90	–/−14	3	−2.0	−1.0
		6 × ≤40 min	Post-exercise hot water immersion	40	–	−0.27	–/−0.43	4	–/−0.69	–/−13	3	−2.0	−2.0
	Zurawlew *et al.* (2018b)	6 × ≤40 min	Post-exercise hot water immersion	40	–	−0.17	–/−0.36	12	–/−0.67	–/−14	3	−2.0	−1.0
		6 × ≤40 min	Post-exercise hot water immersion	40	–	−0.23	–/−0.47	−3	–/−0.75	–/−13	3	−2.0	−2.0
	Zurawlew *et al.* (2019)	6 × ≤40 min	Post-exercise hot water immersion	40	–	−0.32	–/−0.42	−7	–/−0.81	–/−6	3	−1.3	−0.8

*T_{amb}, ambient temperature; RH, relative humidity; T_{core}, core body temperature; WBSR, whole-body sweat rate; T_{sk}, mean skin temperature; HR, heart rate; RPE, rating of perceived exertion; TS, thermal sensation; VO_{2max}, maximal oxygen consumption; VO_{2peak}, peak oxygen consumption; HR_{max}, maximal heart rate; W_{max}, maximal work capacity. *Denotes a value that has been estimated from a figure.*

2.3.3 Adaptation timeframe

Heat acclimation is a relatively rapid process with adaptations developing from the first exposure (Périard *et al.*, 2015). Indeed, Pandolf (1998) reports that 65–75% of adaptation can be observed after the first 4–6 exposures. The development of these adaptations can be categorised as short-term acclimation (≤7 exposures), medium-term acclimation (8–14 exposures), and long-term acclimation (≥15 exposures; Tyler *et al.*, 2016). In contrast, Horowitz (2014) argues that heat acclimation is a biphasic process (Figure 2.5). The initial transient phase occurs over ~5 days and is characterised by accelerated efferent activity to compensate for the insufficient peripheral responsiveness. The longer second phase (3–4 weeks) is more stable and involves central changes, including a reduction in the temperature threshold for activation of effector organs with a concomitant increase in their sensitivity (Horowitz, 2014).

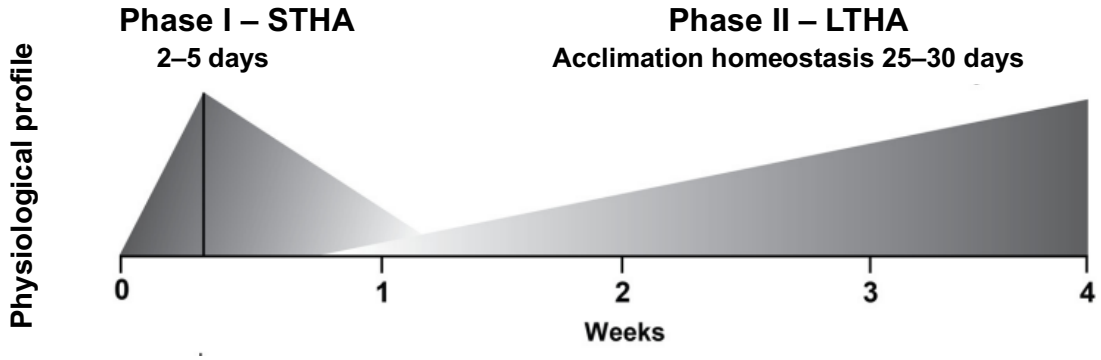

Figure 2.5. Biphasic process of heat acclimation illustrating the physiological profile during short-term (STHA) and long-term heat acclimation (LTHA). Taken from Horowitz (2016).

Most adaptations (improvements in heart rate, resting and exercising T_{core}, T_{sk}, and sweat rate) develop quickly within the first week of heat acclimation and more slowly in the subsequent 2 weeks (Figure 2.6; Périard *et al.*, 2015). Cardiovascular adaptations develop most rapidly, with significant improvements observed after four to five exposures (Garrett *et al.*, 2012; James *et al.*, 2017a). Indeed, the reduction in heart rate is virtually complete after six exposures (Daanen *et al.*, 2011). Similarly, most of the improvements in T_{core} and T_{sk} during exercise-heat-stress have also occurred after seven exposures (Périard *et al.*, 2015). The thermoregulatory benefits of heat acclimation are generally considered to be complete after 10 exposures (Moss *et al.*,

2020). Increasing the number of heat exposures (e.g., ≥10 exposures) is considered to confer cellular adaptations, which provide cellular protection against subsequent heat exposure; as such, heat acclimation interventions involving ≥10 heat exposures are viewed more favourably as they provide a more complete state of heat acclimation (McClung *et al.*, 2008; Yamada *et al.*, 2008).

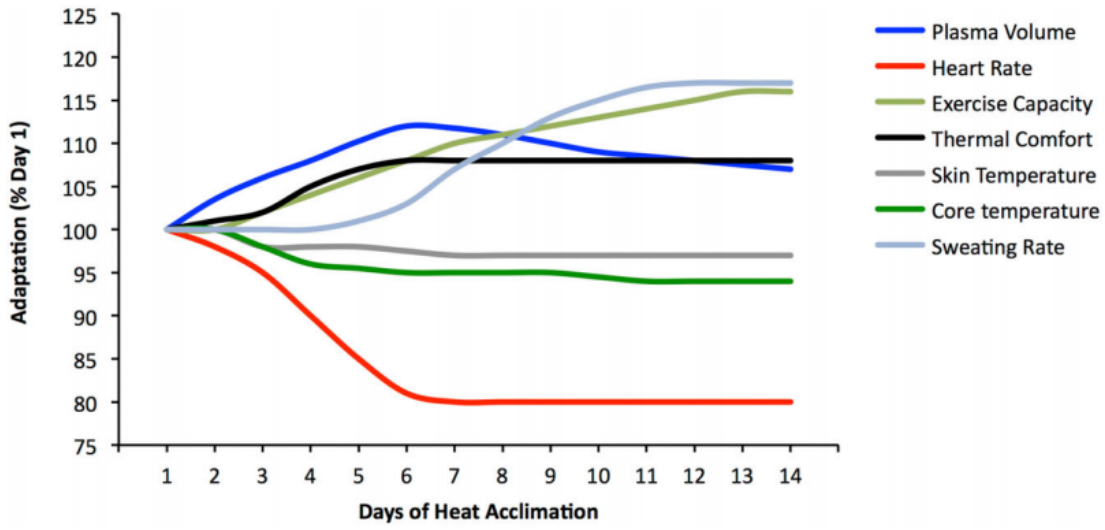

Figure 2.6. Heat acclimation timeframe. Taken from Périard *et al.* (2015).

Despite the apparent physiological and cellular benefits, prolonged heat acclimation interventions place additional financial and logistical burdens on sporting and military bodies. Extended conventional protocols interfere more with normal training, particularly during tapering in the lead-up to a sporting event (Schmit *et al.*, 2018; Saunders *et al.*, 2019). The stepwise recovery during tapering is essential to enable the consolidation of physiological adaptations after a period of strenuous training, and heat acclimation should not interfere with this process (Bosquet *et al.*, 2007). Furthermore, military personnel often must deploy to a hot climate at short notice, hence completing a long-term heat acclimation intervention is not always possible. In such circumstances, short-term heat acclimation becomes the more viable option for improving military persons' ability to sustain exercise-heat-stress before moving to a hot climate. However, as previously mentioned short interventions only provide partial physiological adaptation (Tyler *et al.*, 2016). Consequently, attempts have been made to further reduce the duration of heat acclimation interventions. Willmott *et al.* (2016) investigated the

efficacy of a twice-daily intervention for inducing heat acclimation adaptations. Participants completed a 45-min cycle at 50% $\dot{V}O_{2peak}$ in a hot/humid environment (35°C, 60% RH) in the morning and afternoon on two consecutive days. The authors observed no significant differences in exercising T_{core}, heart rate or 3-km running performance from pre- to post-intervention. The authors suggest that the intervention was ineffective owing to the short session duration, and resultant insufficient thermal stimulus (small rise in T_{core}). Arguably, increasing session duration may have initiated an enhanced adaptive response, however, undergoing an intervention that involves twice-daily bouts of prolonged exercise-heat-stress will likely have detrimental effects, including fatigue and compromised immunity (Gleeson, 2007; Schmit *et al.*, 2018). More research is warranted to evaluate the efficacy of rapid heat acclimation protocols, with a focus on practical passive heating strategies.

To obtain the greatest benefit, consensus recommendations state that heat acclimation should be scheduled at the anticipated clock-time of future exercise-heat-stress (Périard *et al.*, 2015). This recommendation is based on research demonstrating that 4 h of passive heat exposure conducted in the afternoon (1400 h to 1800 h) for 9–10 consecutive days reduced resting T_{core} only between 1400 and 1800 h (Shido *et al.*, 1999). However, the recent findings of an investigation by Zurawlew *et al.* (2018a) challenged this popular belief, showing that post-exercise hot water immersion completed in the morning initiates a similar magnitude of heat acclimation adaptation throughout the day. Specifically, the authors observed that reductions in resting (morning, −0.34 ± 0.24°C; afternoon, −0.27 ± 0.23°C) and end-exercise rectal temperature (morning, −0.47 ± 0.33°C; afternoon, −0.43 ± 0.29°C) were not different in the morning or afternoon. These findings are relevant for when the time of day of future exercise-heat-stress is unknown, for example, in military and other occupational settings.

2.3.4 Heat acclimation maladaptation

It is common for athletes to experience minor fatigue and acute reductions in performance as consequences of the normal training process. This decrement in performance may also be accompanied with related physiological and/or psychological signs and symptoms of maladaptation. Following an adequate recovery period that may take from several days to weeks, functional overreaching will eventually result in an improvement in performance (Meeusen *et al.*, 2013). However, when the balance between training stress and recovery is disproportionate, non-functional overreaching can occur. Non-functional overreaching is defined as a stagnation or decrease in performance with or without related physiological and

psychological signs and symptoms of maladaptation after intensified training in which restoration of performance may take several weeks or months. In more severe cases, overtraining syndrome can develop, which can reportedly take months to recover from, however, this is based on anecdotal evidence (Halson & Jeukendrup, 2004). Becoming overreached can have severe adverse effects on athletes and military personnel beyond that of decrements in performance and mission readiness, including immune perturbations and a number of diseases such as metabolic syndrome, insulin resistance, and hypertension (Tsatsoulis & Fountoulakis, 2006; Svendsen *et al.*, 2016).

Evidence suggests that overreaching can also be detrimental to immune function (Hausswirth *et al.*, 2014; Svendsen *et al.*, 2016). Several studies have observed that even relatively short periods (1–3 weeks) of intensified training cause marked reductions in neutrophil function, lymphocyte proliferation, salivary-immunoglobulin A, and circulating number of T-cells producing interferon-γ (Gleeson, 2000; Lancaster *et al.*, 2004; Svendsen *et al.*, 2016). Furthermore, the immune perturbations associated with overreaching may also increase the susceptibility to infection (Hausswirth *et al.*, 2014), which is an independent risk factor of exertional heat illness (Epstein *et al.*, 1999).

It is difficult to quantify the prevalence of overreaching and overtraining in athletic populations owing to the disparity in reported incidence. These equivocal findings are likely a consequence of the varying durations of assessment. Studies that evaluated indicators of overreaching during a single training season/cycle had findings in the lower range (~10–20%; Morgan *et al*., 1987; O'Connor *et al*., 1989); whereas a study that assessed the entire careers of 139 Swiss athletes found higher rates (30%) of non-functional overreaching and overtraining (Birrer *et al.*, 2013). Overreaching also occurs in the military owing to personnel having to endure intense training, sleep deprivation, inadequate nutrition, and the stress of deployment (Szivak & Kraemer, 2015). Tanskanen *et al.* (2011) showed that after 8 weeks of basic training, 11 out of 35 (31%) male recruits were classified as overreached. Participants were deemed overreached if they fulfilled three of the five criteria, which included physiological, psychological, and biochemical parameters. Evidently, overreaching is a problem for athletes and military personnel.

Despite the incidence of overreaching likely being exacerbated when susceptible populations are required to undergo heat acclimation in preparation for events/deployment in hot environments, overreaching is often overlooked in heat acclimation research. The only

investigation to date that directly assessed markers of overreaching after a heat acclimation intervention is a study by Schmit *et al.* (2018). In this study, the authors compared exercise performance and physiological responses during exercise-heat-stress after 5 days of low or high intensity heat acclimation and again after a 7-day taper. The authors observed that the performance response was maximised after low intensity heat acclimation, whereas after high intensity heat acclimation, performance declined and was accompanied by increased perceived fatigue, indicative of overreaching. After tapering, supercompensation was evident in both groups but to a lesser extent in the participants who completed the high intensity heat acclimation protocol. These findings highlight that intensified training during heat acclimation may be detrimental to performance, at least in the short-term. Furthermore, it is conceivable that the magnitude of adaptations observed in previous investigations, particularly those that employed long-term exercise heat acclimation, may have been underestimated owing to overlooking the possibility of the participants being overreached. Findings from Schmit *et al.* (2018) indicate that athletes and military personnel should carefully consider the timing of heat acclimation to ensure adequate recovery before performing/operating in the heat. Future heat acclimation studies should incorporate markers of overreaching (Meeusen *et al.*, 2013) into their testing battery (e.g., endurance capacity, mood, cognitive performance, and sleep efficiency) and, where possible, conduct follow-up testing after a period of recovery to distinguish between functional- and non-functional overreaching.

2.3.5 Decay and re-acclimation

The physiological adaptations associated with heat acclimation are transient and gradually disappear if consistent heat exposure is not maintained. However, there is a lack of consensus regarding the rate of decay of heat acclimation. This issue is mainly owing to a dearth of available data and is exacerbated by the lack of standardization in the protocols for heat acclimation and decay. Givoni and Goldman (1973) suggest that one day of acclimation is lost for every 2 days spent without heat stress. This "rule of thumb" continues to be widely used despite contradicting findings from numerous studies, which show that thermal and cardiovascular heat acclimation adaptations are much more stable (Pandolf *et al.*, 1977; Weller *et al.*, 2007; Daanen *et al.*, 2011; Flouris *et al.*, 2014; Gerrett *et al.*, 2021). For example, Weller *et al.* (2007) observed that there was minimal decay in resting and end-exercise T_{core} reductions after 26 days without exposure to heat stress. A recent systematic review and meta-analysis of 12 studies that investigated heat acclimation decay revealed that reductions in end-exercise

T_{core} and heart rate are lost at a rate of 2.6% and 2.3% per decay day, respectively (Daanen *et al.*, 2018). The decay rate of sweat rate, however, has proven more difficult to quantify owing to the variability in sweat rate adaptations during heat acclimation and the day to day variation within participants (Daanen *et al.*, 2018). Similarly, the decay rate of T_{sk} adaptations after heat acclimation are just as elusive owing to conflicting findings. Weller *et al.* (2007) observed a slow decay of 8% after 12 days and 56% after 26 days, whereas Neal *et al.* (2016b) found no significant changes after 7 days of decay. In contrast, Daanen *et al.* (2011) reported a fast a decay rate of 57% after only 6 days.

To date, there have been only two studies that have assessed performance decay after heat acclimation (Garrett *et al.*, 2009; Gerrett *et al.*, 2021). Garrett *et al.* (2009) observed that time to exhaustion in the heat increased by 14% after short-term heat acclimation and remained higher after one (10%), but not 2 (2%) or 3 weeks without heat exposure. The decay in performance paralleled reductions in end-exercise T_{core} and heart rate, which remained for only one week after heat acclimation. More recently, Gerrett *et al.* (2021) found that time to exhaustion returned to pre-heat acclimation values after a 28-day decay period. However, in contrast with previous work, Gerrett *et al.* (2021) observed that reductions in resting and end-exercise T_{core} and end-exercise heart rate after 10 days of controlled hyperthermia were maintained for at least 28 days without heat exposure. This disparity is likely owing to the longer duration of heat acclimation employed by (Gerrett *et al.*, 2021). Evidently, more research is necessary to elucidate the timeframe for heat acclimation decay, particularly performance decay, in which there is a scarcity of available literature.

Following a period of decay, evidence suggests that re-acclimation induces reductions in end-exercise T_{core} and heart rate at a faster rate than heat acclimation (Daanen *et al.*, 2018; Racinais & Périard, 2020). Ashley *et al.* (2015) found that after 2 or 4 weeks without heat stress, re-acclimation was attained after 4 and 5 days, respectively. Moreover, a study by Weller *et al.* (2007) reported the re-induction of heat acclimation adaptions after only 2 and 4 days after 12 and 26 decay days, respectively. Interestingly, Saat *et al.* (2005) and Weller *et al.* (2007) both demonstrate enhanced adaptation with re-acclimation after a period of decay. Saat *et al.* (2005) showed that following just 3 days of re-acclimation after 14 days of decay, end-exercise T_{core} and heart rate were lower than at the end of a 14-day heat acclimation protocol. Furthermore, these reductions were even more pronounced after an additional 7 days of re-acclimation. Together, these studies highlight that re-acclimation can potentiate adaptations faster and potentially to a greater extent than the initial heat acclimation regime.

2.3.6 Alternative heat acclimation strategies

Conventional exercise heat acclimation protocols can be costly, impractical and the physical demands of daily exercise in the heat may disrupt training and lead to fatigue (Schmit *et al.*, 2018; Saunders *et al.*, 2019). Consequently, athlete engagement with long-term exercise heat acclimation is poor (Périard *et al.*, 2017). To reduce the time commitment, sport scientists have designed short-term heat acclimation interventions; however, research investigations report inconsistent adaptations (Table 2.2). Hence, there is a pressing need for an alternative, more accessible heat acclimation intervention that reduces thermal strain and improves the physical capability of athletes and military personnel performing in the heat.

Recent attempts into alternative heat acclimation strategies include, post-exercise sauna (Scoon *et al.*, 2007) or hot water immersion (Zurawlew *et al.*, 2016; Zurawlew *et al.*, 2018a, b, 2019), and overdressing in temperate conditions (Ely *et al.*, 2018; Stevens *et al.*, 2018). The latter is a simple and cost-effective strategy, but adaptations were modest compared to those conferred by exercise heat acclimation. In contrast, several studies support the effectiveness of post-exercise sauna (Scoon *et al.*, 2007), and hot water immersion (Zurawlew *et al.*, 2016; Zurawlew *et al.*, 2018a, b, 2019) for reducing thermal strain and improving endurance performance during subsequent heat challenges. Scoon *et al.* (2007) demonstrated that 12 sessions of post-exercise sauna bathing over 3 weeks increased run time to exhaustion in thermoneutral conditions by 32%, equivalent to a meaningful 2% improvement in time trial performance. The authors attributed this improvement to an expansion in plasma volume. Unfortunately, no thermal measures were recorded during this study, thus the efficacy of this method for preparing athletes and military personnel for exercise in the heat remains unconfirmed.

Previous studies have consistently demonstrated that short-term post-exercise hot water immersion offers a practical and effective heat acclimation strategy that reduces thermal strain in recreationally active (Zurawlew *et al.*, 2016; Zurawlew *et al.*, 2018a, 2019) and trained runners (Zurawlew *et al.*, 2018b). Unlike exercise-based heat acclimation protocols, post-exercise hot water immersion can be integrated into regular training, for example, as part of a post-training washing routine. This approach allows athletes to train as normal and reduces interference with tapering before competition. The practical advantages are especially apparent when considering team sports and sports in which conventional exercise heat acclimation modes can alter technique (e.g., race walking). Six days of post-exercise hot water provides reductions in thermal strain that compare favourably with exercise-based interventions (Table

2.2). However, a direct comparison between post-exercise hot water immersion and conventional exercise heat acclimation is yet to be made. Furthermore, findings on the effectiveness of post-exercise hot water immersion is limited to 6 days. Further research is required to establish whether shortening the 6-day intervention provides meaningful adaptations and whether extending it achieves a more complete state of heat acclimation.

2.4 Summary and recommendations for future research

In summary, humans regulate their T_{core} to survive in environmental extremes. However, endurance performance is impaired in hot environments and the accumulative effects of exercise and heat put athletes and military personnel at risk of exertional heat illness. Fortunately, repeated exposure to heat stress, whether via active or passive regimes or a combination of both, potentiates a multitude of perceptual, physiological, and cellular adaptations, which in turn reduce exertional heat strain and can improve endurance performance in hot environments. Heat acclimation adaptations develop and decay at different rates and are not restricted to the clock-time of daily heat exposures. This literature review has highlighted the practical difficulties of conventional exercise heat acclimation and potential for prolonged or intensified protocols to lead to symptoms of overreaching, including decrements in performance, low mood and compromised immune function. Moreover, the effectiveness of short-term exercise heat acclimation has been questioned. Consequently, post-exercise heat stress interventions may provide more practical and effective alternatives to exercise-based approaches for preparing for exercise in the heat.

Further research is warranted into practical and accessible heat acclimation solutions for use in sporting and occupational settings. In addition, future investigations into the temporal patterning of post-exercise hot water immersion heat acclimation and how this compares with conventional exercise heat acclimation would benefit athletes, military personnel, and occupational workers. Finally, a better understanding of the mechanism for the reduction in thermal strain after post-exercise hot water immersion heat acclimation is required.

The current recommendations for athletes, military personnel, and occupational workers on how best to prepare for exercise-heat-stress may be outdated. Hence, the primary aim of this thesis is to investigate post-exercise hot water immersion heat acclimation as an alternative preparatory strategy to conventional exercise heat acclimation. The findings of this research will inform subsequent revisions of Ministry of Defence heat acclimation guidance within JSP 375.

CHAPTER THREE

General Methods

3.1 Ethical approval

Each study received ethical approval from the Ministry of Defence Research Ethics Committee and the School of Sport, Health and Exercise Sciences at Bangor University (**Chapters 4, 5 and 6**) and was conducted in accordance with the Declaration of Helsinki (2013), except for registration in a database.

3.2 Participants

Following a full briefing of experimental procedures, all participants provided full written informed consent (**Appendix A**), completed a medical screening questionnaire (**Appendix B**), and underwent a 12-lead electrocardiogram. Participants were healthy, non-smokers, free from any known cardiovascular or metabolic diseases and were not taking any medication. Additionally, all individuals were non-heat acclimatised, i.e. had not been regularly (> once a week) exposed to the heat (including sauna and hot bath use) in the 6 weeks before commencing testing.

3.3 Fitness assessment and speed verification

3.3.1 Peak oxygen uptake

Peak oxygen uptake ($\dot{V}O_{2peak}$) was assessed using a continuous maximal incremental exercise test performed on a motorised treadmill (HP Cosmos Mercury 4.0, Nussdorf-Traunstein, Germany) in a temperate laboratory (19°C) to volitional exhaustion (**Chapters 4, 5 and 6**). Firstly, participants completed a series of 4-min stages, which started at a running speed of 8 $km \cdot h^{-1}$ (1% gradient) and increased by 1 $km \cdot h^{-1}$ each stage, until reaching their anaerobic threshold (blood lactate concentration ≥ 4 $mmol \cdot L^{-1}$; Lactate Pro 2™, Arkray, Australia). Following a 15-min recovery period, participants recommenced running at the speed equivalent to their anaerobic threshold (1% gradient) for 2 min. The gradient was then increased by 1% every 1 min until volitional exhaustion. Expired gas was analysed continuously, and heart rate

and blood lactate were recorded upon cessation of exercise. $\dot{V}O_{2peak}$ was determined as the highest oxygen uptake attained over a 30-s period.

3.3.2 Speed verification

A running speed that elicited 65% $\dot{V}O_{2peak}$ in temperate conditions was determined by the interpolation of the running speed–$\dot{V}O_2$ relationship. This speed was then verified 30 min following the $\dot{V}O_{2peak}$ test with a 60-s expired gas sample collected by Douglas bag method during steady state exercise. This individualised running speed was used for both the submaximal exercise during experimental trials and daily intervention (**Chapters 4, 5 and 6**).

3.4 Physiological measurements

3.4.1 Body temperatures

Rectal core temperature (T_{re}) was measured using a flexible, sterile, disposable thermistor (Henleys Medical Supplies Ltd., Herts, UK) self-inserted 10 cm beyond the anal sphincter and recorded using a data logger (YSI model 4000A; YSI, Dayton, Ohio, USA; **Chapters 4, 5 and 6**). Prior to insertion of the T_{re} thermistor, a bead was affixed 10 cm from the inserted end to ensure the thermistor remained inserted to the correct depth. Skin thermistors (Grant EUS-U, Cambridge, UK) were attached 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) and recorded using a data logger (Grant SQ2020, Cambridge, UK). Mean skin temperature (T_{sk}) was calculated (**Chapters 4 and 6**) using the following four-site weighted equation (Ramanathan, 1964):

$$T_{sk} = 0.3(T_{chest} + T_{arm}) + 0.2(T_{thigh} + T_{calf})$$

3.4.2 Sweating responses

The change in dry nude body mass during experimental trials and intervention sessions was used to estimate whole-body sweat rate (**Chapters 4 and 6**). Local forearm sweat rate was measured by dew point hygrometry. Anhydrous compressed nitrogen was passed through a 5-cm^2 capsule affixed to the lower arm ventral surface (halfway between the antecubital fossa and carpus) and connected to a hygrometry system (DS2000; Alpha Moisture Systems, UK). Local forearm sweating rate was calculated using the difference in water content between

effluent and influent air and the flow rate (1 $L \cdot min^{-1}$), and normalised for the skin surface area under the capsule (expressed in milligrams per square centimetre per minute). T_{re} at sweating onset was determined by plotting the relationship between local forearm sweat rate and T_{re} (recorded at 20-s intervals) before using segmented linear regression to identify the breakpoint in the two line segments (**Chapters 4 and 6**; Cheuvront *et al*., 2009).

3.4.3 Heart rate

Heart rate was monitored (Polar FT1, Polar Electro, Kempele, Finland) continuously and recorded every 5 min during all exercise, water immersion, rest, and recovery periods (**Chapters 4, 5 and 6**).

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 following 30 min of seated rest (**Chapters 5 and 6**) and at 9–10, 19–20, 29–30 and 39–40 min of exercise (**Chapters 4 and 6**) during experimental trials (James *et al.*, 2017b).

3.4.5 Blood sample collection and analysis

Following 20 min seated rest, venous blood samples were collected on the morning of experimental trials by a trained phlebotomist by venepuncture, from an antecubital vein without stasis into two 6-mL EDTA (ethylenediaminetetraacetic acid) vacutainers (BD, Oxford, UK). Aliquots of whole blood were used for the immediate determination of haemoglobin concentration ($g \cdot dL^{-1}$), in duplicate (Hemocue, Sheffield, UK) and haematocrit (%), in triplicate (capillary tube method; **Chapters 4, 5 and 6**). The remaining whole blood was immediately centrifuged at 4°C and 2500 RPM for 10 min. The resultant plasma was aliquoted into Eppendorf tubes and stored at −80°C until thyroid hormone analysis (**Chapters 5 and 6**).

Plasma concentrations of free and total triiodothyronine (T3) and thyroxine (T4) were measured in duplicate by ELISA (free T3: Cat. No. RE55231, detection limit: 0.1 $pmol \cdot L^{-1}$, IBL International, Hamburg, Germany; free T4: Cat. No. RE55241, detection limit: 0.6 $pmol \cdot L^{-1}$, IBL International; total T3: Cat. No. RE55251, detection limit: 0.2 $nmol \cdot L^{-1}$, IBL International; total T4: Cat. No. RE55261, detection limit: 0.1 $nmol \cdot L^{-1}$, IBL International). The intra-assay coefficient of variation for duplicates were as follows: free T3, 5.1%; free T4, 2.6%; total T3,

5.6%; total T4, 5.9%. Thyroid hormone concentrations were adjusted for plasma volume changes using the following formula (Sherk *et al.*, 2013):

$$\text{Corrected value} = \text{Uncorrected value}*((100 + \%\Delta PV)/100).$$

3.4.6 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 and 6**; 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 & Goldman, 1977) were measured every 10 min during all submaximal runs in experimental trials and intervention sessions (**Chapters 4, 5 and 6**). Additionally, thermal sensation was measured every 5 min at rest, during water immersions, and during recovery from all exercise/immersion protocols (**Chapters 4, 5 and 6**).

3.6 Endurance capacity

Participants completed a time to exhaustion (TTE) on a motorised treadmill at 65% $\dot{V}O_{2peak}$ (**Chapters 4 and 6**). Participants were instructed to "run for as long as possible". TTE was terminated when participants stopped running owing to volitional exhaustion, thermal discomfort, or when T_{re} exceeded 39.5°C. No fluids were consumed, no feedback or encouragement was provided, and T_{re} and heart rate were monitored continuously.

3.7 Markers of overreaching

3.7.1 Mood

Participants completed the abbreviated Profile of Mood States questionnaire (**Appendix C**; Grove & Papavessis, 1992) at rest in a temperate environment (19°C, 45% RH) on the morning of experimental trials (**Chapters 4 and 6**). Total mood disturbance was calculated by summing the total for the negative subscales and then subtracting the totals for the positive subscales (Grove & Prapavessis, 1992). Energy index represented the difference between the scores of vigour and fatigue (Kenttä *et al.*, 2006).

3.7.2 Stroop test

Participants performed a modified Stroop test (Dupuy *et al.*, 2014) on a computer following submaximal exercise in the heat during experimental trials (**Chapters 4 and 6**). The test consisted of a series of trials, in which participants were instructed to name the colour of a colour-word, the meaning of the word being congruent or incongruent with the colour itself. Participants named the colour by tapping on the corresponding key on a QWERTY keyboard. Red, green, blue, and yellow were mapped to letters "s", "d", "k", and "l", respectively. Participants completed one practice block of 24 trials, where they were presented with 12 congruent trials (50%) and 12 incongruent trials (50%) intermixed randomly for the participant to familiarise themselves with the test. During the practice block an "ERROR" sign appeared following each incorrectly answered trial. Following a 10,000-ms break, one block of 60 trials was presented with 30 congruent trials (50%) and 30 incongruent trials (50%) intermixed randomly. To decrease expectancy effects, the inter-trial interval varied randomly between 800 ms, 1000 ms, and 1200 ms, with a mean interval of 1000 ms. Reaction time and accuracy were recorded.

3.7.3 Sleep efficiency

Sleep efficiency was assessed using an Actigraph (Actigraph GT3X Version 4.4.0, Actigraph, Pensacola, USA), worn on the non-dominant arm with a 1-min epoch length (**Chapters 4 and 6**). 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) using Actilife+Sleep Version 6 (Actigraph, Pensacola, USA).

CHAPTER FOUR

A comparison of short-term heat acclimation by post-exercise hot water immersion and exercise in the heat

4.1 Summary

This study aimed to compare heat acclimation adaptations after 3 and 6 days of either post-exercise hot water immersion (HWI) or exercise heat acclimation (EHA) in recreationally active individuals. Post-exercise HWI involved a daily 40-min treadmill-run at 65% $\dot{V}O_{2peak}$ in temperate conditions (19°C, 45% RH) followed by HWI (≤ 40 min, 40°C water; n = 9). Daily EHA involved a ≤60-min treadmill-run in the heat (65% $\dot{V}O_{2peak}$; 33°C, 40% RH; n = 9), chosen to elicit a similar endogenous thermal stimulus to HWI. A thermoneutral exercise intervention (TNE, 19°C, 45% RH; n = 9), work-matched to EHA, was also included to determine thermoregulatory adaptations to daily exercise in temperate conditions. An exercise-heat-stress test was performed before and after 3 and six intervention days and involved a 40-min treadmill-run and TTE at 65% $\dot{V}O_{2peak}$ in the heat (33°C, 40% RH). ANCOVA, using baseline values as the covariate, revealed no interaction effects but significant group effects demonstrated that compared to EHA, HWI elicited larger reductions in resting T_{re} (P = 0.021), T_{re} at sweating onset (P = 0.011), and end-exercise T_{re} during exercise-heat-stress (−0.47°C; P = 0.042). Despite a similar endogenous thermal stimulus to HWI, EHA elicited a modest reduction in end-exercise T_{re} (−0.26°C), which was not different from TNE (−0.25°C, P = 1.000). There were no main effects or interaction effects for end-exercise T_{sk}, heart rate, physiological strain index, RPE, thermal sensation, plasma volume, or TTE (all $P \geq 0.154$). Compared with conventional short-term exercise heat acclimation, short-term post-exercise hot water immersion elicited larger thermal adaptations.

4.2 Introduction

In preparation for competing or working in the heat, athletes, military personnel and occupational workers who reside in temperate conditions are advised to complete a period of heat acclimation (Racinais *et al.*, 2015a; Périard *et al.*, 2017). Heat acclimation adaptations, that improve endurance capacity and reduce susceptibility to exertional heat illness (Périard *et al.*, 2015), include an earlier onset of cutaneous vasodilatation and sweating, an increase in sweating rate, and a reduction in resting and exercising body temperature (Lorenzo *et al.*, 2010; Taylor, 2014). Recommendations to maximise adaptations are to complete ≥ 15 daily exercise heat acclimation exposures (long-term heat acclimation), which initiate profuse sweating and maintain an elevated body temperature for ≥ 60 min (Périard *et al.*, 2015; Saunders *et al.*, 2019). However, protocols can be costly, impractical, ineffective as trained individuals are considered partially heat acclimatised (Gisolfi & Robinson, 1969; Shvartz *et al.*, 1977), and the physical demands of daily exercise-heat-stress can disrupt training and lead to fatigue (Schmit *et al.*, 2018; Saunders *et al.*, 2019). As a consequence, athlete engagement with long-term exercise heat acclimation is poor (Périard *et al.*, 2017). To reduce the time commitment, sport scientists have designed short-term heat acclimation interventions, supported by the premise that ~80% of adaptations occur in 7 days (Robinson *et al.*, 1943). However, research investigations report inconsistent reductions in core body temperature at rest and during exercise-heat-stress after short-term exercise heat acclimation (Tyler *et al.*, 2016).

Post-exercise passive heating, such as sauna bathing (Scoon *et al.*, 2007) and hot water immersion (HWI; Zurawlew *et al*., 2016; Zurawlew *et al*., 2018a, b, 2019) provide alternative, more accessible and time efficient heat acclimation strategies compared to conventional exercise-based approaches. These exposures to hot air/water can be incorporated into normal training, e.g., HWI as part of a post-exercise washing routine, and may also support muscle recovery (Versey *et al.*, 2013). Six days of post-exercise HWI presents a short-term heat acclimation strategy, which provides reductions in thermal strain that compare favourably with long-term interventions (Tyler *et al.*, 2016). HWI exposes individuals to a large dual thermal stimulus (i.e. elevated core and skin temperatures), which is purported to induce a more complete state of heat acclimation (Regan *et al.*, 1996). Furthermore, exposure to high skin temperatures has been shown to accelerate heat acclimation adaptation in females (Mee *et al.*, 2018).

The primary aim of the current study was to compare thermal adaptations from 3 and 6 days of post-exercise HWI with exercise heat acclimation (EHA) in recreationally active males. We also aimed to compare the effect of these interventions on endurance capacity in the heat and markers of overreaching. In addition, by including a work-matched thermoneutral exercise intervention (TNE), we investigated the individual contributions of daily submaximal exercise and heat stress to adaptation after EHA. We hypothesised that post-exercise HWI would accelerate the speed of adaptation compared to EHA, and that the benefits of EHA beyond that of TNE would be modest. We also hypothesised that the high physical demands of daily exercise-heat-stress during EHA would lead to increased markers of overreaching compared to post-exercise HWI and TNE.

4.3 Methods

4.3.1 Participants

Twenty-seven recreationally active and non-heat-acclimatised males provided written informed consent to participate (**Section 3.2**). The study received local and Ministry of Defence Research Ethics Committee approval and was conducted following the Declaration of Helsinki (2013; although was not pre-registered) and received Defence Science and Technology Laboratory permission to publish. Participants were matched for fitness characteristics in groups of three and randomly assigned to either HWI, EHA, or TNE (randomiser.org; see Table 4.1 for participant characteristics). HWI involved a 40-min treadmill-run in temperate conditions (19°C) followed by hot water immersion (≤ 40-min, 40°C water). To elicit a similar endogenous thermal stimulus to HWI (i.e. area under the curve, AUC, time and magnitude T_{re} was >38.5°C, °C·min^{-1}; Table 4.2), EHA involved a ≤ 60-min treadmill-run in the heat (33°C, 40% RH). Pilot data demonstrated a similar AUC from post-exercise HWI[12] vs a 60-min treadmill run in the heat (65% $\dot{V}O_{2peak}$; 33°C, 40% RH). We deemed it unnecessary to include a thermoneutral water immersion intervention as we have previously demonstrated that it provides no heat acclimation benefits (Zurawlew *et al.*, 2016). We did however include a thermoneutral exercise intervention (TNE) to account for the effect of daily submaximal exercise on thermoregulatory adaptations. To enable work-matching with EHA, TNE participants completed the same external work ≥ 1-day after EHA participants.

Table 4.1. Participant characteristics of post-exercise hot water immersion (HWI), exercise heat acclimation (EHA) and thermoneutral exercise (TNE).

	HWI	**EHA**	**TNE**
Age (years)	22 ± 3	20 ± 2	21 ± 2
Height (cm)	177 ± 5	181 ± 5	178 ± 6
Body mass (kg)	73 ± 7	74 ± 7	70 ± 7
$\dot{V}O_{2peak}$ (mL·kg^{-1}·min^{-1})	53 ± 6	54 ± 3	53 ± 4

Data are displayed as mean ± SD; n = 9, each group.

Table 4.2. The daily thermal stimulus for adaptation and physical activity during days 1–3 and days 6–8 of thermoneutral exercise (TNE), exercise heat acclimation (EHA) and post-exercise hot water immersion (HWI).

	1–3			**6–8**		
	TNE	**EHA**	**HWI**	**TNE**	**EHA**	**HWI**
Duration $T_{re} \geq 38.5$°C (min) ##	9 ± 15	36 ± 12 §§	35 ± 5 §§	7 ± 11	37 ± 10 §§	39 ± 7 §§
AUC (°C·min^{-1}) ##	2 ± 5	18 ± 9 §§	16 ± 4 §§	2 ± 4	16 ± 7 §§	18 ± 3 §§
End intervention T_{re} (°C) ##	38.2 ± 0.5	39.2 ± 0.3 §§	39.2 ± 0.2 §§	38.2 ± 0.5	39.2 ± 0.2 §§	39.3 ± 0.1 §§
Physical activity > 3 METS (min)	121 ± 80	138 ± 81	138 ± 55	120 ± 46	125 ± 43	135 ± 66

T_{re}; rectal core temperature, AUC; area under the curve for $T_{re} > 38.5$°C. Data are displayed as mean ± SD of days 1–3 and days 6–8. ##$P < 0.01$ denotes main effect of group; §§$P < 0.01$ denotes group different from TNE.

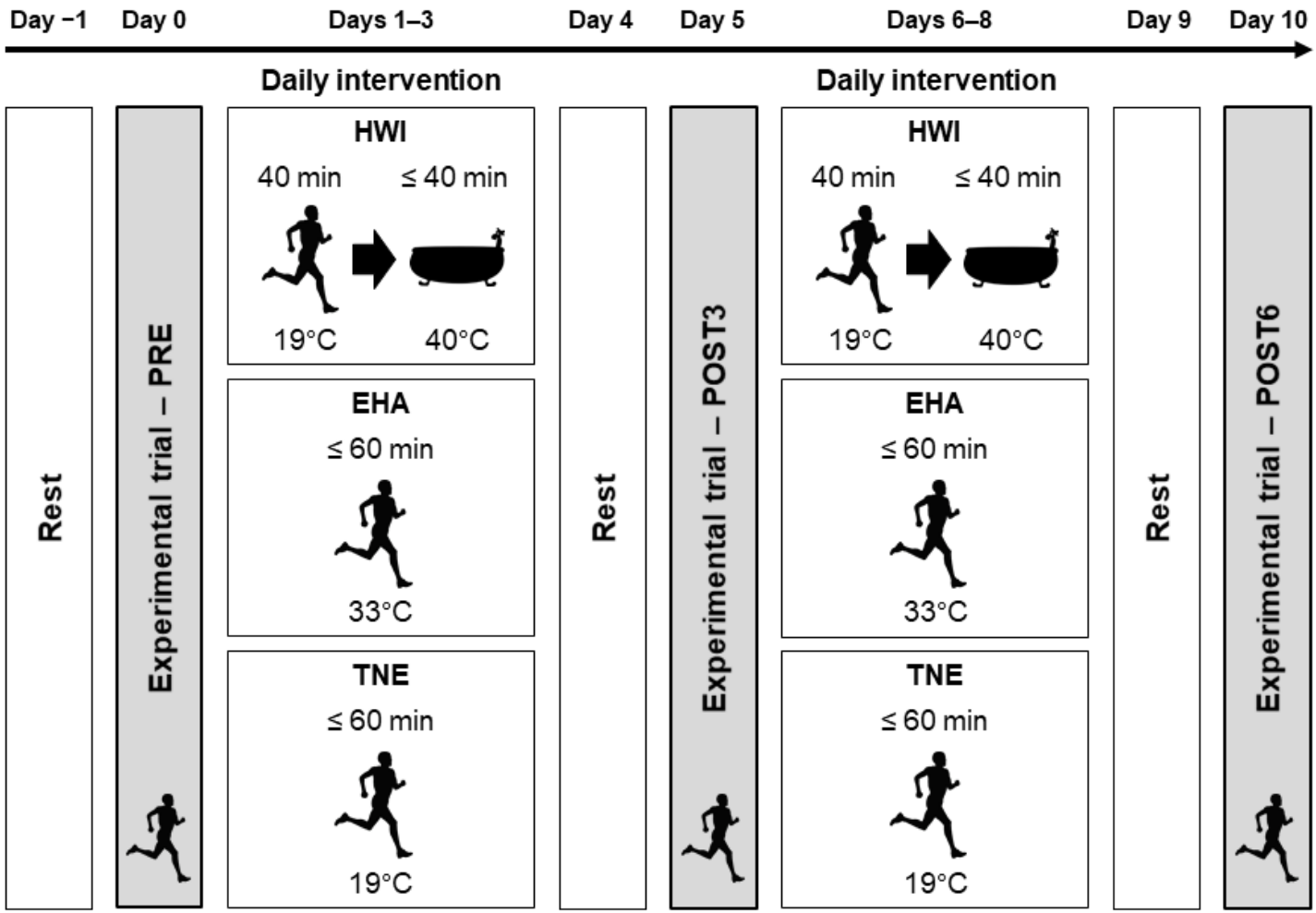

Figure 4.1. Schematic of the study design. HWI; post-exercise hot water immersion, EHA; exercise heat acclimation and TNE; work-matched thermoneutral exercise.

4.3.2 Fitness assessment

Participants completed a fitness assessment within a week before their first experimental trial. $\dot{V}O_{2peak}$ was assessed using a continuous maximal incremental exercise test performed on a motorised treadmill in a temperate laboratory (19°C) to volitional exhaustion (**Section 3.3.1**). $\dot{V}O_{2peak}$ was determined as the highest oxygen uptake attained over a 30-s period. A running speed that elicited 65% $\dot{V}O_{2peak}$ in temperate conditions was determined by the interpolation of the running speed–$\dot{V}O_2$ relationship (**Section 3.3.2**). Participants were then familiarised with the treadmill running speed and experimental trial procedures. As temperate training influences heat acclimation adaptations (Gisolfi & Robinson, 1969), participants' physical activity time (> 3 METS) for the duration of the study was assessed using a wrist-based accelerometer (Fitbit Flex, San Francisco, USA).

4.3.3 Experimental trials

Participants completed three experimental trials: before and after 3, and 6 days of their assigned intervention (Figure 4.1). Twenty-four hours before experimental trials, participants refrained from exercise, alcohol, diuretics, and caffeine. Before the first experimental trial, participants completed a diet diary and replicated this food and fluid intake before subsequent experimental trials. To ensure a similar circadian pattern, participants were instructed to sleep between 2200 h and 0700 h before experimental trials; sleep duration and efficiency were confirmed (Actigraph wGT3X-BT, Actigraph, Pensacola, USA).

On the day of the experimental trials (Figure 4.1), participants arrived at the laboratory at 0730 h and were provided with a standardised breakfast (2091 kilojoules, 71 g carbohydrate, 18 g fat, 17 g protein) and a bolus of water (7 $mL \cdot kg^{-1}$ of body mass). Following 20-min seated rest in temperate conditions (19°C, 45% RH), participants completed the Profile of Mood States questionnaire (Grove & Prapavessis, 1992) to determine total mood disturbance and energy index (**Section 3.7.1**), to detect perceived training-induced fatigue. A venous blood sample was then taken without stasis (**Section 3.4.5**) and total haemoglobin mass, blood volume, and plasma volume were assessed using the optimised carbon monoxide rebreathing technique (Schmidt & Nicole, 2005). Briefly, aliquots of whole blood were used for the immediate determination of haemoglobin concentration ($g \cdot dL^{-1}$), in duplicate (Hemocue, Sheffield, UK) and haematocrit (%), in triplicate (capillary tube method). Total haemoglobin mass was estimated from the percentage change in carboxyhaemoglobin concentration (ABL80 CO-OX

Flex hemoximeter Radiometer; Copenhagen, Denmark) measured in duplicate from earlobe capillary blood samples collected before and after rebreathing a mixed bolus of (0.8 mL·kg^{-1} body mass) carbon monoxide (99.9%) and oxygen (3 L, 99.5%). Total haemoglobin mass, haemoglobin concentration and haematocrit (%) was used to calculate blood volume (mL; [haemoglobin mass / haemoglobin concentration] × 100) and red cell mass (mL; blood volume × [haematocrit / 100]) for the calculation of plasma volume (mL; = blood volume − red cell mass; Schmidt & Nicole, 2005). A urine sample was analysed using a handheld refractometer (**Section 3.4.6**); exercise began when USG was < 1.03 (Armstrong, 2005). A rectal thermistor fitted 10 cm beyond the anal sphincter, and a data logger provided a measure of T_{re} (**Section 3.4.1**). A pre-exercise nude body mass was recorded using a digital platform scale (Model 703; Seca, Hamburg, Germany). Skin thermistors were attached on the right side of the body for the determination of T_{sk}, as previously described (**Section 3.4.1**; Ramanathan, 1964). Following instrumentation, participants rested for a further 30 min in temperate conditions (19°C, 45% RH) to establish baseline measures.

At 0945 h, dressed in shorts, socks, and trainers, participants entered the environmental chamber (Delta Environmental Systems, Chester, UK; 33°C, 40% RH; 0.2 m·s^{-1} wind velocity) to complete a 40-min treadmill run at 65% $\dot{V}O_{2peak}$. No fluids were consumed and T_{re}, skin temperatures, and heart rate (**Section 3.4.3**) were monitored continuously. Local forearm sweat rate was measured by dew point hygrometry for the determination of T_{re} at sweating onset (**Section 3.4.2**; Cheuvront *et al*., 2009). RPE (Borg, 1970), thermal sensation (Hollies & Goldman, 1977), $\dot{V}O_2$ and RER, assessed by the Douglas bag method (**Section 3.4.4**), were recorded every 10 min. On completion of the exercise, participants rested for 20-min in temperate conditions (19°C, 45% RH), during which they completed a modified Stroop test (**Section 3.7.2**; Dupuy *et al*., 2014), and provided a nude body mass to estimate whole-body sweat rate.

Participants then re-entered the environmental chamber and completed a time to exhaustion (TTE) on a motorised treadmill at 65% $\dot{V}O_{2peak}$ (**Section 3.6**). Following the cessation of exercise, capillary blood lactate concentrations were assessed (Lactate Pro 2™, Arkray, Australia) as a marker of short-term overreaching (Le Meur *et al.*, 2013; Schaal *et al.*, 2015). Participants were provided with a bolus of water and were free to leave the laboratory when T_{re} ≤ 38.5°C.

4.3.4 Daily intervention

Each participant completed two, three consecutive day blocks of their assigned intervention (Figure 4.1), during which they consumed their normal diet and fluid intake. Each day, participants arrived at the laboratory (0600 h and 1300 h), fitted a rectal thermistor to monitor T_{re}, and completed a 15-min seated rest in temperate conditions. Participants commenced their assigned intervention dressed in shorts, socks, and trainers. A bolus of water (5 mL·kg^{-1} of nude body mass) was consumed during the first 20 min of exercise.

HWI involved a 40-min treadmill run in temperate conditions (65% $\dot{V}O_{2peak}$; 19°C, 45% RH; 0.2 m·s^{-1} wind velocity) followed by a semi-recumbent ≤ 40-min hot water immersion (40°C) to the neck, as described (Zurawlew *et al.*, 2016). EHA and TNE involved a ≤ 60-min treadmill run at the predetermined speed that reflected 65% $\dot{V}O_{2peak}$ (in temperate conditions) in hot (33°C, 40% RH; 0.2 m·s^{-1} wind velocity) or temperate conditions (19°C, 45% RH; 0.2 m·s^{-1} wind velocity). Intervention sessions were terminated if maximal immersion or exercise duration was reached, at the participant's volition, or if T_{re} exceeded 39.5°C.

4.3.5 Statistical analysis

A sample size estimation (G*Power 3.1.9; Faul *et al*., 2007) was performed using data from post-exercise HWI (−0.36°C; Zurawlew *et al*., 2016), exercise heat acclimation (−0.22°C; Gibson *et al*., 2015) and thermoneutral exercise (0.00°C), with a pooled SD of 0.2°C. A one-way analysis of variance (ANOVA; alpha = 0.05, power = 0.8, correlation = 0.7) estimated that eight participants per group was required to detect a difference in the change in end-exercise T_{re} between groups. However, following statistical advice during the review process, a two-way mixed-methods analysis of covariance (ANCOVA) was considered the more appropriate and statistically powerful approach for comparing the effectiveness of interventions. To ensure adequate power and allowing for dropout, nine participants per group were recruited. All data were checked for normality and sphericity, presented as mean and standard deviation (SD), and statistical significance was accepted at $P < 0.050$. Uncertainty in the true (population) values of effects is presented as 95% confidence intervals (CI). A two-way mixed model ANCOVA with baseline (PRE) as the covariate was used to compare hallmark heat acclimation adaptations (e.g., end-exercise T_{re}) across time (post 3 days vs post 6 days) and between groups (HWI vs EHA vs TNE). The endogenous thermal stimulus and physical activity during each of the daily interventions was compared using a two-way mixed model ANOVA. Bonferroni-adjusted

pairwise comparisons were used where appropriate to determine where differences occurred. The magnitude of effect was reported using Cohen's *d*, where 0.2, 0.5, and 0.8 represent small, medium, and large effects, respectively (Cohen, 1988). Pearson's correlations determined the strength of the relationship between hallmark adaptations and changes in TTE. Pearson correlation coefficients of 0.00–0.19 were regarded as very weak, 0.20–0.39 as weak, 0.40–0.59 as moderate, and 0.60–0.79 as strong relationships. To assess endogenous thermal stimulus, the AUC was performed on the daily intervention T_{re} (time and magnitude T_{re} was >38.5°C) in each group using the trapezoid method (Cheuvront *et al.*, 2008). A statistically meaningful change in end-exercise T_{re} was defined as −0.34°C based on the large beneficial effect observed in a recent meta-analysis (Tyler *et al.*, 2016). Data were analysed using SPSS version 27 (IBM Corporation, NY, USA), or GraphPad Prism Version 9 (GraphPad Software Inc. La Jolla, USA).

4.4 Results

4.4.1 Daily intervention

All participants completed 6 days of their assigned intervention. Differences in the daily endogenous thermal stimulus were observed between groups (main effect of group, $f = 29.756$, $P < 0.001$; Table 4.2), for example, mean daily AUC for $T_{re} > 38.5$°C was similar in HWI and EHA (HWI, 17 ± 3°C·min^{-1}; EHA, 17 ± 7°C·min^{-1}; $P = 1.000$) but lower in TNE (2 ± 3°C·min^{-1}; $P < 0.001$; Table 4.2). The daily endogenous thermal stimulus was maintained throughout the 6-day intervention (main effect of time, $f = 0.035$, $P = 0.853$; interaction effect, f=1.019, $P = 0.376$), owing to an increase (main effect of time, $f = 7.897$, $P = 0.010$) in mean daily HWI (days 1–3, 31 ± 6 min; days 6–8, 35 ± 5 min) and EHA duration (days 1–3, 49 ± 9 min; days 6–8, 54 ± 8 min) . Following a significant main effect of group ($f = 4.315$, $P = 0.025$), *post hoc* pairwise comparisons revealed that total external work was lower in HWI (37 ± 6 km) compared to EHA (48 ± 9 km, $P = 0.026$), but no differences were detected between TNE (45 ± 9 km) and EHA ($P = 1.000$), or between TNE and HWI ($P = 0.169$). No differences were observed for daily physical activity time (> 3 METS) throughout the study protocol, evidenced by no main effects of time or group, and no interaction effect (all $P \geq 0.423$; Table 4.2).

4.4.2 Hallmark heat acclimation adaptations

No differences were detected between groups for sleep duration (6 ± 1 h), sleep efficiency (86 ± 9%) or USG (1.019 ± 0.007) before experimental trials, evidenced by no main effects of time or group, and no interaction effects (all $P \geq 0.336$). A two-way mixed model ANCOVA, with baseline as the covariate, detected a main effect of group for resting T_{re} ($f = 6.438$, $P = 0.006$, Figure 4.2A), end-exercise T_{re} ($f = 5.299$, $P = 0.013$, Figure 4.2B), T_{re} at sweating onset ($f = 7.633$, $P = 0.003$), and whole-body sweat rate ($f = 7.633$, $P = 0.001$, Table 4.3); there were no main effects of time or interaction effects (all $P \geq 0.144$). *Post hoc* pairwise comparisons revealed that HWI elicited a larger reduction in resting T_{re} (baseline-adjusted: −0.38 ± 0.23°C, CI: −0.26 to −0.49°C, $d = 1.6$) compared to EHA (−0.14 ± 0.23°C, CI: −0.03 to −0.26°C, $P = 0.021$, $d = 0.6$) and TNE (−0.12 ± 0.23°C, CI: −0.01 to −0.24°C, $P = 0.011$, $d = 0.5$; Figure 4.2A). Similarly, the reduction in end-exercise T_{re} was larger after HWI (−0.47 ± 0.23°C, CI: −0.36 to −0.58°C, $d = 2.1$) compared to EHA (−0.26 ± 0.24°C, CI: −0.15 to −0.38°C, $P = 0.042$, $d = 1.1$) and TNE (−0.25 ± 0.23°C, CI: −0.14 to −0.37°C; $P = 0.025$, $d = 1.1$; Figure 4.2B). Furthermore, HWI elicited a statistically meaningful decrease in end-exercise T_{re} (i.e. ≥0.34°C

reduction; Tyler *et al*., 2016) after only 3 days. No differences were observed between EHA and TNE for resting T_{re} ($P = 1.000$) or end-exercise T_{re} ($P = 1.000$). Additional *post hoc* analyses revealed resting and end-exercise T_{re} were lower after 3 days of HWI (main effects of time, $P \leq 0.012$) but not EHA ($P \geq 0.063$) or TNE ($P \geq 0.080$). T_{re} at sweating onset was reduced in accordance with resting T_{re}, with reductions being larger after HWI compared with EHA ($P = 0.011$) and TNE ($P = 0.005$; Table 4.3); no differences were observed between EHA and TNE ($P = 1.000$). Whole-body sweat rate was greater after HWI ($P = 0.001$) and EHA ($P = 0.009$) compared to TNE, but no difference was detected between HWI and EHA ($P = 0.950$; Table 4.3). The change in T_{re} during the 40-min treadmill-run in the heat was lower at POST6 compared to at POST3, evidenced by a main effect of time ($f = 4.444$, $P = 0.046$, Table 4.3); however, no differences were detected between groups (main effect of group, $f = 1.046$, $P = 0.368$; interaction effect, $f = 1.046$, $P = 0.368$). No main effects of time or group, and no interaction effects were detected for end-exercise T_{sk}, heart rate, physiological strain index, RPE, thermal sensation, plasma volume, blood volume, total haemoglobin mass, mean $\dot{V}O_2$ or mean RER (all $P \geq 0.154$; Table 4.3).

4.4.3 Endurance capacity and markers of overreaching

Seven participants were removed from the TTE analysis owing to: reaching the T_{re} ethical cut-off (HWI, $n = 2$); going to the toilet (EHA, $n = 1$); lower limb discomfort (TNE, $n = 1$); exercise-induced bronchoconstriction (TNE, $n = 1$); nausea (TNE, $n = 1$); and an obvious lack of effort without markers of overreaching at rest (TNE, $n = 1$). No main effects of time or group, and no interaction effects (all $P \geq 0.416$) were observed in the remaining 20 participants who completed the TTE protocol (7 HWI; 8 EHA; 5 TNE; Figure 4.3). Correlational analysis revealed that the change in TTE was moderately associated with the magnitude of adaptation in end-exercise T_{re} ($r = -0.47$, $P = 0.019$), end-exercise physiological strain index ($r = -0.54$, $P = 0.008$), and whole-body sweat rate ($r = 0.49$, $P = 0.013$). There were no main effects of time or group, and no interaction effects detected for markers of short-term overreaching (all $P \geq 0.172$), including: mood disturbance, energy index, Stroop reaction time, Stroop accuracy, end-TTE heart rate, end-TTE blood lactate, and sleep efficiency. Although interestingly, the three EHA participants who experienced no improvement or a decline in endurance capacity (Figure 4.3) showed some signs of overreaching, evidenced by an increase in total mood disturbance (+29), and decreases in energy index (−15), sleep efficiency (−13%), and Stroop accuracy (−3%).

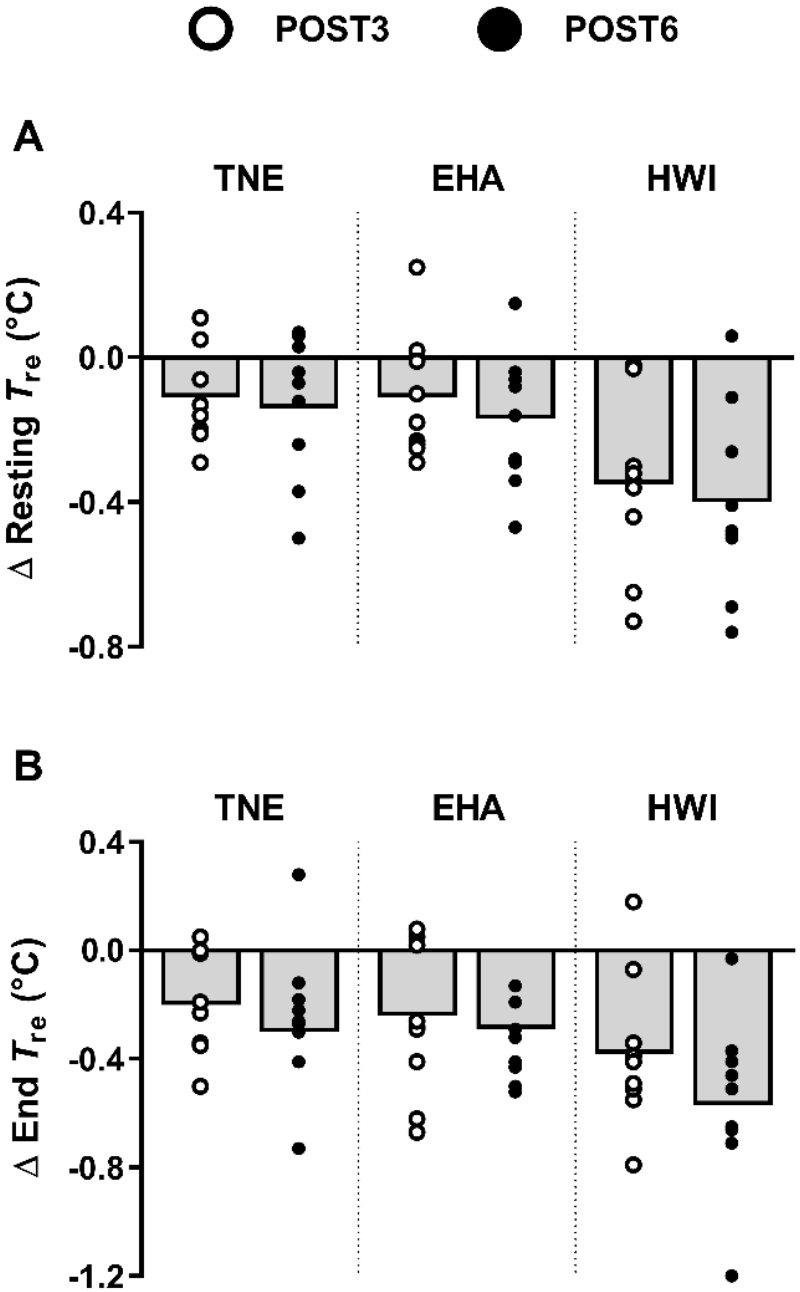

Figure 4.2. Influence of 3 (POST3) and 6 days (POST6) of thermoneutral exercise (TNE), exercise heat acclimation (EHA), or post-exercise hot water immersion (HWI) on resting rectal core temperature (T_{re}; A) and end-exercise T_{re} following a 40-min treadmill run at 65% $\dot{V}O_{2peak}$ in the heat (33˚C, 40% RH; B). Bars represent the baseline-adjusted mean change from baseline; circles represent individual participant responses.

Table 4.3. Influence of 3 (POST3) and 6 days (POST6) of thermoneutral exercise (TNE), exercise heat acclimation (EHA), or post-exercise hot water immersion (HWI) on heat acclimation adaptations at rest and during a 40-min treadmill run at 65% $\dot{V}O_{2peak}$ in the heat (33°C, 40% RH).

	TNE			EHA			HWI		
	PRE	POST3	POST6	PRE	POST3	POST6	PRE	POST3	POST6
Resting T_{re} (°C) $^{\#\#}$	37.05 ± 0.25	36.97 ± 0.19	36.94 ± 0.22	37.08 ± 0.29	36.96 ± 0.19	36.90 ± 0.22	37.10 ± 0.24	36.72 ± 0.19$^{\dagger, \S}$	36.68 ± 0.22$^{\dagger, \S}$
End-exercise T_{re} (°C) $^{\#}$	38.68 ± 0.49	38.62 ± 0.26	38.52 ± 0.21	38.98 ± 0.31	38.58 ± 0.26	38.53 ± 0.21	38.80 ± 0.27	38.44 ± 0.26$^{\dagger, \S}$	38.25 ± 0.20$^{\dagger, \S}$
T_{re} at sweating onset (°C) $^{\#\#}$	36.99 ± 0.25	36.92 ± 0.18	36.89 ± 0.20	37.03 ± 0.32	36.93 ± 0.18	36.83 ± 0.20	37.08 ± 0.27	36.70 ± 0.18$^{\dagger, \S\S}$	36.58 ± 0.20$^{\dagger, \S\S}$
Δ T_{re} during exercise (°C) *	1.63 ± 0.49	1.66 ± 0.26	1.58 ± 0.28	1.91 ± 0.19	1.62 ± 0.26	1.64 ± 0.29	1.69 ± 0.31	1.72 ± 0.25	1.57 ± 0.28
Whole-body sweat rate (L·h^{-1}) $^{\#\#}$	0.84 ± 0.12	0.85 ± 0.08	0.83 ± 0.06	0.91 ± 0.16	0.94 ± 0.08§§	0.93 ± 0.06§§	0.92 ± 0.20	0.95 ± 0.08§§	0.97 ± 0.06§§
End-exercise T_{sk} (°C)	35.63 ± 0.62	35.15 ± 0.49	35.06 ± 0.45	35.78 ± 0.79	35.22 ± 0.50	34.97 ± 0.46	35.19 ± 0.62	34.91 ± 0.51	34.86 ± 0.47
End-exercise heart rate (beats·min^{-1})	182 ± 16	177 ± 6	173 ± 6	189 ± 11	175 ± 6	170 ± 6	183 ± 11	173 ± 6	168 ± 6
End-exercise physiological strain	7.6 ± 1.2	7.4 ± 0.6	7.1 ± 0.5	8.5 ± 0.9	7.2 ± 0.6	7.0 ± 0.5	8.0 ± 0.4	7.1 ± 0.5	6.6 ± 0.5
Plasma volume (mL)	3031 ± 365	3139 ± 269	3197 ± 228	3203 ± 248	3287 ± 270	3314 ± 228	3108 ± 659	3169 ± 268	3249 ± 227
Blood volume (mL)	5647 ± 604	5711 ± 343	5771 ± 328	5914 ± 338	5864 ± 347	5930 ± 331	5622 ± 1067	5819 ± 344	5925 ± 329
Total haemoglobin mass (g)	861 ± 90	847 ± 49	851 ± 46	891 ± 70	878 ± 50	869 ± 46	832 ± 155	866 ± 49	873 ± 46
Mean $\dot{V}O_2$ (L·min^{-1})	2.72 ± 0.26	2.79 ± 0.13	2.79 ± 0.15	3.01 ± 0.35	2.86 ± 0.13	2.85 ± 0.15	2.90 ± 0.50	2.84 ± 0.13	2.84 ± 0.14
Mean RER	0.92 ± 0.03	0.91 ± 0.04	0.91 ± 0.03	0.92 ± 0.08	0.93 ± 0.05	0.93 ± 0.03	0.93 ± 0.04	0.91 ± 0.05	0.93 ± 0.03
End-exercise RPE (6–20 scale)	16 ± 2	15 ± 2	15 ± 1	15 ± 3	14 ± 2	14 ± 1	15 ± 2	14 ± 2	14 ± 1
End-exercise thermal sensation (1–13 scale)	11 ± 1	11 ± 1	11 ± 1	11 ± 1	10 ± 1	10 ± 1	10 ± 1	10 ± 1	10 ± 1
Time to exhaustion (s)	1300 ± 349	1428 ± 497	1512 ± 664	1156 ± 423	1531 ± 515	1766 ± 687	1821 ± 936	1708 ± 532	2090 ± 710

*T_{re}, rectal core temperature; T_{sk}, mean skin temperature; RER, respiratory exchange ratio; RPE, rating of perceived exertion. Data are mean ± SD at PRE and baseline-adjusted mean ± SD at POST3 and POST6. *$P < 0.05$ denotes main effect of time; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$; denotes main effect of group; $^{\dagger}P < 0.05$ denotes HWI different from EHA; $^{\S}P < 0.05$, $^{\S\S}P < 0.01$ denotes group different from TNE.*

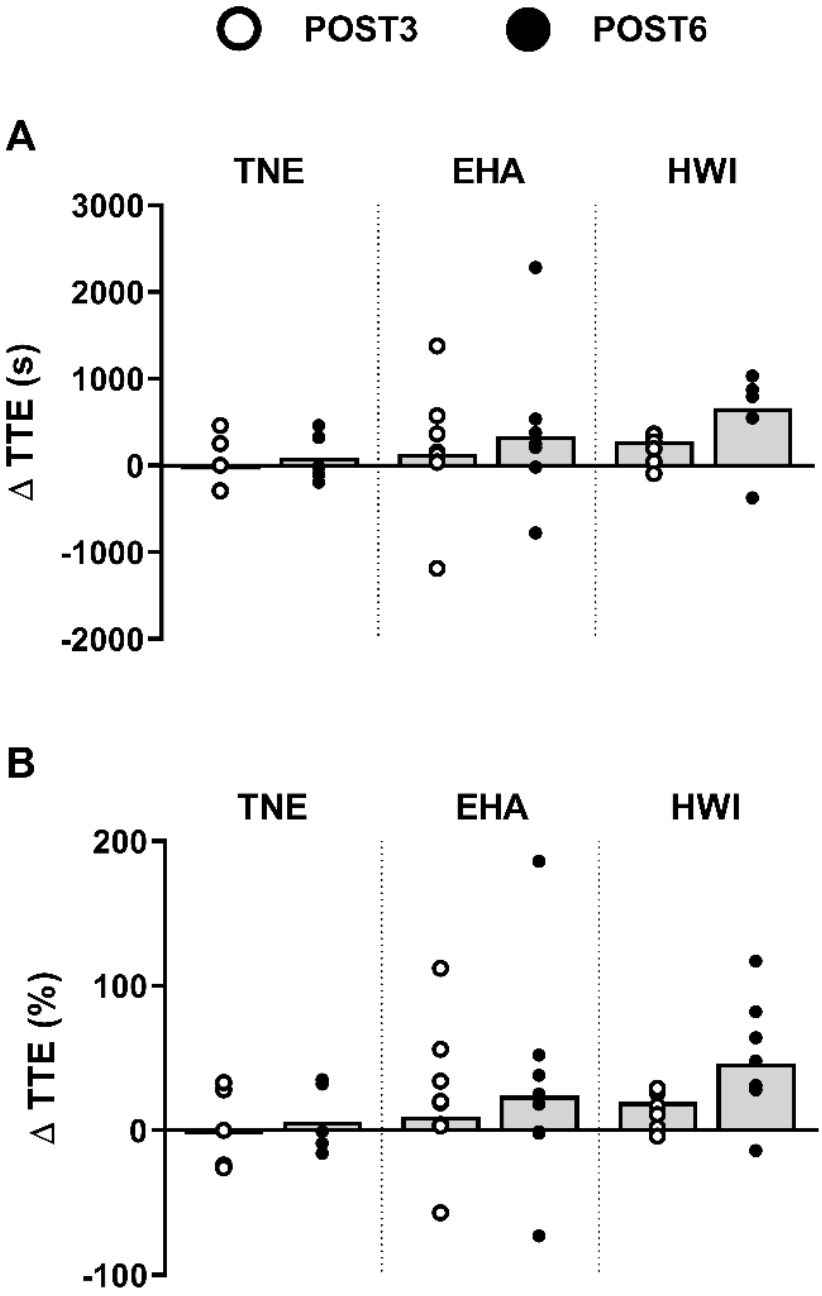

Figure 4.3. Influence of 3 (POST3) and 6 days (POST6) of thermoneutral exercise (TNE, *n* = 5), exercise heat acclimation (EHA, *n* = 8) or post-exercise hot water immersion (HWI, *n* = 7) on treadmill (65% $\dot{V}O_{2peak}$) time to exhaustion (TTE) in the heat (33°C, 40% RH). Bars represent the baseline-adjusted mean change (A) and percentage change (B) from baseline; circles represent individual participant responses.

4.5 Discussion

The current study sought to compare adaptations after 3 and 6 days of post-exercise HWI and EHA in recreationally active males. In addition, the individual contributions of daily submaximal exercise and heat stress to the adaptations after EHA were investigated. The novel finding is that short-term post-exercise HWI elicits larger thermal adaptations compared with short-term EHA. For example, resting T_{re} was lower after HWI (−0.38°C) compared to EHA (−0.14°C), which translated to a lower end-exercise T_{re} (−0.47°C) during exercise-heat-stress. Despite a similar daily endogenous thermal stimulus during HWI and EHA (Table 4.2), the benefits of exercising in the heat beyond exercising in temperate conditions appear modest (end-exercise T_{re} reduction: EHA, −0.26°C; TNE, −0.25°C).

Post-exercise HWI initiated a large reduction in resting T_{re} (−0.38°C), which accounted for most of the reduction in end-exercise T_{re} during exercise-heat-stress (~81%). The induction of a large reduction in resting T_{re} after HWI is likely due to exposure to a large dual thermal stimulus (average end-immersion T_{re} was 39.3°C and T_{sk} was 40°C), which is purported to induce a more complete state of heat acclimation (Regan *et al.*, 1996). We contend that this dual thermal stimulus is necessary for meaningful heat acclimation adaptations to arise; a recent post-exercise HWI study eliciting an end-intervention T_{re} of only 38.4°C observed no further benefit compared to exercise in the heat alone (Stevens *et al.*, 2020). Furthermore, the HWI protocol in the current study likely elicited a greater peripheral stimulus as skin temperature was continuously elevated for the whole immersion duration. In the present study, EHA had no effect on resting T_{re} beyond that of exercise in temperate conditions, despite eliciting a similar endogenous thermal stimulus to HWI. The larger reductions in resting T_{re}, end-exercise T_{re} and T_{re} at sweating onset after HWI compared to EHA are likely due to a higher skin temperature during the daily intervention (40°C vs ~35°C). This finding is supported by previous work that demonstrated an accelerated rate of phenotypic adaptation when a high skin temperature was employed in conjunction with conventional exercise heat acclimation (Mee *et al.*, 2018). Research has linked the repeated elevation in skin temperature and activation of warm-sensitive neurons to the induction of hypothalamic neural network changes that reduce resting core temperature (Tan *et al.*, 2016). Accordingly, the induction of meaningful heat acclimation benefits is dependent upon the magnitude of both the endogenous thermal stimulus and skin temperature (Regan *et al.*, 1996). In addition, HWI may elicit haematological adaptations (e.g., plasma volume expansion) distinct from conventional exercise heat

acclimation (Kissling *et al.*, 2019); however, no differences were observed in plasma volume after the interventions.

The inclusion of a work-matched thermoneutral exercise intervention allows for insights into the individual influence of daily submaximal exercise and heat stress on adaptations in recreationally active participants. For example, after adjusting for baseline, ~96% of the reduction in end-exercise T_{re} after 6 days of EHA was observed from daily thermoneutral exercise alone (−0.26°C vs −0.25°C). Although the magnitude of thermal adaptation elicited by EHA is consistent with previous short-term exercise-based heat acclimation (Table 2.2), few studies have included an appropriate thermoneutral exercise control intervention. Also aligning with previous research (Tyler *et al.*, 2016), EHA had a larger effect on increasing whole-body sweat rate compared to daily exercise in temperate conditions, but this did not translate to an improvement in endurance capacity in the heat. It is however worth noting that three EHA participants experienced either a decline or no change in TTE, coinciding with evidence of short-term overreaching (e.g., total mood disturbance and sleep efficiency; Meeusen *et al*., 2013). The combined stressors of daily exercise-heat-stress could have exhibited these abnormal training responses within our recreationally active population (Meeusen *et al.*, 2013). As such, interventions should be undertaken with caution, while ensuring adequate time for recovery to minimise fatigue and ensure adaptations are fully realised (Saunders *et al.*, 2019).

The reduction in end-exercise T_{re} after 6 days of post-exercise HWI in the present study (−0.47°C) exceeds that previously reported (−0.36°C; Zurawlew *et al*., 2016). This is likely explained by the lower aerobic fitness of the participants in the present study (53 $mL \cdot kg^{-1} \cdot min^{-1}$ vs 61 $mL \cdot kg^{-1} \cdot min^{-1}$; Zurawlew *et al*., 2018); endurance-trained individuals are considered partially heat acclimatised and to have a reduced adaptation potential (Gisolfi & Robinson, 1969; Shvartz *et al.*, 1977). Furthermore, the current study involved an additional exercise-heat exposure (experimental trial) and rest-day after 3 days (Figure 4.1). It is possible that previous research, adopting a limited recovery period between the final heat acclimation bout and the exercise-heat-stress test, may underplay heat acclimation benefits, as the full effects may manifest after adequate recovery (Daanen *et al.*, 2011).

4.5.1 Limitations

While the data clearly demonstrate heat acclimation adaptations, findings on the effectiveness of the interventions for improving TTE should be considered with caution owing to the small sample size. Indeed, a *post hoc* power estimation (G*Power) revealed that the TTE analysis was underpowered. As such, future work with a larger sample size is required to confirm (or reject) whether post-exercise HWI provides favourable improvements in endurance capacity in the heat compared to EHA. We also recognise that while the approach used in the current study allowed us to examine the importance of the dual thermal stimulus for heat acclimation adaptation, mean body temperature (calculated from core and skin temperature) was likely higher during HWI compared to EHA. Hence, further research is required to determine whether post-exercise HWI compares favourably to EHA when mean body temperature is matched. Furthermore, increasing the duration and/or intensity of daily exercise-heat-stress sessions in line with certain previous protocols (Febbraio *et al*., 1994; Fujii *et al*., 2012) may elicit larger heat acclimation adaptations than were observed in the current study; however, doing so would likely lead to greater fatigue and exacerbate the incidence of overreaching.

4.5.2 Practical implications

Taking a hot bath submerged to the neck, for up to 40 min, following habitual training in temperate conditions, presents a practical and economical heat acclimation intervention — eliminating the requirement for an increased training load, access to an environmental chamber or relocation to a hot climate. To facilitate adaptations from the post-exercise hot bath intervention, exposure to a large dual thermal stimulus is required. Further, although HWI alone has been shown to provide some thermal adaptations (Bailey *et al.*, 2016), to acquire more complete heat acclimation, it is recommended that exercise in a thermoneutral environment is completed immediately before HWI as this provides an additional training stimulus and initial increase in T_{core} (~1°C), which contributes to the dual thermal stimulus.

4.5.3 Conclusions

Short-term post-exercise HWI intervention elicited larger thermal adaptations compared with conventional short-term exercise heat acclimation. In addition, the thermal benefits of conventional short-term exercise heat acclimation beyond exercising in temperate conditions appear modest.

Chapter Five

The role of thyroid hormones in short-term heat acclimation thermal adaptations

5.1 Summary

This study aimed to investigate whether the reduction in resting T_{re} following short-term heat acclimation is associated with a reduction in circulating thyroid hormone concentrations. Fifty-seven males completed experimental trials before and after 6 days of post-exercise hot water immersion (HWI; n = 32), exercise heat acclimation (EHA, n = 9), or thermoneutral exercise (TNE; n = 16). Post-exercise HWI involved a daily 40-min treadmill-run at 65% $\dot{V}O_{2peak}$ in temperate conditions (19°C; 45% RH) followed by a ≤40-min HWI (40°C water). EHA and TNE involved a daily ≤60-min treadmill-run at 65% $\dot{V}O_{2peak}$ in hot (33°C, 40% RH) or temperate conditions (19°C; 45% RH), respectively. During experimental trials, resting T_{re} was recorded, and plasma samples were collected for the determination of free and total triiodothyronine (T3) and thyroxine (T4). No differences were detected in the endogenous thermal stimulus between heat acclimation methods (mean daily AUC for T_{re} > 38.5°C: HWI, 23 ± 10°C·min^{-1}; EHA, 17 ± 7°C·min^{-1}; P = 0.610). ANCOVA, using baseline values as the covariate, revealed no interaction effects but a significant group effect demonstrated that resting T_{re} was lower following 6 days of HWI (−0.33°C) compared to TNE (−0.10°C, P = 0.002) but not EHA (−0.17 ± 0.20°C; P = 0.133). No differences between interventions were detected for plasma volume-corrected free T3, free T4, total T3, or total T4 (all $P \geq 0.214$). A weak correlation was detected between the endogenous thermal stimulus and the change in total T4 following HWI and EHA (r = −0.32, P = 0.021), however, the reduction in resting T_{re} following 6 days of heat acclimation was not associated with changes in plasma thyroid hormone concentrations (all $r \leq 0.2$, $P \geq 0.099$). This study provides evidence that the endogenous thermal stimulus from short-term heat acclimation is related to plasma total T4. However, reductions in circulating thyroid hormone concentrations were not related and therefore, may not be the mechanism for the reduction in resting T_{re} following short-term heat acclimation.

5.2 Introduction

Repeated exposure to hot environments initiates phenotypic adaptations known collectively as heat acclimation, which alleviates thermal strain during subsequent heat challenges (Casa *et al.*, 2015; Périard *et al.*, 2015). Recent data demonstrates that taking a hot bath following exercise in a temperate environment provides favourable thermal adaptations compared with conventional short-term exercise heat acclimation (**Chapter 4**). The reduction in thermal strain following post-exercise hot water immersion (HWI) is predominantly a result of a large reduction in resting core temperature (−0.35°C after 3 days; **Chapter 4**). The underlying mechanism for this reduction in resting core temperature is currently unknown but may involve either a reduction in resting metabolic heat production via reduced thyroid hormone secretion (Buguet *et al.*, 1988), a decrease in the thermoregulatory balance point (Romanovsky, 2007), or hypothalamic neural network remodelling (Tan *et al.*, 2016; Zhao *et al.*, 2017).

Thyroid hormone is essential for growth, normal development, neural differentiation, and metabolic regulation in mammals (Williams, 2008; Cheng *et al.*, 2010; Iwen *et al.*, 2018). Release of thyroid stimulating hormone by the anterior pituitary gland stimulates the release of two protein-iodine-bound hormones: T3 and T4. When unbound, free thyroid hormones are metabolically active and stimulate glucose uptake, gluconeogenesis, lipolysis, and thermogenesis via modulating the heat generation capacity of brown adipose tissue (BAT; Iwen *et al*., 2018). Hence, a reduction in circulating thyroid hormone concentrations can affect BAT activity or centrally reduce sympathetic nervous system activation (Law *et al.*, 2019). Rats treated with propylthiouracil, an antithyroid drug that reduces serum T3 and T4, had impaired metabolic thermogenesis and a −0.5°C reduction in core temperature at rest and during heat stress (Bauer *et al.*, 1992; Yang & Gordon, 1997). Further, several studies have demonstrated that total T3 and T4 concentrations were reduced in heat acclimated rats (Horowitz *et al.*, 1986; Mirit *et al.*, 2000). However, no study to date has investigated the influence of short-term (< 7 days) heat acclimation on thyroid hormone concentrations in humans. Moreover, whether changes in thyroid hormone concentrations are related to the thermal adaptations from heat acclimation is unknown.

Despite the importance of the heat acclimation-induced reduction in resting core temperature for alleviating thermal strain, the mechanism responsible for this adaptation is yet to be elucidated. As such, this study aimed to investigate whether the reduction in resting core temperature after heat acclimation is associated with changes in circulating thyroid hormone

concentrations. We hypothesised that short-term post-exercise HWI would reduce plasma thyroid hormone concentrations to a greater extent than exercise heat acclimation (EHA) or thermoneutral exercise (TNE), and that these changes would be related to reductions in resting core temperature.

5.3 Methods

5.3.1 Study design

The analysis of thyroid hormone concentrations was performed on data collected from 57 participants from four previously published heat acclimation studies in our laboratory (Zurawlew *et al*., 2016; Zurawlew *et al*., 2018a; Zurawlew *et al*., 2018b; **Chapter 4**). All participants completed an experimental trial before (PRE) and after 6 days (POST6) of their assigned intervention. Eighteen participants (HWI, n = 9; TNE, n = 9) from **Chapter 4** completed an additional exercise-heat exposure (experimental trial) and rest day after the third intervention session (data not shown).

5.3.2 Participants

All participants were male, healthy, non-smokers, free from any known cardiovascular or metabolic diseases, were not taking any medication, and had not been regularly (> once a week) exposed to the heat (including sauna and hot bath use) in the 6 weeks before commencing testing (see Table 5.1 for participant characteristics). All studies received local ethical approval and were conducted in accordance with the Declaration of Helsinki (2013), except for registration in a database.

Table 5.1. Participant characteristics of post-exercise hot water immersion (HWI), exercise heat acclimation (EHA), and thermoneutral exercise (TNE).

	HWI (n = 32)	**EHA** (n = 9)	**TNE** (n = 16)
Age (years)	23 ± 3	20 ± 2	22 ± 3
Height (cm)	178 ± 6	181 ± 5	178 ± 6
Body mass (kg)	72 ± 8	74 ± 7	71 ± 6
$\dot{V}O_{2peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	58 ± 6	54 ± 3	56 ± 7

Data are displayed as mean ± SD.

5.3.3 Preliminary measurements

Participants completed a fitness assessment within a week before their first experimental trial. $\dot{V}O_{2peak}$ was assessed using a continuous maximal incremental exercise test performed on a motorised treadmill in a temperate laboratory (19°C), as described (**Section 3.3.1**). A running speed that elicited 65% $\dot{V}O_{2peak}$ in temperate conditions was subsequently determined by the interpolation of the running speed–$\dot{V}O_2$ relationship (**Section 3.3.2**). The individualised running speed was used for the exercise during the daily intervention sessions.

5.3.4 Experimental trials

Twenty-four hours before PRE, participants were instructed to refrain from exercise, alcohol, diuretics, and caffeine and to complete a diet diary. Twenty-four hours before POST6, participants were instructed to replicate this food and fluid intake. To ensure a similar circadian pattern, participants were instructed to sleep between 2200 h and 0700 h before experimental trials.

On the day of experimental trials, participants arrived at the laboratory at 0730 h and were provided with a standardised breakfast (2201 kilojoules, 71 g carbohydrate, 18 g fat, 17 g protein) and a bolus of water (7 $mL \cdot kg^{-1}$ of nude body mass). At 0800 h, dressed in a t-shirt, shorts, socks and trainers, participants rested for 20 min in temperate laboratory conditions (19°C, 45% RH). Following the seated rest, a venous blood sample was taken without stasis for the determination of plasma volume and plasma concentrations of free T3, free T4, total T3, and total T4 as previously described (**Section 3.4.5**). A urine sample was then analysed for USG to confirm euhydration (**Section 3.4.6**; Armstrong *et al*., 2005) and a flexible, sterile, single-use rectal thermistor was self-inserted 10 cm beyond the anal sphincter to measure T_{re} (**Section 3.4.1**). Resting T_{re} was recorded after a further 30-min seated rest in temperate laboratory conditions (19°C, 45% RH) using a data logger (YSI model 4000A; YSI, Dayton, Ohio, USA). $\dot{V}O_2$ and RER were assessed from a 60-s expired gas sample collected by Douglas bag method at 29–30 min of seated rest and used to estimate resting metabolic heat production as follows (Gagge & Gonzalez, 2011):

$$\text{Metabolic heat production} = [0.23(\text{RER}) + 0.77] \cdot [5.873(\dot{V}O_2)] \cdot (60 / \text{body surface area}).$$

5.3.5 Daily intervention

All participants completed 6 days of their assigned intervention. During the intervention, participants were instructed to consume their normal diet and fluid intake, including caffeine and alcohol (≤ 3 units per day). Participants arrived at the laboratory each day between 0600 h and 1300 h. Before exercise, a nude body mass was taken, and a rectal thermistor and heart rate monitor were fitted. Following instrumentation, participants completed a 15-min seated rest in a temperate laboratory (19°C, 45% RH) to establish baseline measures, before commencing their assigned intervention protocol. A bolus of water (5 $mL \cdot kg^{-1}$ of nude body mass) was consumed during the first 20 min of exercise.

Participants assigned to HWI completed a 40-min treadmill run in temperate conditions (65% $\dot{V}O_{2peak}$, 19°C, 45% RH; 0.2 $m \cdot s^{-1}$ wind velocity) dressed in shorts, socks, and trainers. Following exercise (2–3 min transition), dressed in shorts, participants began a semi-recumbent ≤ 40-min HWI (40°C) to the neck, as previously described (**Chapter 4**). Participants assigned to EHA completed a ≤ 60-min treadmill run in an environmental chamber (65% $\dot{V}O_{2peak}$, 33°C, 40% RH; 0.2 $m \cdot s^{-1}$ wind velocity). All heat acclimation sessions were terminated if the maximal immersion/exercise duration was reached, at the participant's volition, or if T_{re} exceeded 39.5°C. Upon removal from the hot water/environmental chamber, participants rested in a temperate laboratory, were provided with a bolus of water, and were free to leave the laboratory when $T_{re} \leq 38.5$°C. Participants assigned to TNE completed a daily submaximal treadmill run in temperate conditions (65% $\dot{V}O_{2peak}$; 19°C, 45% RH; 0.2 $m \cdot s^{-1}$ wind velocity). Mean daily TNE duration was 45 ± 7 min.

5.3.6 Blood sample collection and analysis

Venous blood samples were collected from an antecubital vein without stasis into two 6-mL EDTA vacutainers (**Section 3.4.5**). Aliquots of whole blood were used for the immediate determination of haemoglobin in duplicate (Hemocue, Sheffield, UK) and haematocrit in triplicate (capillary tube method). The change in plasma volume was estimated by correcting the initial plasma volume at PRE for the percentage change in plasma volume (%ΔPV) at POST6, as previously described (Dill & Costill, 1974). The remaining whole blood was then centrifuged, and the plasma frozen at −80°C for later analysis. Using ELISA, plasma concentrations of free and total T3 and T4 as previously described (**Section 3.4.5**). Thyroid

hormone concentrations were adjusted for plasma volume changes (**Section 3.4.5**; Sherk *et al*., 2013).

5.3.7 Statistical analysis

A sample size estimation (G*Power 3.1.9; Faul *et al*., 2007) with an alpha of 0.05 and a power of 0.95 determined that 34 participants were required to detect a moderate (0.5) correlation coefficient between a reduction in resting T_{re} and a reduction in plasma thyroid hormone concentrations. All data were checked for normality and sphericity; plasma free T4 data was reciprocal transformed to address statistical assumptions of sphericity. Data are presented as untransformed mean and SD, and statistical significance was accepted at $P < 0.05$. Uncertainty in the true (population) values of effects is presented as 95% CI. The mean daily endogenous thermal stimulus and external work during HWI, EHA, and TNE were compared using a one-way ANOVA. A one-way ANCOVA, with baseline (PRE) as the covariate, was used to detect group differences in resting T_{re}, resting metabolic heat production, and plasma thyroid hormone concentrations following 6 days of HWI, EHA, or TNE. Bonferroni-adjusted pairwise comparisons were used where appropriate to determine where differences occurred. To assess endogenous thermal stimulus, an AUC calculation was performed on the daily intervention T_{re} (time and magnitude T_{re} was >38.5°C) in each group using the trapezoid method (Cheuvront *et al.*, 2008). Pearson's correlations determined the strength of the relationship between changes in plasma thyroid hormone concentrations and resting T_{re}, and the total AUC for $T_{re} > 38.5$°C after 6 days of heat acclimation by HWI and EHA. Pearson correlation coefficients of 0.00–0.19 were regarded as very weak, 0.20–0.39 as weak, 0.40–0.59 as moderate, and 0.60–0.79 as strong relationships. Data were analysed using SPSS version 27 (IBM Corporation, NY, USA) or GraphPad Prism Version 9.1 (GraphPad Software Inc. La Jolla, USA).

5.4 Results

5.4.1 Daily intervention

All participants completed 6 days of their assigned intervention. A one-way ANOVA detected a main effect of group for mean daily duration T_{re} > 38.5°C (f = 51.632, P < 0.001), AUC for T_{re} > 38.5°C (f = 35.585, P < 0.001), end-intervention T_{re} (f = 83.424, P < 0.001) and external work (f = 3.737, P = 0.030). Follow-up pairwise comparisons revealed that the endogenous thermal stimulus was similar between HWI and EHA (all $P \geq 0.167$) but lower in TNE (Table 5.2; all P < 0.001). No differences were detected for mean daily external work between HWI and EHA (P = 0.066), HWI and TNE (P = 0.161), or EHA and TNE (Table 5.2; P = 1.000).

Table 5.2. The daily endogenous thermal stimulus and external work during thermoneutral exercise (TNE), exercise heat acclimation (EHA), and post-exercise hot water immersion (HWI).

	TNE	**EHA**	**HWI**
Duration $T_{re} \geq 38.5$°C (min)	7 ± 8	37 ± 10 **	41 ± 13 **
AUC (°C·min^{-1})	1 ± 2	17 ± 7 **	23 ± 10 **
End intervention T_{re} (°C)	38.3 ± 0.4	39.2 ± 0.2 **	39.3 ± 0.2 **
External work (km)	7.3 ± 1.3	7.7 ± 1.5	7.0 ± 1.1 **

*T_{re}; rectal core temperature, AUC; area under the curve for T_{re} > 38.5°C. Data are displayed as mean ± SD. **P < 0.01 denotes group different from TNE.*

5.4.2 Experimental trials

A one-way ANCOVA, with baseline as the covariate, revealed that resting T_{re} was different between interventions (f = 7.432, P = 0.001). *Post hoc* pairwise comparisons revealed that resting T_{re} was lower following HWI (−0.33 ± 0.20°C, CI: −0.26 to −0.40°C) compared to TNE (−0.10 ± 0.20°C, CI: 0.01 to −0.20°C, P = 0.002), but not EHA (−0.17 ± 0.20°C, CI: −0.03 to −0.31°C, P = 0.133; Figure 5.1A). No differences were detected for resting T_{re} between EHA and TNE (P = 1.000). Differences were also observed between interventions for resting metabolic heat production (main effect of group, f = 3.281, P = 0.045), although *post hoc* analysis detected no significant differences between HWI (−6 ± 16 W, CI: −10 to 1) and EHA (+9 ± 16 W, CI: −1 to 20; P = 0.072), HWI and TNE (−6 ± 16 W, CI: −14 to 1; P = 1.000), or EHA and TNE (P = 0.060; Figure 5.1B). The reduction in resting T_{re} after 6 days of heat acclimation was associated with a reduction in resting metabolic heat production (r = 0.30, P = 0.031). No between-group differences were observed for plasma volume (HWI, +4 ± 6 %; EHA, +5 ± 6 %; TNE, +2 ± 6 %; f = 0.829, P = 0.442).

After correcting for individual changes in plasma volume, ANCOVA, with baseline as the covariate, detected no significant between-group differences in free T3 (f = 0.149, P = 0.862, Figure 5.1C), reciprocal transformed free T4 (f = 2.976, P = 0.060; Figure 5.1D), total T3 (f = 0.497, P = 0.611, Figure 5.1E), or total T4 (f = 1.585, P = 0.214, Figure 5.1F).

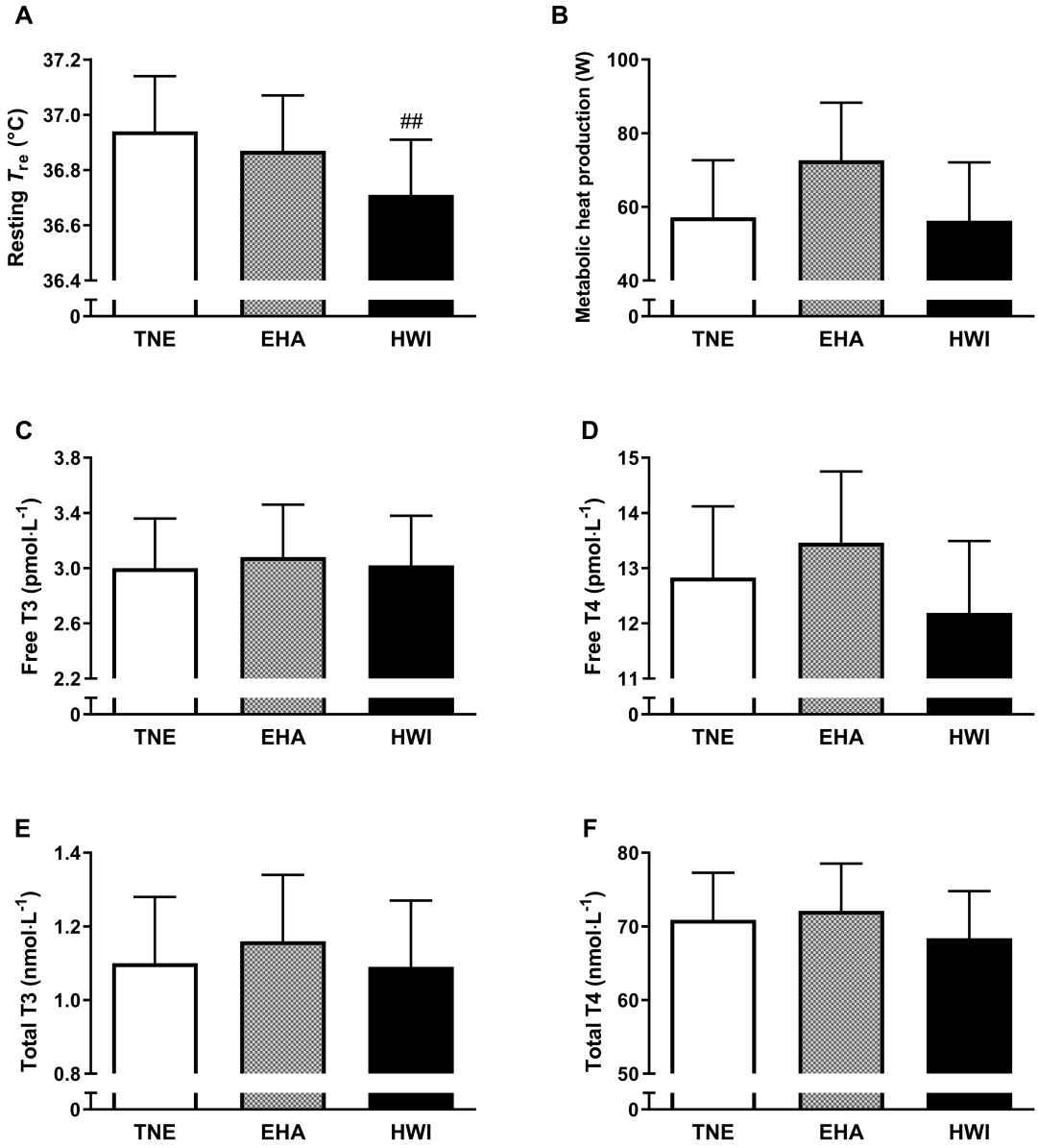

Figure 5.1. Influence of 6 days of thermoneutral exercise (TNE), exercise heat acclimation (EHA), or post-exercise hot water immersion (HWI) on resting rectal core temperature (T_{re}, A), resting metabolic heat production (B), and plasma concentrations of free triiodothyronine (T3; C), free thyroxine (T4; D), total T3 (E), and total T4 (F). Bars show baseline-adjusted mean ± SD. $^{\#\#}P < 0.01$ denotes HWI lower than TNE.

A weak correlation was detected between the endogenous thermal stimulus (total AUC for T_{re} > 38.5°C) and the change in plasma total T4 (r = −0.32, P = 0.021), whereby a larger endogenous thermal stimulus resulted in a greater reduction in total T4. Conversely, very weak and non-significant relationships were observed between total AUC for T_{re} > 38.5°C and the change in free T3 (r = −0.15, P = 0.171), free T4 (r = −0.13, P = 0.206), or total T3 (r = 0.10, P = 0.271).

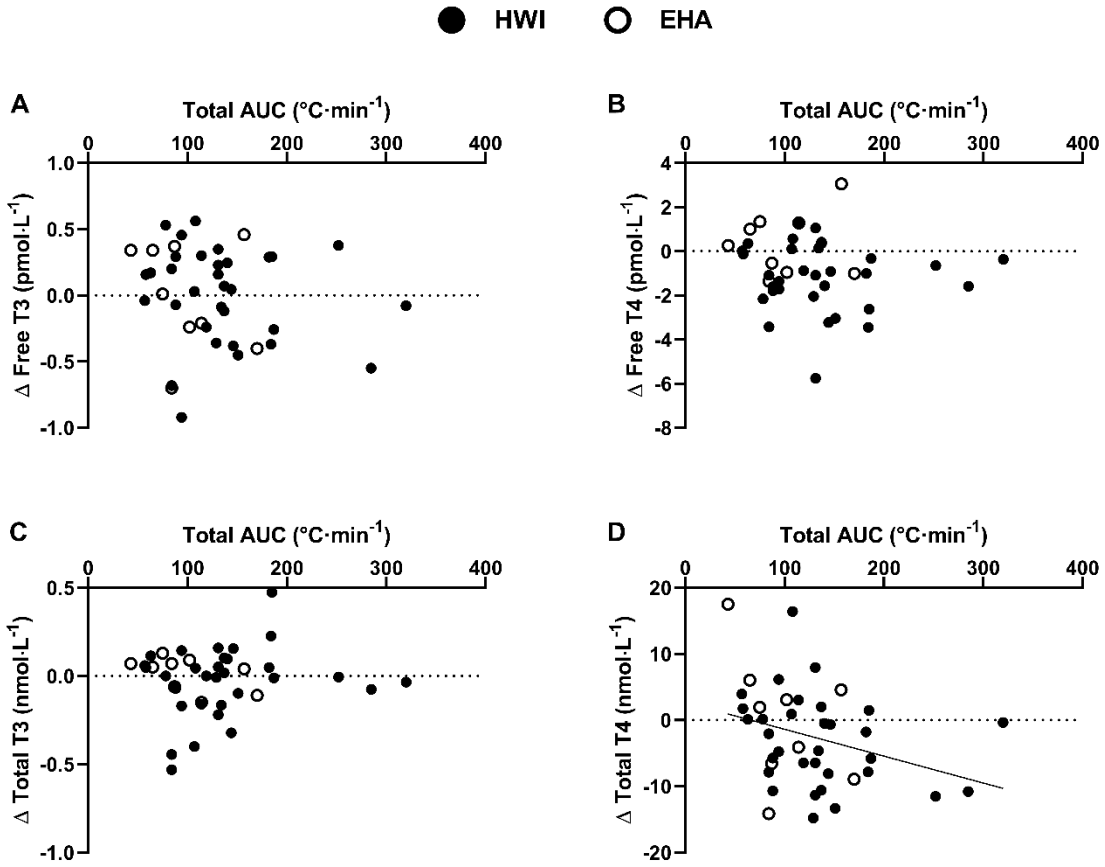

Figure 5.2. Relationship between total area under the curve (AUC) for T_{re} > 38.5°C and the change in plasma concentrations of free triiodothyronine (T3; A), free thyroxine (T4; B), total T3 (C), and total T4 (D) following 6 days of post-exercise hot water immersion (HWI) or exercise heat acclimation (EHA).

The reduction in resting T_{re} following 6 days of heat acclimation by HWI or EHA was not associated with changes in plasma concentrations of free T3 (r = −0.05, P = 0.374, Figure 5.2A), free T4 (r = 0.13, P = 0.209, Figure 5.2B), total T3 (r = 0.20, P = 0.099, Figure 5.2C) or total T4 (r = −0.15, P = 0.179, Figure 5.2D).

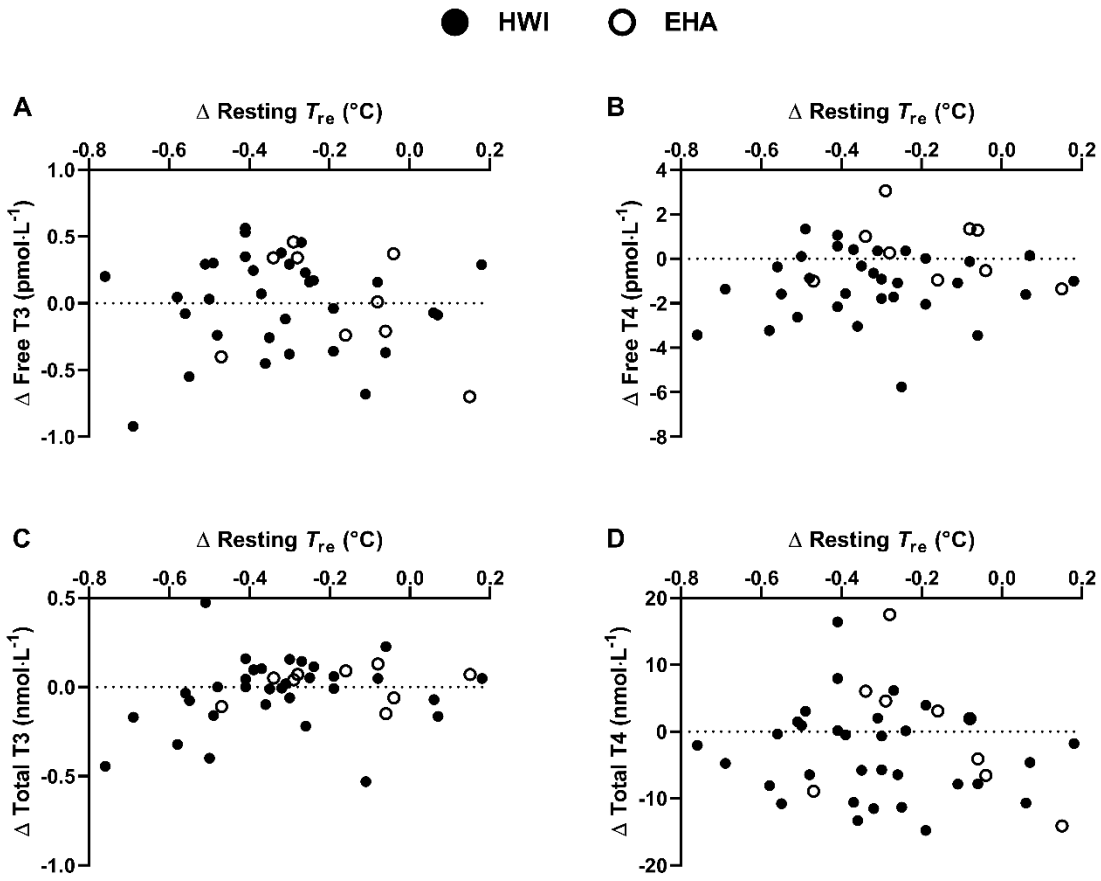

Figure 5.3. Relationship between the change in resting T_{re} and the change in plasma concentrations of free triiodothyronine (T3; A), free thyroxine (T4; B), total T3 (C), and total T4 (D) following 6 days of post-exercise hot water immersion (HWI) or exercise heat acclimation (EHA).

5.5 Discussion

The current study is the first to investigate the effect of short-term heat acclimation by post-exercise HWI and EHA on plasma thyroid hormone concentrations. There are two new and noteworthy findings from this study. Firstly, the endogenous thermal stimulus from short-term heat acclimation was partly related to plasma thyroid hormones. We observed a weak relationship between the total endogenous thermal stimulus and the change in total T4 following 6 days of heat acclimation. Secondly, the reduction in resting T_{re} following short-term heat acclimation was associated with a reduction in resting metabolic heat production; however, in contrast to the hypothesis, resting T_{re} was not associated with a reduction in circulating thyroid hormones. Six days of heat acclimation by post-exercise HWI or EHA also had no effect on plasma thyroid hormone concentrations beyond that of exercise in a temperate environment (Figure 5.1). Together, these findings do not support thyroid hormones as the mechanism for the reduction in resting T_{re} following short-term heat acclimation.

Our findings align with previous work in rats that demonstrated that short-term heat acclimation did not affect thyroid hormone concentrations (Mirit *et al.*, 2000). We did, however, observe a weak relationship between the total endogenous thermal stimulus and the change in total T4 following 6 days of heat acclimation, suggesting that a larger stimulus may be required to initiate a significant reduction in plasma thyroid hormone concentrations. Indeed, Mirit *et al.* (2000) found that total T3 and total T4 concentrations were only significantly lower than the control after 3 weeks of heat acclimation. In addition, previous research has also demonstrated reductions in thyroid hormones in humans following chronic heat exposure. For example, in environmentally exposed metal shop workers, total T3 was 10% lower in summer when temperatures ranged 30–36°C than in winter when temperatures ranged 14–21°C (Gertner *et al.*, 1983). In combination, these findings suggest reductions in thyroid hormone concentrations are more characteristic of long-term heat acclimation adaptation.

We found that the reduction in resting T_{re} following short-term heat acclimation was not associated with a reduction in plasma thyroid hormone concentrations. Previous research in rats has demonstrated the considerable effect a reduction in total T3 and T4 can have on core temperature, with a −0.5°C reduction in core temperature evident after 3 days of treatment with the antithyroidal drug, propylthiouracil (Yang & Gordon, 1997). However, the administration of propylthiouracil elicited much greater reductions in serum total T3 (−60%) and total T4 (−95%) than were observed in the current study after 6 days of post-exercise HWI (total T3,

−3%; total T4, −5%; Figure 5.1). Therefore, whilst a reduction in thyroid hormones can result in a reduction in resting core temperature, our findings do not support thyroid hormones as the mechanism for the reduction in resting T_{re} following short-term heat acclimation.

A possible alternative mechanism for the reduction in resting T_{re} following short-term heat acclimation is a leftward shift in the thermoregulatory balance point. The term balance point designates the regulated core body temperature, which is determined by independent thermoeffector loops (Romanovsky, 2007). These thermoeffector loops can be categorised as cold defence effectors or heat defence effectors and are controlled by specific warmth-sensitive neurons, each with a distinct temperature threshold (Romanovsky, 2018). A recent study found that 7 days of controlled hyperthermia HWI heat acclimation reduced the mean body temperature required to activate skin sympathetic nerve activity, resulting in lower activation thresholds for cutaneous vasodilation and sweat production (Barry *et al.*, 2020). These findings demonstrate that short-term heat acclimation elicits sympathetic neural adjustments that activate heat loss thermoeffectors. It follows that short-term post-exercise HWI heat acclimation could also lead to a reduction in the core body temperature thresholds for heat gain mechanisms, such as BAT thermogenesis. A lower temperature threshold for BAT thermogenesis would inevitably result in a lower resting T_{re} in a temperate environment. However, future research is required to confirm (or reject) this hypothesis.

5.5.1 Strengths and limitations

This study has several strengths. Firstly, given the similar intervention methodologies and endogenous thermal stimulus between HWI and EHA, we were able to pool data from several previous studies, which provided a large sample size for our primary aim. Secondly, including the TNE intervention controlled for the influence of daily submaximal exercise, thereby enabling robust conclusions on the effect of short-term heat acclimation on thyroid hormone concentrations. Thirdly, correcting thyroid hormone concentrations for changes in plasma volume provides confidence that our findings reflect the true influence of heat acclimation on circulating thyroid hormones rather than a consequence of plasma volume expansion. We also recognise that this study is not without limitation. Whilst changes in plasma thyroid hormones was hypothesised as the most likely mechanism for heat acclimation thermal adaptations, it is possible that other hormones may be involved. For example, insulin, glucagon, and adrenaline may affect thermogenesis; however, do not appear to have a primary regulatory role (Silva,

2006). Hence, as samples obtained from previous studies were limited, plasma thyroid hormones were prioritised for analysis due to their central role in thermoregulation

Although efforts were made to maximise the accuracy of the estimation of resting metabolic heat production by controlling the time of day, diet, and environmental conditions, we recognise the weakness of using an expired gas sample duration of only 60 s. As such, future research should verify the effect of heat acclimation on resting metabolic heat production using a measurement duration of 10 min (Compher *et al.*, 2006).

5.5.2 Conclusions

We found that the endogenous thermal stimulus from short-term heat acclimation is partly associated with plasma thyroid hormone concentrations. This finding is in accordance with previous research demonstrating reductions in circulating thyroid hormones following long-term heat acclimation and adds to the limited literature in humans on this topic. This study also provides evidence that changes in circulating thyroid hormones are not the mechanism for the reduction in resting T_{re} following short-term heat acclimation. Although this study does not offer a conclusive answer to the question of how heat acclimation reduces resting T_{re}, our findings broaden the understanding of heat acclimation adaptation by providing preliminary evidence that the mechanism for the reduction in core temperature following short-term protocols appears to be different from that of long-term heat acclimation. Future research should assess the effect of short-term post-exercise HWI heat acclimation on temperature thresholds for cold defence thermoeffectors as a potential mechanism for the reduction in resting core temperature.

Chapter Six

A comparison of medium-term post-exercise hot water immersion and exercise heat acclimation

6.1 Summary

This study aimed to compare heat acclimation adaptations from 12 days of post-exercise hot water immersion (HWI) and exercise heat acclimation (EHA) with thermoneutral exercise (TNE) and investigate the effect of medium-term heat acclimation on plasma thyroid hormone concentrations. Twenty-one males completed experimental trials before and after 3, 6, and 12 days of post-exercise HWI (n = 7), EHA (n = 7), or TNE (n = 7). Post-exercise HWI involved a daily 40-min treadmill run at 65% $\dot{V}O_{2peak}$ in temperate conditions (19°C; 45% RH) followed by HWI (40°C water). EHA and TNE involved a daily treadmill run at 65% $\dot{V}O_{2peak}$ in hot (33°C, 40% RH) or temperate conditions (19°C; 45% RH), respectively. During experimental trials, participants completed a 40-min treadmill run and TTE at 65% $\dot{V}O_{2peak}$ in the heat (33°C, 40% RH), and plasma samples were collected for the determination of free and total T3 and T4. ANCOVA, using baseline values as the covariate, revealed no interaction effects for thermal, endurance capacity, or markers of overreaching ($P \geq 0.198$). Group effects revealed that resting core temperature (T_{re}), end-exercise T_{re}, T_{re} at sweating onset, rating of perceived exertion, and thermal sensation were lower and whole-body sweat rate was higher after HWI than TNE (all $P \leq 0.049$). In contrast, aside from whole-body sweat rate, which was greater after EHA (P = 0.013), no additional heat acclimation adaptations existed between EHA than TNE ($P \geq 0.157$). Further, T_{re} at sweating onset was lower after HWI than EHA (P = 0.015). The endogenous thermal stimulus (r = 0.57, P = 0.017) and the reduction in resting T_{re} (r = 0.47, P = 0.044) were associated with changes in plasma free T3 after 12 days of heat acclimation. Plasma free T3 was lower after 12 days of HWI (−23%) than EHA (+4%, P = 0.008) and TNE (+1%, P = 0.015) and the reduction free T3 was associated with . There were no main effects or interaction effects for resting metabolic heat production, plasma volume, the change in T_{re} during exercise, end-exercise T_{sk}, end-exercise heart rate, exercising $\dot{V}O_2$, exercising RER, TTE, free T4, total T3, or total T4 (all $P \geq 0.072$). In conclusion, post-exercise HWI conferred a more complete heat acclimation than TNE and EHA. Changes in circulating thyroid hormones are unlikely the mechanism for medium-term heat acclimation adaptation.

6.2 **Introduction**

It is well established that exercise in hot and hot-humid environments is detrimental to physical performance (Galloway & Maughan, 1997; Maughan *et al.*, 2012) and may expose individuals to the risk of exertional heat illness (Casa *et al.*, 2015). To reduce these deleterious effects of heat stress on performance, athletes, military personnel, and occupational workers should prepare by completing a period of heat acclimation (Saunders *et al.*, 2019). Previous research from our laboratory demonstrates that taking a hot bath for up to 40 min immediately after submaximal exercise in temperate conditions on 6 days reduces thermal strain during exercise-heat-stress in both recreationally active (Zurawlew *et al*., 2016; **Chapter 4**) and endurance trained individuals (Zurawlew *et al.*, 2018b). Moreover, we recently demonstrated that despite a similar endogenous thermal stimulus for adaptation, short-term (< 7 days) post-exercise hot water immersion (HWI) elicited larger thermal adaptations than conventional exercise heat acclimation (EHA; **Chapter 4**). While the 6-day post-exercise HWI intervention presents an effective, practical, and time-efficient strategy, previous literature suggests that medium- (7–14 days; Moss *et al*., 2020) and long-term (> 14 days; Patterson *et al*., 2004) interventions provide a more complete state of heat acclimation. It is yet to be determined whether extending the 6-day post-exercise HWI intervention provides additional thermal benefits. In contrast to the beneficial adaptations, the physical demands of prolonged heat acclimation interventions can disrupt training and may lead to overreaching (Schmit *et al.*, 2018; Saunders *et al.*, 2019). It is currently unknown if medium-term heat acclimation results in overreaching. In addition, the true benefit of medium-term heat acclimation strategies beyond exercising in thermoneutral conditions is unknown due to the lack of work-matched interventions within the literature and hence further research is warranted. In contrast to the beneficial adaptations, the physical demands of prolonged heat acclimation interventions can disrupt training and may trigger overreaching as recently observed in a 5-day heat acclimation study (Schmit *et al.*, 2018; Saunders *et al.*, 2019). It is currently unknown if medium-term heat acclimation results in overreaching.

The reduction in thermal strain after heat acclimation can be largely attributed to a reduction in resting core temperature (Brazaitis & Skurvydas, 2010; Zurawlew *et al.*, 2016; Charlot *et al.*, 2017). The underlying mechanism for this adaptation is currently unknown but may involve a reduction in circulating thyroid hormones due to their central importance for thermogenesis and metabolism (Iwen *et al.*, 2018). A recent study in our laboratory found that the endogenous thermal stimulus from short-term heat acclimation was partly associated with changes in

plasma total T4, suggesting a larger stimulus, i.e., from medium-term heat acclimation, would likely lead to larger changes in plasma thyroid hormones (**Chapter 5**). Further, reductions in thyroid hormones have been demonstrated after prolonged heat exposure in rats (Horowitz *et al.*, 1986; Mirit *et al.*, 2000). Moreover, previous research shows rats with lower circulating thyroid hormones have a lower core temperature at rest and during heat stress (Yang & Gordon, 1997; Maloyan & Horowitz, 2002). Taken together, these findings suggest that thyroid hormones may be involved in thermal adaptations from medium- to long-term heat acclimation, congruent with the biphasic acclimation model proposed by Horowitz (2014). However, no study to date has investigated the influence of medium-term heat acclimation on circulating thyroid hormones in humans.

The aims of this study were firstly; to assess heat acclimation adaptations, markers of overreaching and endurance capacity after 12 days of post-exercise HWI and compare these with EHA and thermoneutral exercise (TNE) in recreationally active males. Secondly, the study aimed to investigate the influence of medium-term heat acclimation by post-exercise HWI and EHA on plasma thyroid hormone concentrations. Given larger thermal adaptations were observed after short-term post-exercise HWI than short-term EHA (**Chapter 4**), we hypothesised that extending the post-exercise HWI intervention to 12 days would augment thermal adaptations and that these would confer a more complete heat acclimation than with EHA and TNE. In addition, we expected that the high physical demands of daily exercise and heat-stress during HWI and EHA would lead to increased markers of overreaching (evidenced by low mood and physical/cognitive performance decrements) compared to TNE. We also hypothesised that medium-term heat acclimation would elicit reductions in plasma thyroid hormone concentrations and that these reductions would be associated with the thermal adaptations from medium-term heat acclimation.

6.3 **Methods**

6.3.1 Participants

Twenty-one males provided written informed consent to participate in the study (**Section 3.2**). All participants were healthy, non-smokers, free from any known cardiovascular or metabolic diseases, were not taking any medication, and had not been regularly (> once a week) exposed to the heat (including sauna and hot bath use) in the 6 weeks before commencing testing. Participants were matched for fitness characteristics in groups of three and randomly assigned to either post-exercise HWI, EHA, or TNE as previously described (**Section 4.3.1**) see Table 6.1 for participant characteristics).

Table 6.1. Participant characteristics of post-exercise hot water immersion (HWI), exercise heat acclimation (EHA), and thermoneutral exercise (TNE).

	HWI	**EHA**	**TNE**
Age (years)	22 ± 3	21 ± 2	23 ± 2
Height (cm)	176 ± 4	183 ± 5	176 ± 6
Body mass (kg)	70 ± 6	75 ± 6	76 ± 5
$\dot{V}O_{2peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	53 ± 7	54 ± 3	52 ± 2

Data are displayed as mean ± SD; n = 7, each group

6.3.2 Study design

To assess the effect of medium-term heat acclimation on hallmark adaptations and circulating thyroid hormone concentrations, participants performed four experimental trials: before (PRE), and after 3 (POST3), 6 (POST6), twelve (POST12) days of their assigned intervention (Figure 6.1). All experimental trials were conducted at the same time of day and involved resting measures followed by a 40-min submaximal run and TTE in an environmental chamber maintained at 33°C and 40% RH.

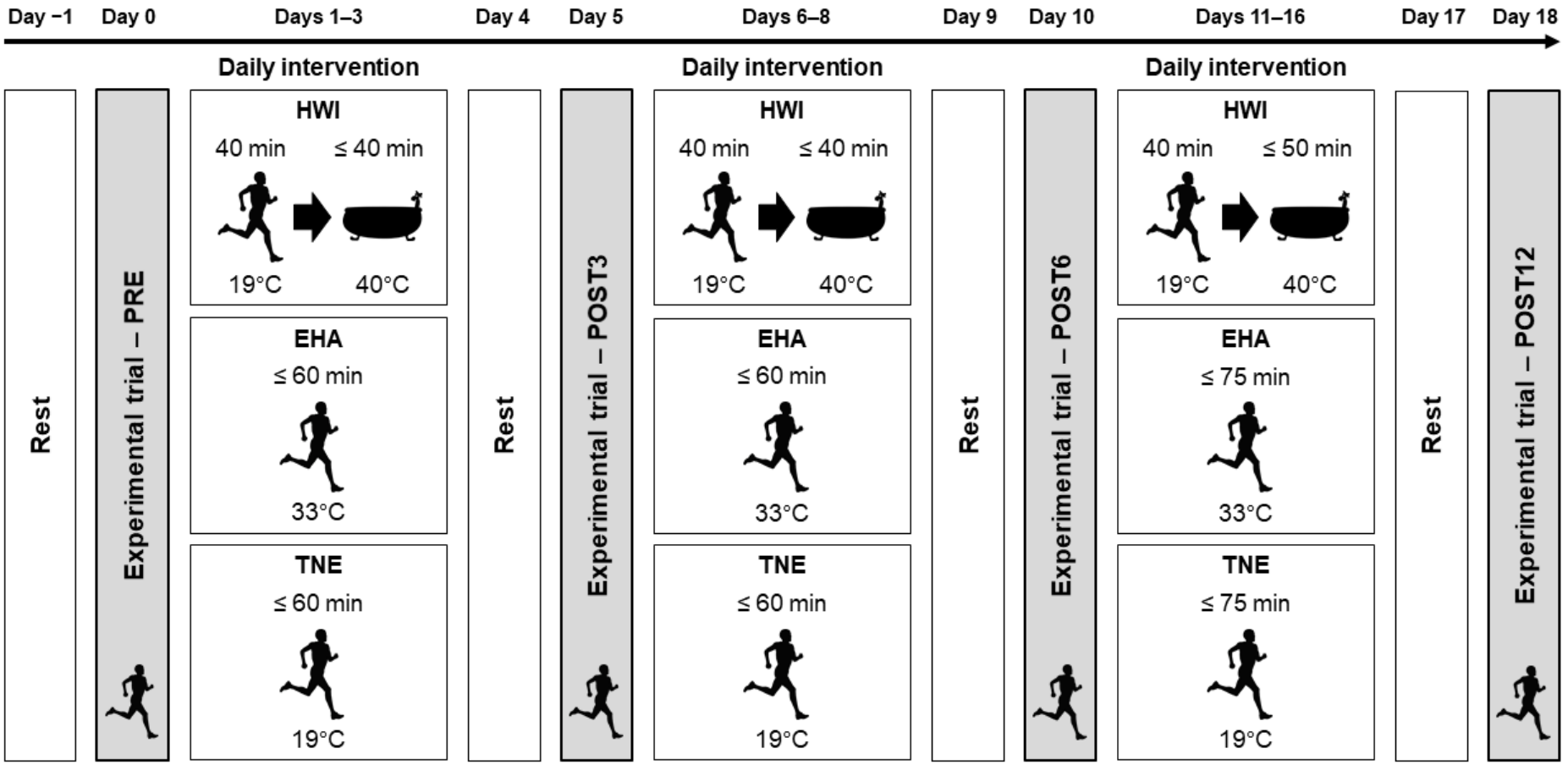

Figure 6.1. Schematic of the study design. HWI; post-exercise hot water immersion, EHA; exercise heat acclimation and TNE; work-matched thermoneutral exercise.

6.3.3 Fitness assessment

Participants completed a fitness assessment within a week before their first experimental trial. $\dot{V}O_{2peak}$ was assessed using a continuous maximal incremental exercise test performed on a motorised treadmill in a temperate laboratory (19°C), as described (**Section 3.3.1**). A running speed that elicited 65% $\dot{V}O_{2peak}$ was subsequently determined by the interpolation of the running speed–$\dot{V}O_2$ relationship (**Section 3.3.2**). The individualised running speed was used for the exercise during the experimental trials and daily intervention sessions. Participants were then familiarised with the treadmill running speed and experimental trial procedures.

6.3.4 Experimental trials

Twenty-four hours before the first experimental trial, participants were instructed to refrain from exercise, alcohol, diuretics, and caffeine and to complete a diet diary. Twenty-four hours before all subsequent experimental trials, participants were instructed to replicate this food and fluid intake. To ensure a similar circadian pattern, participants were instructed to sleep between 2200 h and 0700 h before experimental trials; sleep duration and efficiency were confirmed (Actigraph wGT3X-BT, Actigraph, Pensacola, USA).

On the day of experimental trials, participants arrived at the laboratory at 0730 h and were provided with a standardised breakfast (2201 kilojoules, 71 g carbohydrate, 18 g fat, 17 g protein) and a bolus of water (7 $mL \cdot kg^{-1}$ of nude body mass). At 0800 h, dressed in a t-shirt, shorts, socks and trainers, participants rested for 20 min in temperate laboratory conditions (19°C, 45% RH). Following the seated rest, participants completed the Profile of Mood States questionnaire (Grove & Prapavessis, 1992) to determine total mood disturbance and energy index (**Section 3.7.1**), to detect perceived training-induced fatigue. A venous blood sample was then taken without stasis for the determination of plasma volume and plasma concentrations of free T3, free T4, total T3, and total T4 as previously described (**Section 3.4.5**). A urine sample was then analysed for USG to confirm euhydration (**Section 3.4.6**; Armstrong *et al*., 2005) and a flexible, sterile, single-use rectal thermistor was self-inserted 10 cm beyond the anal sphincter to measure T_{re} (**Section 3.4.1**). A pre-exercise nude body mass was recorded using a digital platform scale (Model 703; Seca, Hamburg, Germany) and skin thermistors were attached on the right side of the body for the determination of T_{sk}, as previously described (**Section 3.4.1**; Ramanathan, 1964). Following instrumentation, participants rested for a further 30-min in temperate conditions (19°C, 45% RH) to establish baseline measures. $\dot{V}O_2$ and RER were

assessed from a 60-s expired gas sample collected by Douglas bag method at 29–30 min of seated rest and used to estimate resting metabolic heat production as described (**Section 5.3.3**; Gagge & Gonzalez, 2011).

At 0945 h, dressed in shorts, socks, and trainers, participants entered the environmental chamber (33°C, 40% RH, 0.2 $m \cdot s^{-1}$ wind velocity) to complete a 40-min treadmill run at 65% $\dot{V}O_{2peak}$. T_{re}, skin temperatures, and heart rate were monitored continuously. Local forearm sweat rate was measured by dew point hygrometry for the determination of T_{re} at sweating onset (**Section 3.4.2**; Cheuvront *et al*., 2009). RPE (Borg, 1970), thermal sensation (Hollies & Goldman, 1977), $\dot{V}O_2$, and RER (James *et al.*, 2017b) were recorded every 10 min (**Section 4.3.3**). On completion of the exercise, participants rested for 20-min in temperate conditions (19°C, 45% RH), during which they completed a modified Stroop test (**Section 3.7.2**; Dupuy *et al*., 2014), and provided a nude body mass to estimate whole-body sweat rate.

Participants then re-entered the environmental chamber and completed a time to exhaustion (TTE) on a motorised treadmill at 65% $\dot{V}O_{2peak}$ (**Section 3.6**). Following the cessation of exercise, capillary blood lactate concentrations were assessed (Lactate Pro 2™, Arkray, Australia) as a marker of short-term overreaching (Le Meur *et al.*, 2013; Schaal *et al.*, 2015). Participants were provided with a bolus of water and were free to leave the laboratory when $T_{re} \leq 38.5$°C.

6.3.5 Daily intervention

All participants completed twelve days of their assigned intervention. Twenty-one participants (HWI, $n = 7$; EHA, $n = 7$; TNE, $n = 7$) completed an additional six consecutive days of their assigned intervention after the third experimental trial. During the intervention, participants were instructed to consume their normal diet and fluid intake, including caffeine and alcohol (≤ 3 units per day). Participants arrived at the laboratory each day between 0600 h and 1300 h. Before exercise, a nude body mass was taken, and a rectal thermistor and heart rate monitor were fitted. Following instrumentation, participants completed a 15-min seated rest in a temperate laboratory (19°C, 45% RH) to establish baseline measures, before commencing their assigned intervention protocol. A bolus of water (5 $mL \cdot kg^{-1}$ of nude body mass) was consumed during the first 20 min of exercise.

Participants assigned to HWI completed a 40-min treadmill run in temperate conditions (65% $\dot{V}O_{2peak}$, 19°C, 45% RH, 0.2 $m \cdot s^{-1}$ wind velocity) dressed in shorts, socks, and trainers. Following exercise (2–3 min transition), dressed in shorts, participants began a semi-recumbent ≤ 40-min HWI (40°C) to the neck, as previously described (Zurawlew *et al.*, 2016). Participants assigned to EHA completed a ≤ 60-min treadmill run in an environmental chamber (65% $\dot{V}O_{2peak}$, 33°C, 40% RH, 0.2 $m \cdot s^{-1}$ wind velocity). To maintain the endogenous thermal stimulus for adaptation, as of the seventh intervention session, maximum immersion and exercise duration increased by 25% to ≤ 50 min and ≤ 75 min, respectively. All intervention sessions were terminated if the maximal immersion/exercise duration was reached, at the participant's volition, or if T_{re} exceeded 39.5°C. Upon removal from the hot water/environmental chamber, participants rested in a temperate laboratory, were provided with a bolus of water, and were free to leave the laboratory when $T_{re} \leq 38.5$°C. Participants assigned to TNE completed a daily submaximal treadmill run in temperate conditions (65% $\dot{V}O_{2peak}$, 19°C, 45% RH, 0.2 $m \cdot s^{-1}$ wind velocity). Mean daily TNE duration was 45 ± 7 min.

6.3.6 Blood sample collection and analysis

Venous blood samples were collected from an antecubital vein without stasis into two 6-mL EDTA vacutainers (**Section 3.4.5**). Aliquots of whole blood were used for the immediate determination of haemoglobin in duplicate (Hemocue, Sheffield, UK) and haematocrit in triplicate (capillary tube method). The change in plasma volume was estimated by correcting the initial plasma volume at PRE for the percentage change in plasma volume (%ΔPV) at POST3, POST6 and POST12, as previously described (Dill & Costill, 1974). The remaining whole blood was then centrifuged, and the plasma frozen at −80°C for later analysis. Using ELISA, plasma concentrations of free and total T3 and T4 as previously described (**Section 3.4.5**). Thyroid hormone concentrations were adjusted for plasma volume changes (**Section 3.4.5**; Sherk *et al*., 2013).

6.3.7 Statistical analysis

A sample size estimation (G*Power 3.1.9; Faul *et al*., 2007) was performed using the reduction in end-exercise core temperature change after post-exercise HWI (−0.36°C; Zurawlew *et al*., 2016), exercise heat acclimation (−0.44°C; Mee *et al*., 2015 and −0.49°C; Gibson *et al*., 2015), and thermoneutral exercise (0.00°C; Zurawlew *et al*., 2016) with a SD of 0.21°C (control group; Zurawlew *et al*., 2016). A mixed-model analysis of covariance (ANCOVA; alpha =

0.05, power = 0.80) estimated that a total sample size of 21 participants was required to detect a significant difference in the change in end-exercise T_{re} between heat acclimation and thermoneutral exercise interventions. All data were checked for normality and sphericity, presented as mean and SD, and statistical significance was accepted at $P < 0.050$. Uncertainty in the true (population) values of effects is presented as 95% CI. The mean daily endogenous thermal stimulus and external work during HWI, EHA, and TNE were compared using a two-way methods ANOVA. A two-way mixed-methods ANCOVA with baseline as the covariate was used to detect differences in heat acclimation adaptations, endurance capacity, markers of overreaching, and plasma thyroid hormone concentrations between groups (HWI vs EHA vs TNE) and across time (POST3 vs POST6 vs POST12). Bonferroni-adjusted pairwise comparisons were used where appropriate to determine where differences occurred. Pearson's correlations determined the strength of the relationship between changes in plasma thyroid hormone concentrations and resting T_{re}, and the total AUC for $T_{re} > 38.5$°C after twelve days of heat acclimation by HWI and EHA. Pearson correlation coefficients of 0.00–0.19 were regarded as very weak, 0.20–0.39 as weak, 0.40–0.59 as moderate, and 0.60–0.79 as strong relationships. Data were analysed using SPSS version 27 (IBM Corporation, NY, USA) or GraphPad Prism Version 9.1 (GraphPad Software Inc. La Jolla, USA).

6.4 **Results**

6.4.1 Participant flow, drop out and exclusion

Participant flow, drop out, and exclusion prior to biochemical and statistical analysis is summarised in Figure 6.2.

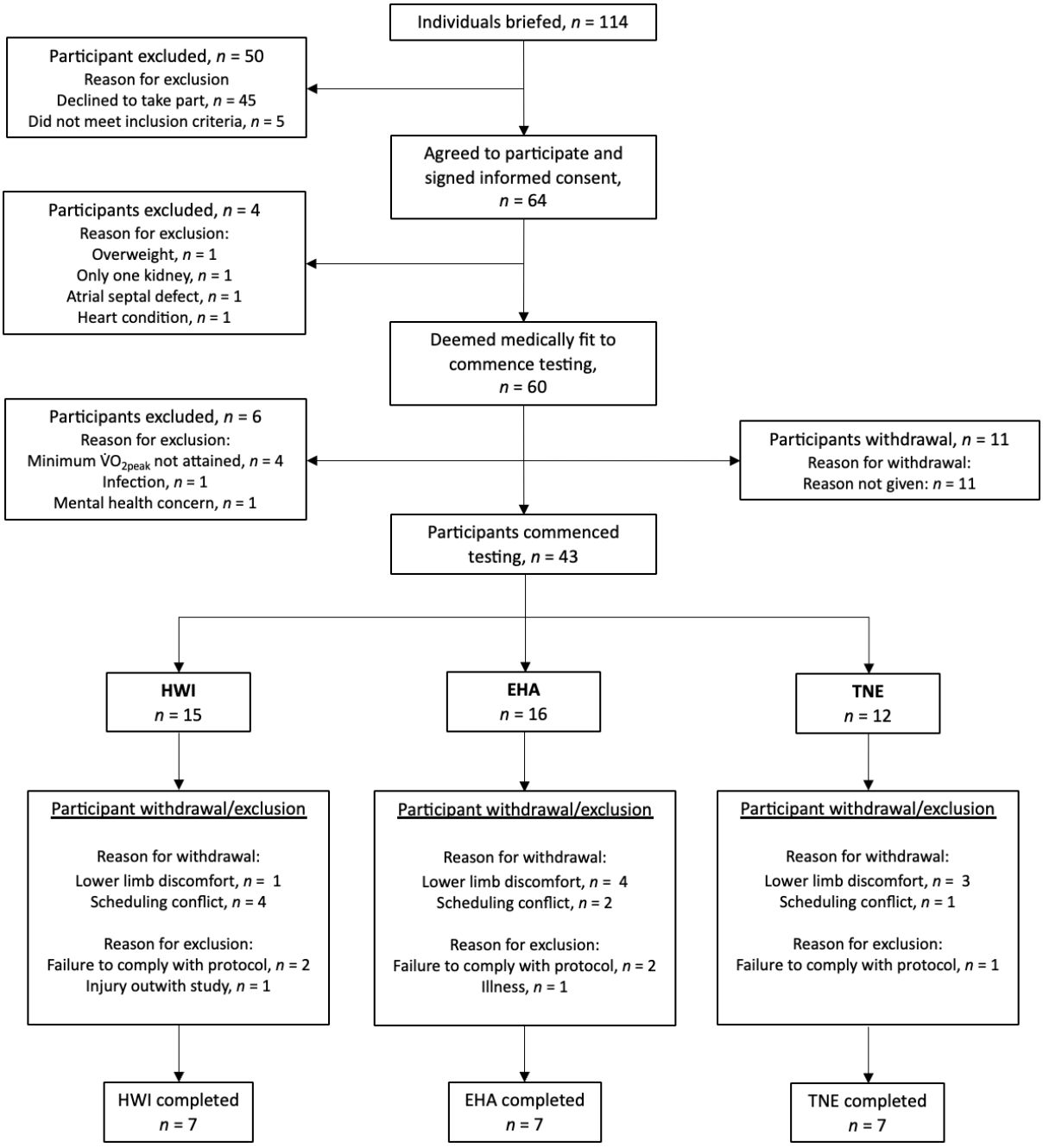

Figure 6.2. Flow diagram indicating the numbers of participants recruited, deemed medically fit, and completed. HWI; post-exercise hot water immersion, EHA; exercise heat acclimation and TNE; work-matched thermoneutral exercise.

6.4.2 Daily intervention

All participants completed twelve days of their assigned intervention. The daily endogenous thermal stimulus was different between groups, evidenced by a main effect of group for duration T_{re} > 38.5°C (f = 29.971, P < 0.001), AUC of T_{re} > 38.5°C (f = 29.843, P < 0.001), and end-intervention T_{re} (f = 44.086, P < 0.001). *Post hoc* pairwise comparisons revealed that the daily endogenous thermal stimulus was similar between HWI and EHA (all $P \geq 0.407$), but lower in TNE (all $P < 0.001$; Table 6.2). The daily endogenous thermal stimulus was maintained throughout the 12-day heat acclimation interventions, evidenced by no main effects of time or interaction effects (all $P \geq 0.252$). The maintenance of the daily endogenous thermal stimulus was likely owing to an increase in mean daily HWI and EHA duration, evidenced by an interaction effect (f = 3.185, P = 0.024), with follow-up analysis detecting differences between days 1–3 (33 ± 4 min) and days 7–12 (39 ± 5 min; P = 0.043) of HWI, and between days 1–3 (51 ± 9 min) and days 7–12 (61 ± 11 min; P = 0.001), and days 4–6 (55 ± 8 min) and days 7–12 (P = 0.026) of EHA. Between-group differences were also observed for mean daily external work (main effect of group, f = 5.744, P = 0.012). *Post hoc* pairwise comparisons revealed that mean daily external work was significantly lower in HWI than EHA (P = 0.006) and tended to be lower than TNE (P = 0.053; Table 6.2).

Table 6.2. The daily endogenous thermal stimulus and external work during thermoneutral exercise (TNE), exercise heat acclimation (EHA), and post-exercise hot water immersion (HWI) interventions.

	Days 1–3			Days 6–8			Days 11–16		
	TNE	**EHA**	**HWI**	**TNE**	**EHA**	**HWI**	**TNE**	**EHA**	**HWI**
Duration T_{re} ≥38.5°C (min)	7 ± 12	35 ± 14 $^{\#\#}$	36 ± 5 $^{\#\#}$	8 ± 12	38 ± 11 $^{\#\#}$	38 ± 6 $^{\#\#}$	7 ± 10	38 ± 13 $^{\#\#}$	39 ± 8 $^{\#\#}$
AUC (°C·min^{-1})	1 ± 3	17 ± 10 $^{\#\#}$	17 ± 5 $^{\#\#}$	2 ± 4	16 ± 8 $^{\#\#}$	18 ± 4 $^{\#\#}$	1 ± 1	12 ± 6 $^{\#\#}$	20 ± 6 $^{\#\#}$
End intervention T_{re} (°C)	38.2 ± 0.3	39.2 ± 0.3 $^{\#\#}$	39.2 ± 0.2 $^{\#\#}$	38.2 ± 0.5	39.1 ± 0.2 $^{\#\#}$	39.3 ± 0.1 $^{\#\#}$	38.2 ± 0.2	39.0 ± 0.3 $^{\#\#}$	39.3 ± 0.2 $^{\#\#}$
External work (km)	7.4 ± 1.1	7.7 ± 1.6	6.1 ± 1.1 †	7.6 ± 1.7	8.1 ± 1.7	6.1 ± 1.1 †	8.7 ± 1.7	9.0 ± 1.5	6.1 ± 1.1 †

T_{re}; rectal core temperature, AUC; area under the curve for T_{re} >38.5°C. Data are displayed as mean ± SD of days 1–3, days 6–8 and days 11–16. $^{\#\#}P < 0.01$ denotes group different from TNE; $^{\dagger}P < 0.05$ denotes group different from EHA.

6.4.3 Hallmark heat acclimation adaptations

No differences were detected between groups for sleep duration (6 ± 1 h), sleep efficiency (86 ± 9%) or urine specific gravity (1.020 ± 0.007) before experimental trials, evidenced by no main effects of time or group, and no interaction effects (all $P \geq 0.184$). Compared to exercise in thermoneutral conditions, post-exercise HWI elicited larger reductions in resting T_{re} and end-exercise T_{re} following exercise-heat-stress, whereas EHA provided no further benefits to TNE. Main effects of group were detected for resting T_{re} ($f = 6.832$, $P = 0.007$) and end-exercise T_{re} ($f = 3.836$, $P = 0.042$); there were no main effects of time or interaction effects (all $P \geq 0.645$). *Post hoc* pairwise comparisons revealed that resting T_{re} was lower after HWI (baseline-adjusted: −0.41 ± 0.15°C, CI: −0.29 to −0.53°C) compared to TNE (−0.12 ± 0.15°C, CI: 0.00 to −0.24°C; $P = 0.007$), but not EHA (−0.20 ± 0.15°C, CI: −0.08 to −0.32°C; $P = 0.061$; Figure 6.2A). No differences were observed between EHA and TNE for resting T_{re} ($P = 0.936$). The reduction in resting T_{re} was related to the reduction in resting metabolic heat production after 6 ($r = 0.47$, $P = 0.045$) and 12 days ($r = 0.66$, $P = 0.005$) but not 3 days ($r = 0.42$, $P = 0.072$) of heat acclimation; however, there were no main effects of time or group, and no interaction effects detected for resting metabolic heat production (all $P \geq 0.515$; Table 6.3). End-exercise T_{re} was lower after HWI (−0.50 ± 0.13°C, CI: −0.40 to −0.61°C) compared to TNE (−0.33 ± 0.13°C, CI: −0.22 to −0.43°C; $P = 0.049$), but not EHA (−0.37 ± 0.13°C, CI: −0.26 to −0.47°C; $P = 0.196$; Figure 6.2B). No differences were observed between EHA and TNE for end-exercise T_{re} ($P = 1.000$).

Differences between interventions were also detected for T_{re} at sweating onset ($f = 9.604$, $P = 0.002$), whole-body sweat rate ($f = 9.003$, $P = 0.002$), RPE ($f = 4.035$, $P = 0.037$), and TS ($f = 4.171$, $P = 0.034$). T_{re} at sweating onset was lower after HWI (−0.43 ± 0.12°C) compared to EHA (−0.22 ± 0.12°C; $P = 0.015$) and TNE (−0.16 ± 0.12°C; $P = 0.002$). Conversely, exercising in the heat did not augment the reduction in T_{re} at sweating onset compared to exercise in temperate conditions ($P = 1.000$; Figure 6.2C). Whole-body sweat rate was greater after HWI (+0.08 $L \cdot h^{-1}$; $P = 0.003$) and EHA (+0.06 $L \cdot h^{-1}$; $P = 0.013$) than TNE (−0.06 $L \cdot h^{-1}$), but no difference was detected between HWI and EHA ($P = 1.000$; Figure 6.2D). In accordance with thermal adaptations, perceptual responses to exercise-heat-stress were lower after HWI (RPE, −1.9 ± 1.2; TS, −1.1 ± 1.0) compared to TNE (RPE, 0.0 ± 1.2, $P = 0.036$; TS, +0.3 ± 0.9, $P = 0.047$) but not EHA (RPE, −1.3 ± 1.1, $P = 0.951$; TS, −0.6 ± 0.9, $P = 1.000$); no differences were observed between EHA and TNE ($P \geq 0.157$; Figures 6.2E and 6.2F). There

were no main effects of time or group, and no interaction effects detected for plasma volume, the change in T_{re} during the 40-min submaximal treadmill run in the heat, end-exercise T_{sk}, end-exercise heart rate, exercising $\dot{V}O_2$, or exercising RER (all $P \geq 0.105$; Table 6.3).

6.4.4 Endurance capacity and markers of overreaching

Five participants were removed from the TTE analysis owing to: reaching the T_{re} ethical cut-off (HWI, $n = 2$); going to the toilet (EHA, $n = 1$); exercise-induced bronchoconstriction (TNE, $n = 1$); and an obvious lack of effort without markers of overreaching at rest (TNE, $n = 1$). Analysis of the remaining 16 participants (HWI, $n = 5$; EHA, $n = 6$; TNE, $n = 5$) who completed the TTE revealed no statistical differences between interventions or across time ($P \geq 0.219$; Table 6.3). Furthermore, there was no evidence to suggest that heat acclimation induced overreaching to a greater extent that exercise in temperate conditions, with no main effects of time or group, and no interaction effects detected for total mood disturbance, energy index, Stroop reaction time, Stroop accuracy, end-TTE heart rate, end-TTE blood lactate, or sleep efficiency (all $P \geq 0.198$; Table 6.4).

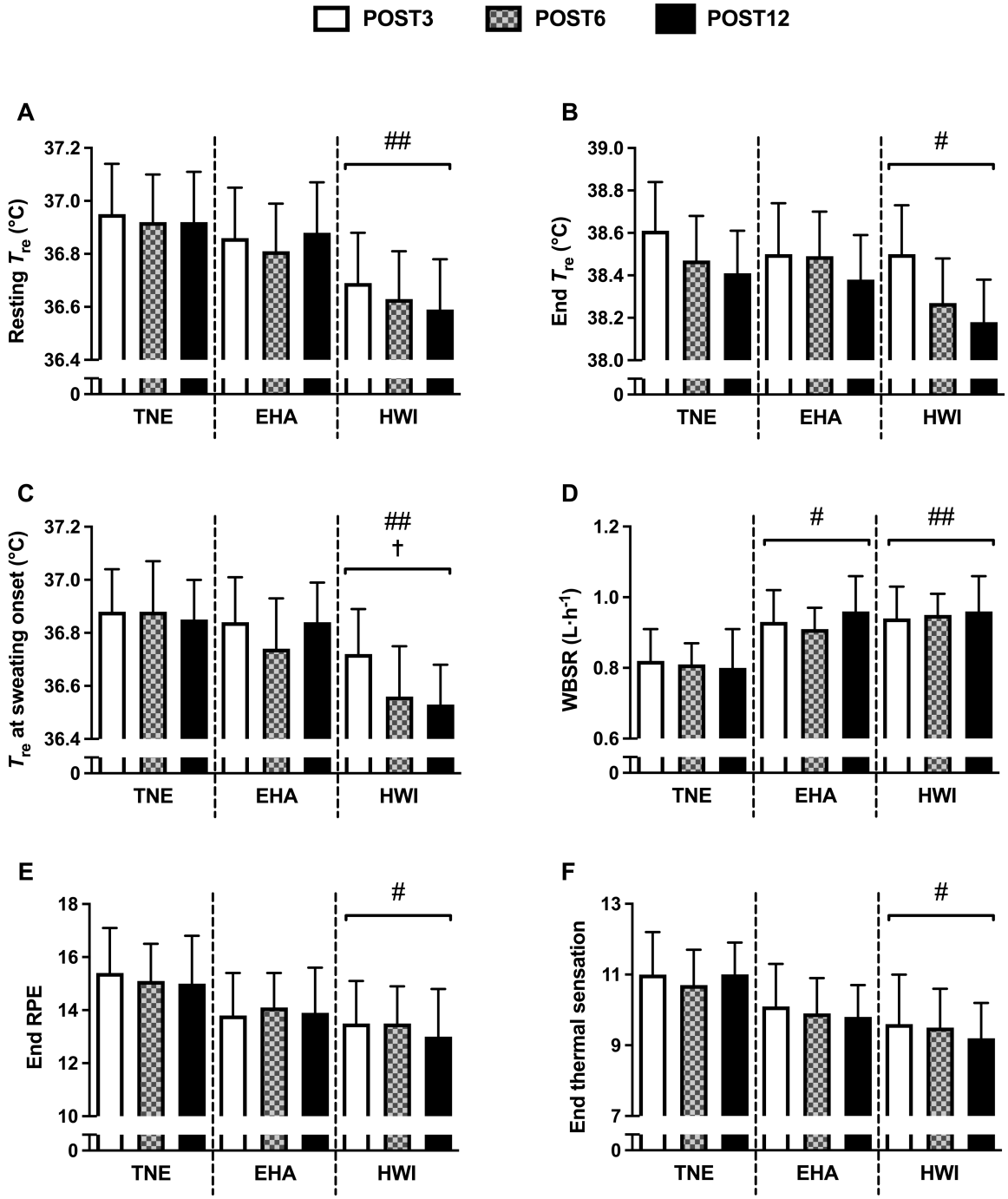

Figure 6.3. Influence of 3 (POST3), 6 (POST6) and 12 days (POST12) of thermoneutral exercise (TNE), exercise heat acclimation (EHA), or post-exercise hot water immersion (HWI) on resting rectal core temperature (T_{re}, A), end-exercise T_{re} (B), T_{re} at sweating onset (C), whole-body sweat rate (D), end-exercise rating of perceived exertion (RPE, E), and end-exercise thermal sensation (F). Bars show baseline-adjusted mean ± SD. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ denotes group lower than TNE; $^{\dagger}P < 0.05$ denotes HWI lower than EHA.

Table 6.3. Influence of 3 (POST3), 6 (POST6), and 12 days (POST12) of thermoneutral exercise (TNE), exercise heat acclimation (EHA), or post-exercise hot water immersion (HWI) on heat acclimation adaptations and endurance capacity in the heat (33°C, 40% RH).

	TNE			EHA			HWI		
	POST3	POST6	POST12	POST3	POST6	POST12	POST3	POST6	POST12
Rest									
Resting T_{re} (°C)	−0.10 ± 0.19	−0.13 ± 0.18	−0.13 ± 0.19	−0.19 ± 0.19	−0.24 ± 0.18	−0.17 ± 0.19	−0.35 ± 0.19 $^{\#\#}$	−0.41 ± 0.18 $^{\#\#}$	−0.46 ± 0.19 $^{\#\#}$
Resting metabolic heat production (W)	7 ± 20	−9 ± 15	4 ± 17	2 ± 20	4 ± 15	9 ± 17	3 ± 21	0 ± 15	3 ± 17
Plasma volume (%)	0 ± 4	2 ± 3	0 ± 4	1 ± 4	3 ± 3	3 ± 4	2 ± 4	2 ± 3	1 ± 4
Submaximal exercise									
End-exercise T_{re} (°C)	−0.21 ± 0.23	−0.36 ± 0.21	−0.41 ± 0.20	−0.32 ± 0.24	−0.33 ± 0.21	−0.44 ± 0.21	−0.32 ± 0.23 $^{\#}$	−0.56 ± 0.21 $^{\#}$	−0.64 ± 0.20 $^{\#}$
Δ T_{re} during exercise (°C)	−0.10 ± 0.26	−0.22 ± 0.29	−0.28 ± 0.29	−0.16 ± 0.28	−0.09 ± 0.30	−0.29 ± 0.30	−0.06 ± 0.27	−0.15 ± 0.30	−0.18 ± 0.30
T_{re} at sweating onset (°C)	−0.15 ± 0.16	−0.15 ± 0.19	−0.18 ± 0.15	−0.19 ± 0.17	−0.29 ± 0.19	−0.19 ± 0.15	−0.30 ± 0.17 $^{\#\#,\dagger}$	−0.47 ± 0.19 $^{\#\#,\dagger}$	−0.50 ± 0.15 $^{\#\#,\dagger}$
Whole-body sweat rate ($L \cdot h^{-1}$)	−0.05 ± 0.09	−0.05 ± 0.06	0.07 ± 0.11	0.06 ± 0.09 #	0.04 ± 0.06 $^{\#}$	0.09 ± 0.10 $^{\#}$	0.08 ± 0.09 $^{\#\#}$	0.08 ± 0.06 $^{\#\#}$	0.10 ± 0.10 $^{\#\#}$
End-exercise T_{sk} (°C)	−0.38 ± 0.49	−0.38 ± 0.46	−0.73 ± 0.54	−0.39 ± 0.50	−0.66 ± 0.47	−0.95 ± 0.55	−0.50 ± 0.52	−0.60 ± 0.48	−1.15 ± 0.57
End-exercise heart rate ($beats \cdot min^{-1}$)	−8 ± 5	−12 ± 7	−14 ± 8	−12 ± 5	−15 ± 7	−20 ± 8	−11 ± 5	−17 ± 7	−20 ± 8
Mean $\dot{V}O_2$ ($L \cdot min^{-1}$)	−0.10 ± 0.13	−0.10 ± 0.15	−0.16 ± 0.13	−0.01 ± 0.13	0.00 ± 0.15	−0.06 ± 0.13	−0.04 ± 0.13	−0.04 ± 0.14	−0.06 ± 0.12
Mean RER	−0.01 ± 0.04	−0.02 ± 0.03	−0.03 ± 0.04	−0.02 ± 0.04	−0.02 ± 0.03	−0.01 ± 0.04	−0.02 ± 0.04	−0.02 ± 0.03	−0.02 ± 0.04
End-exercise RPE (6–20 scale)	0.2 ± 1.7	−0.1 ± 1.4	−0.3 ± 1.8	−1.4 ± 1.6	−1.2 ± 1.3	−1.3 ± 1.7	−1.8 ± 1.6 $^{\#}$	−1.8 ± 1.4 $^{\#}$	−2.2 ± 1.8 $^{\#}$
End-exercise thermal sensation (1–13 scale)	0.4 ± 1.2	0.1 ± 1.0	0.5 ± 0.9	−0.4 ± 1.2	−0.6 ± 1.0	−0.7 ± 0.9	−0.9 ± 1.4 $^{\#}$	−1.0 ± 1.1 $^{\#}$	−1.3 ± 1.0 $^{\#}$
Endurance capacity									
TTE (s)	−27 ± 676	75 ± 808	212 ± 991	101 ± 627	539 ± 749	323 ± 919	321 ± 743	686 ± 888	1030 ± 1089

T_{re}, rectal core temperature; T_{sk}, mean skin temperature; RER, respiratory exchange ratio; RPE, rating of perceived exertion. Data are baseline-adjusted mean change ± SD change at POST3, POST6, and POST12. $^{\#\#}P < 0.01$, $^{\#}P < 0.05$ denotes group different from TNE; $^{\dagger}P < 0.05$ denotes group different from EHA.

Table 6.4. Influence of 3 (POST3), 6 (POST6), and 12 days (POST12) of thermoneutral exercise (TNE), exercise heat acclimation (EHA), or post-exercise hot water immersion (HWI) on markers of overreaching.

	TNE			EHA			HWI		
	POST3	POST6	POST12	POST3	POST6	POST12	POST3	POST6	POST12
Total mood disturbance	5 ± 10	2 ± 12	2 ± 10	5 ± 10	7 ± 12	2 ± 10	4 ± 10	4 ± 12	2 ± 10
Energy index	−3 ± 4	−2 ± 6	−3 ± 5	−3 ± 4	−5 ± 6	−3 ± 5	−2 ± 4	−4 ± 6	−3 ± 5
Stroop reaction time (ms)	−29 ± 58	−25 ± 40	−11 ± 64	−13 ± 58	−32 ± 40	−15 ± 63	−16 ± 62	−18 ± 43	−28 ± 68
Stroop accuracy (%)	0 ± 2	−1 ± 3	1 ± 4	−1 ± 3	−1 ± 3	−2 ± 4	2 ± 3	1 ± 3	0 ± 4
End-TTE heart rate (beats·min^{-1})	−8 ± 8	−10 ± 7	−16 ± 10	−10 ± 8	−12 ± 7	−20 ± 10	−8 ± 8	−10 ± 7	−14 ± 10
End-TTE blood lactate (beats·min^{-1})	0.2 ± 1.4	−0.1 ± 0.7	0.2 ± 0.6	0.5 ± 1.3	−0.2 ± 0.7	−0.9 ± 0.6	−0.2 ± 1.3	−0.1 ± 0.7	0.2 ± 0.6
Sleep efficiency (%)	0 ± 9	−2 ± 7	−1 ± 8	−6 ± 9	−5 ± 7	1 ± 8	−2 ± 9	2 ± 7	−2 ± 8

TTE, time to exhaustion. Data are baseline-adjusted mean change ± SD change at POST3, POST6, and POST12.

6.4.5 Thyroid hormones

A larger endogenous thermal stimulus from 12 days of heat acclimation (total AUC for T_{re} > 38.5°C) was associated with a larger reduction in plasma free T3 ($r = -0.57$, $P = 0.017$; Figure 6.4A). No significant relationships were observed between the endogenous thermal stimulus and changes in free T4 ($r = -0.15$, $P = 0.307$; Figure 6.4B), total T3 ($r = -0.33$, $P = 0.123$; Figure 6.4C), or total T4 ($r = -0.18$, $P = 0.264$; Figure 6.4D).

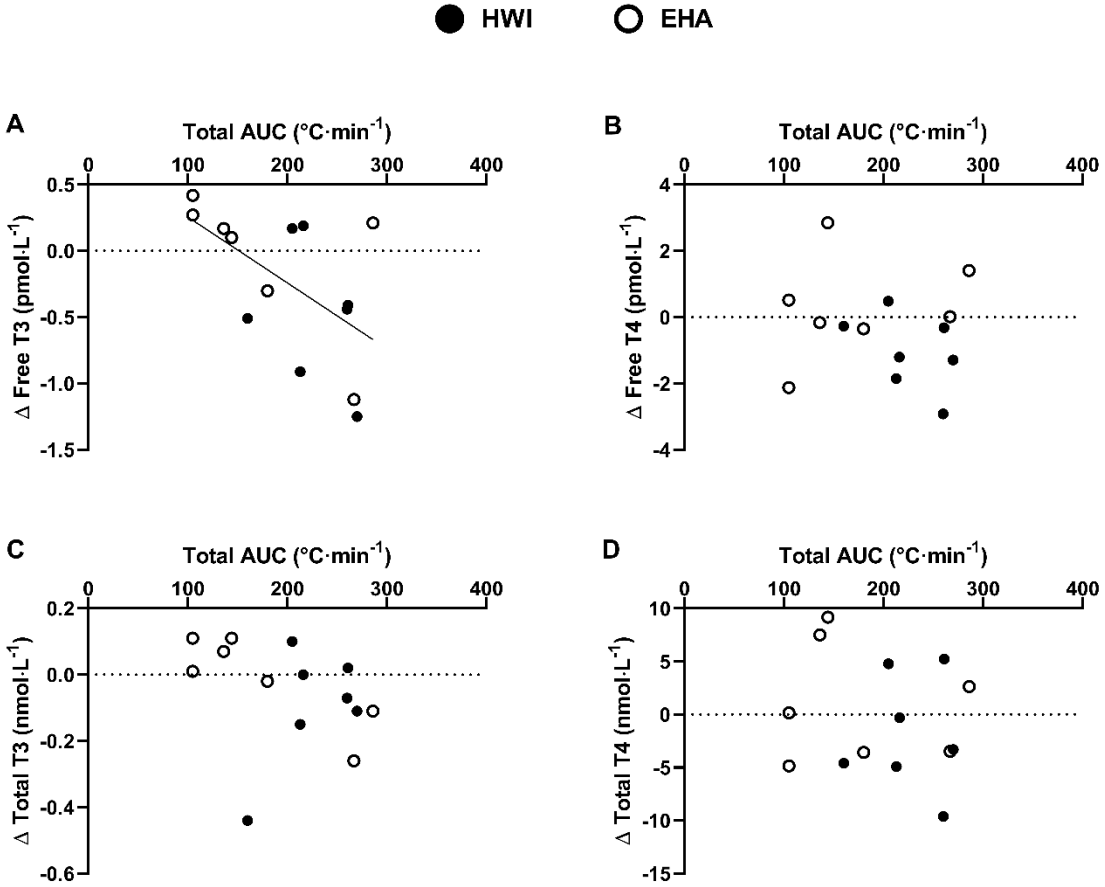

Figure 6.4. Relationship between total area under the curve (AUC) for T_{re} > 38.5°C and the change in plasma concentrations of free triiodothyronine (T3; A), free thyroxine (T4; B), total T3 (C), and total T4 (D) after 12 days of post-exercise hot water immersion (HWI) or exercise heat acclimation (EHA).

A moderate relationship was observed between the change in resting T_{re} and the change in the plasma concentration of free T3 after 12 days of heat acclimation by HWI or EHA (r = 0.47, P = 0.044; Figure 6.5A). In contrast, the change in resting T_{re} was not associated with changes in plasma concentrations of free T4 (r = 0.34, P = 0.120, Figure 6.5B), total T3 (r = 0.19, P = 0.253, Figure 6.5C), or total T4 (r = 0.15, P = 0.310, Figure 6.5D). No significant relationships were observed between changes in plasma thyroid hormone concentrations and changes in resting metabolic heat production (all $r \leq 0.30$, $P \geq 0.150$), end-exercise T_{re} (all $r \leq 0.34$, $P \geq 0.115$), T_{re} at sweating onset (all $r \leq 0.45$, $P \geq 0.054$), end-exercise T_{sk} (all $r \leq 0.18$, $P \geq 0.269$).

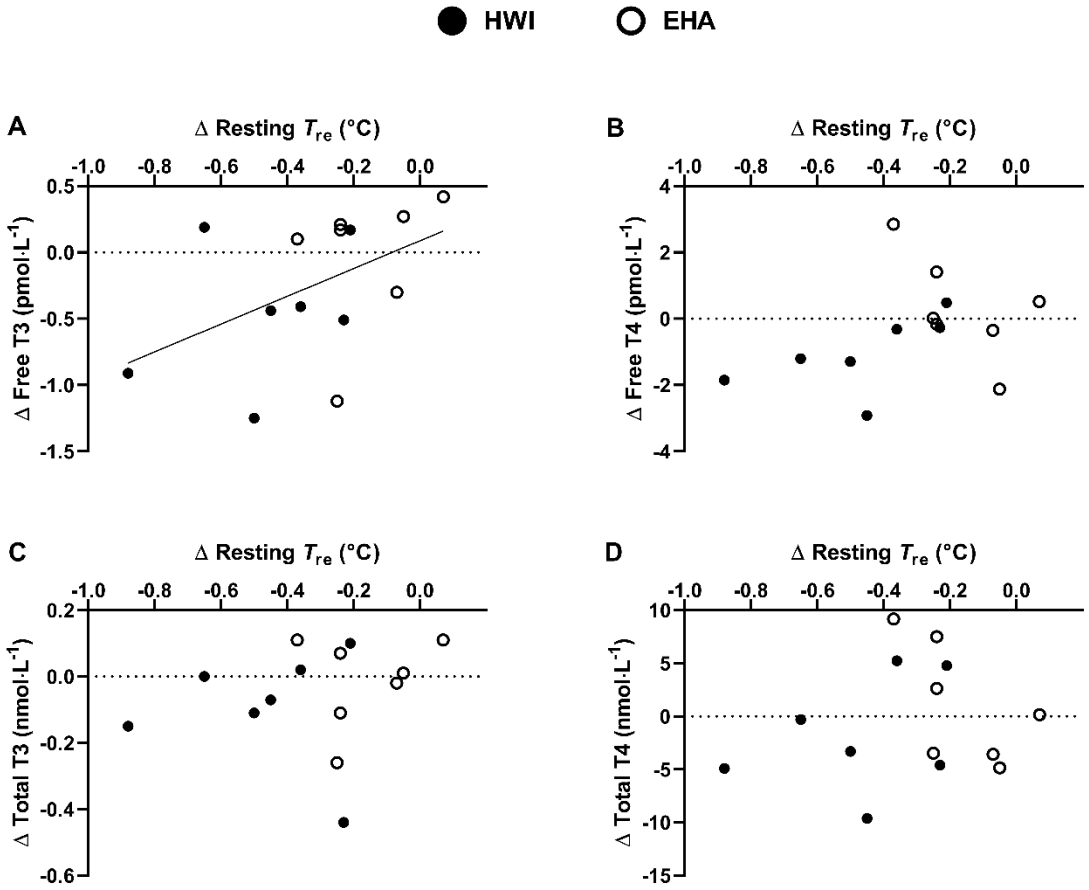

Figure 6.5. Relationship between the change in resting T_{re} and the change in plasma concentrations of free triiodothyronine (T3; A), free thyroxine (T4; B), total T3 (C), and total T4 (D) after 12 days of post-exercise hot water immersion (HWI) or exercise heat acclimation (EHA).

ANCOVA, with baseline as the covariate, detected an interaction effect for plasma concentrations of free T3 ($f = 4.299$, $P = 0.006$). Follow-up analyses revealed that free T3 was lower after 12 days of HWI (−23%) compared to EHA (+4%, $P = 0.008$) and TNE (+1%, $P = 0.015$; Figure 6.6A). No differences were detected between EHA and TNE ($P = 1.000$). Additionally, there was a trend towards a main effect of group for plasma concentrations of total T3 ($f = 3.406$, $P = 0.057$), with *post hoc* pairwise comparisons revealing a trend towards lower total T3 after HWI (−12%) than EHA (+3%; $P = 0.059$; Figure 6.6C). No differences in total T3 were observed between HWI and TNE (−12%, $P = 0.980$), or EHA and TNE ($P = 0.365$). There were no main effects of time or group, and no interaction effects detected (all $P \geq 0.148$) for free T4 (Figure 6.6B) or total T4 (Figure 6.6D).

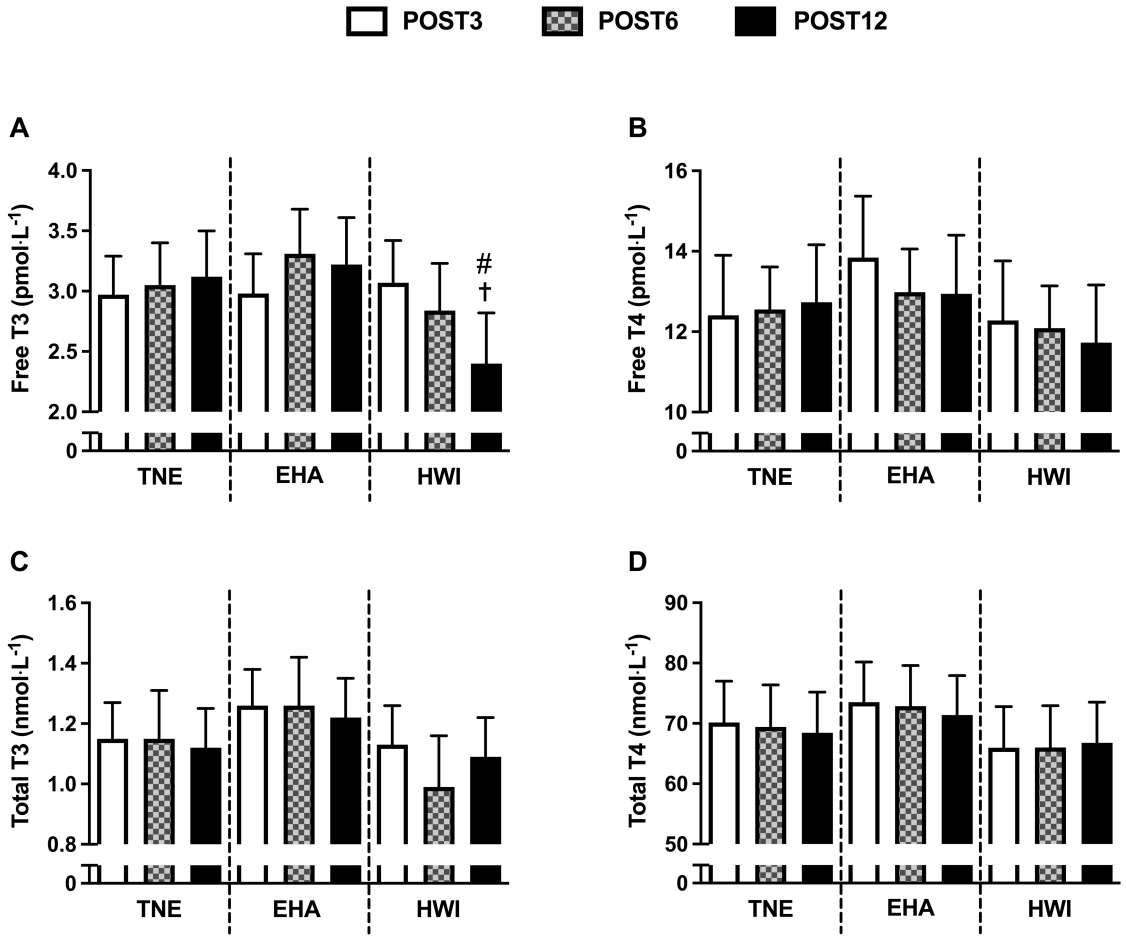

Figure 6.6. Influence of 3 (POST3), 6 (POST6) and 12 days (POST12) of thermoneutral exercise (TNE), exercise heat acclimation (EHA), or post-exercise hot water immersion (HWI) on plasma concentrations of free triiodothyronine (T3; A), free thyroxine (T4; B), total T3 (C), and total T4 (D). Bars show baseline-adjusted mean ± SD. $^{\#}P < 0.05$ denotes HWI lower than TNE; $^{\dagger}P < 0.05$ denotes HWI lower than EHA.

6.5 **Discussion**

The current study is the first to compare thermal adaptations, endurance capacity and markers of overreaching after 12 days of heat acclimation by post-exercise HWI and EHA with a work-matched thermoneutral exercise intervention, and to examine the influence of plasma thyroid hormone concentrations on the thermal adaptations. In line with our hypothesis, compared to exercise in temperate conditions, post-exercise HWI elicited larger and a greater number of thermal adaptations and reductions in perceived strain during exercise-heat-stress, whereas EHA provided only modest further benefits to TNE. Contrary to our hypothesis, despite large reductions in resting and end-exercise T_{re} after 3, 6 and 12 days of post-exercise HWI, a reduction in plasma thyroid hormone concentrations (free T3) was only observed after 12 days. Further, total T3, free and total T4 were unaltered by medium-term heat acclimation and unrelated to thermal adaptations (change in resting T_{re}, resting metabolic heat production, end-exercise T_{re}, T_{re} at sweating onset and end-exercise T_{sk}). The temporal and intervention disconnects between changes in thyroid hormones and thermal adaptations indicate that thyroid hormone changes are unlikely the cause of medium-term heat acclimation adaptations.

Previous research from our laboratory has demonstrated that short-term post-exercise HWI provides heat acclimation adaptations in recreationally active (Zurawlew *et al*., 2016; **Chapter 4**) and endurance trained individuals (Zurawlew *et al.*, 2018b). The current study furthers this work by examining for the first time heat acclimation adaptations and endurance capacity during exercise-heat-stress after 12 days of post-exercise HWI. Despite a doubling of the endogenous thermal stimulus, extending the 6-day intervention to 12 days provided no further thermal benefits (e.g., end-exercise T_{re}: POST6, −0.56°C vs POST12, −0.64°C) or improvements in endurance capacity. Our findings align with previous research demonstrating that, compared to short-term heat acclimation, medium-term interventions provide no further thermal adaptations in males, even when a progressive heat acclimation method is employed (Gibson *et al.*, 2015b).

Compared to TNE, medium-term post-exercise HWI lead to a greater number of heat acclimation adaptations (i.e. reduced resting T_{re}, end-exercise T_{re}, T_{re} at sweating onset, RPE, thermal sensation, and increased whole-body sweat rate) than EHA (i.e. increased whole-body sweat rate). Conversely, the change in T_{re} during exercise in the heat was similar between groups; hence, the larger reduction in thermal strain after post-exercise HWI can be attributed to the larger reduction in resting T_{re}. The inclusion of a work-matched thermoneutral exercise

intervention allowed the independent effectiveness of the exercise and heat stress stimuli to be determined. We found that, aside from an increase in whole-body sweat rate, which was greater after EHA, no additional heat acclimation adaptations existed between EHA than TNE. Our data align with investigations by Shvartz *et al.* (1977), Kampmann *et al.* (2008) and, more recently, Ravanelli *et al.* (2020), who demonstrated that aerobic training in temperate conditions confers heat acclimation adaptations in recreationally active individuals. In turn, the benefits of conventional exercise-based heat acclimation beyond work-matched exercise in temperate conditions appear modest and may increase the risk of dehydration, a risk factor of exertional heat illness (Knapik & Epstein, 2019). Moreover, external work was ~35% lower during the 12-day post-exercise HWI intervention than during EHA (Table 6.2). Although no differences were observed between interventions for markers of overreaching, fewer post-exercise HWI participants withdrew from the study due to lower limb discomfort (1 of 15; 7%) than EHA (4 of 16; 25%) and TNE participants (3 of 12; 25%; Figure 6.2).

The current study found that the endogenous thermal stimulus of medium-term heat acclimation was partly related to a reduction in plasma thyroid hormone concentrations (free T3; Figure 6.4A). This finding aligns with previous research in our laboratory (**Chapter 5**) and other published work in rats (Horowitz *et al.*, 1986; Mirit *et al.*, 2000) and humans (Gertner *et al.*, 1983) that demonstrated a reduction in thyroid hormones after prolonged heat exposure. We also observed a moderate relationship between the reduction in plasma free T3 and the reduction in resting T_{re} after 12 days of heat acclimation by HWI and EHA (Figure 6.5A), which is in accordance with previous literature demonstrating a lower core temperature in hypothyroid compared to control rats (Yang & Gordon, 1997; Maloyan & Horowitz, 2002). While these findings cannot infer causation, they do support the hypothesis that changes in circulating thyroid hormones concentrations are associated with medium-term thermal adaptation. However, the current study design allowed the temporal patterning to be determined and shows that, despite there being a large reduction in resting T_{re} after 3 (−0.35°C) and 6 days (−0.41°C) of post-exercise HWI, no changes in plasma thyroid hormone concentrations were detected until after 12 days. Further, similar thermal adaptations occurred after both post-exercise HWI and EHA, however, no changes in thyroid hormones were observed after medium-term EHA. Moreover, no association was observed between thyroid hormones and heat acclimation adaptations, including, metabolic heat production, end-exercise T_{re}, T_{re} at sweating onset, and end-exercise T_{sk}. Taken together, these findings indicate that

changes in circulating thyroid hormones cannot be the mechanism for the observed heat acclimation adaptations.

6.5.1 Limitations

The most notable limitation of the current study, as with the majority of heat acclimation research, is that we tested only males. While we demonstrated no further reduction in thermal strain or improvement in endurance capacity by extending the 6-day heat acclimation interventions to 12 days, Mee *et al.* (2015) found that 10 days of heat acclimation was required to establish thermal and cardiovascular adaptations in females; suggesting that there are sex differences in the temporal patterning of heat acclimation. This slower rate of adaptation in females was, however, negated when sauna bathing was incorporated into the heat acclimation protocol (Mee *et al.*, 2018). The combination of sauna bathing and exercise in the heat likely accelerated the rate of adaptation by exposing participants to a larger dual thermal stimulus, which is purported to induce a more complete state of heat acclimation (Regan *et al*., 1996; **Chapter 4**). Therefore, while females are likely to benefit more from extended conventional exercise-based heat acclimation interventions, the large dual thermal stimulus from short-term post-exercise HWI heat acclimation should be sufficient to elicit thermal adaptations. However, future research is required to confirm this hypothesis. Findings regarding the direct comparison of heat acclimation adaptations between post-exercise HWI and EHA should also be considered with caution as a *post hoc* power analysis (G*Power) revealed that this analysis was statistically underpowered to detect small to medium effects. Consequently, whilst there may have been statistical differences between these interventions other than T_{re} at sweating onset, a larger sample size would have been required to detect them. Another limitation of this study is that we did not assess cellular markers of thermotolerance and so may have missed cellular adaptations caused from extending the duration of post-exercise HWI heat acclimation from 6 to 12 days. Previous research has found that short- and medium-term exercise-based heat acclimation increases Hsp-72 gene expression, a marker of cytoprotective adaptation (Magalhaes *et al.*, 2010a; Gibson *et al.*, 2015a; Gibson *et al.*, 2016). In addition, prolonged heat acclimation interventions may reduce markers of intestinal permeability, such as, LPS and intestinal fatty acid-binding proteins (I-FABP), and inflammatory markers, including, TNF-α, IL-6 and IL-1β (Barberio *et al.*, 2015). These markers are associated with pathological conditions including exertional heat stroke (Bouchama *et al.*, 1991; Leon & Helwig, 2010).

However, such markers of cellular thermotolerance have not been assessed after short- or medium-term post-exercise HWI heat acclimation; hence, further research is required.

6.5.2 Practical implications

Our findings demonstrate that the majority of medium-term heat acclimation adaptation is achieved within the first 6 days, hence, athletes, military personnel, and occupational workers should carefully consider the time-benefit balance of short- vs medium-term heat acclimation. Medium-term post-exercise HWI conferred a more complete heat acclimation than EHA and TNE; however, the magnitude of adaptation between post-exercise HWI and EHA was modest. Therefore, it is more a matter of individual preference and the available facilities that might determine which heat acclimation method is used. Conversely, we observed fewer incidences of lower limb discomfort during the 12-day post-exercise HWI intervention than EHA, suggesting that the former may increase the proportion of athletes and military personnel able to complete heat acclimation, and reduce the medical cost and financial burden of acclimating individuals to the heat.

6.5.3 Conclusions

Firstly, extending the heat acclimation interventions from 6 to 12 days did not lead to further thermal adaptations or improvements in endurance performance in the heat. Secondly, medium-term post-exercise HWI conferred a more complete heat acclimation than EHA and TNE. Finally, changes in circulating thyroid hormones are unlikely the mechanism for the observed thermal adaptations.

CHAPTER SEVEN

General Discussion

7.1 Summary of main findings

This thesis set out to better understand the efficacy of the post-exercise hot water immersion heat acclimation intervention as a preparatory strategy for exercise in hot environments. In **Chapter 4**, it was demonstrated that despite a similar endogenous thermal stimulus, short-term post-exercise hot water immersion elicits larger thermal adaptations compared to conventional exercise heat acclimation. In addition, we observed that post-exercise hot water immersion initiates rapid heat acclimation adaptations, observable after only 3 days. The second empirical chapter provides evidence that reductions in circulating thyroid hormone concentrations are not the mechanism for the reduction in resting core temperature after short-term heat acclimation (**Chapter 5**). Finally, in **Chapter 6**, we found that extending the 6-day post-exercise hot water immersion intervention to 12 days provides no further thermal adaptation but that medium-term post-exercise hot water immersion conferred a more complete heat acclimation than exercise heat acclimation and thermoneutral exercise. In addition, 12 days of post-exercise hot water immersion elicited a reduction in plasma free T3, however, changes in circulating thyroid hormones are unlikely to be the mechanism for medium-term heat acclimation adaptation.

7.2 The induction of heat acclimation adaptation

7.2.1 Aerobic training

The inclusion of the work-matched thermoneutral exercise group in **Chapter 4** allows for interesting insights into the role of aerobic training for inducing heat acclimation adaptation. We observed that the benefits of short-term exercise heat acclimation beyond that of work-matched exercise in thermoneutral conditions were modest, with the only difference being a greater whole-body sweat rate after exercise heat acclimation (Table 4.2). Indeed, the 6-day thermoneutral exercise intervention elicited a reduction in end-exercise T_{re} equivalent to 96%

of that achieved from exercise heat acclimation. Our data align with investigations by Shvartz *et al.* (1977), Kampmann *et al.* (2008) and, more recently, Ravanelli *et al.* (2020), who demonstrated that aerobic training in temperate conditions confers heat acclimation adaptations in recreationally active individuals. However, findings on the heat acclimation benefits of aerobic training are equivocal. Avellini *et al.* (1982) observed aerobic training-induced improvements in heat tolerance only when core temperature was permitted to rise during exercise. However, Zurawlew and colleagues (2016) found that six consecutive daily treadmill runs in temperate conditions had no effect on subsequent thermoregulatory measures at rest or during exercise-heat stress. This disparity is likely explained by the participants in the Zurawlew *et al.* (2016) study being endurance trained ($\dot{V}O_{2max}$ 60 ± 9 mL·kg^{-1}·min^{-1}), hence, already having thermoregulatory adaptations analogous to that of partial heat acclimation (Piwonka *et al.*, 1965; Gisolfi & Robinson, 1969). Finally, in contrast with our findings, research comparing 10 days of exercise heat acclimation with a work-matched control group reported greater adaptations from exercise in the heat (Gibson *et al.*, 2015c). Taken together, aerobic training in temperate conditions induces a rise in core temperature, which in turn confers thermoregulatory adaptations of a similar magnitude to that of short-term exercise heat acclimation in recreationally active individuals but is likely less effective than extended heat acclimation protocols.

7.2.2 Resting core temperature

In **Chapter 4**, we demonstrated that, despite a similar endogenous thermal stimulus, 6 days of post-exercise hot water immersion compared favourably with a short-term fixed intensity exercise heat acclimation intervention and daily thermoneutral exercise. Specifically, post-exercise hot water immersion elicited a larger reduction in thermal strain during exercise-heat stress (Figure 4.2), which translated into a larger, albeit non-significant, improvement in endurance capacity in the heat (Figure 4.3). The change in T_{re} during exercise in the heat was similar between groups (Table 4.2); hence, the larger reduction in thermal strain after post-exercise hot water immersion can be attributed to the larger reduction in resting T_{re}. Indeed, the reduction in resting T_{re} after post-exercise hot water immersion accounts for most of the reduction in thermal strain during exercise-heat stress (Figure 7.1), which is in accordance with previous studies (Zurawlew *et al.*, 2016; Zurawlew *et al.*, 2018a, b, 2019). In contrast, short-term exercise heat acclimation and work-matched exercise in temperate conditions elicited only modest reductions in resting T_{re} (Figure 7.1).

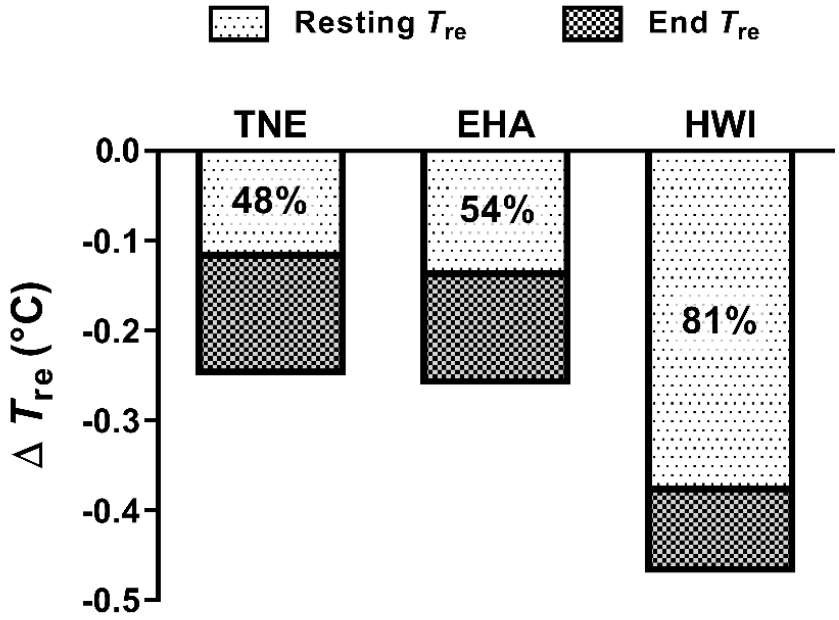

Figure 7.1. Influence of 6 days of thermoneutral exercise (TNE), exercise heat acclimation (EHA) or post-exercise hot water immersion (HWI) on resting rectal core temperature (T_{re}) and end-exercise T_{re} following a 40-min treadmill run at 65% $\dot{V}O_{2peak}$ in the heat (33°C, 40% RH). Bars represent the baseline-adjusted mean change from baseline. The contribution of resting T_{re} to the reduction in end-exercise T_{re} is shown as a percentage.

7.2.3 Dual thermal stimulus

The typical daily rise in T_{re} and T_{sk} during each of the three interventions compared in **Chapter 4** are presented in Figure 7.2. While the endogenous thermal stimulus was similar between the post-exercise hot water immersion and exercise heat acclimation protocols (AUC for T_{re} > 38.5°C; Table 4.1), the former exposed participants to a greater rise in skin temperature (average end T_{sk}, 40°C vs ~35°C, respectively). This large dual thermal stimulus (i.e. maintained elevation in both core temperature and skin temperature) likely explains the larger reduction in resting T_{re} after post-exercise hot water immersion compared to exercise heat acclimation, as it is purported to induce a more complete state of heat acclimation (Regan *et al.*, 1996). For instance, a greater reduction in thermal strain during exercise-heat stress was induced in individuals who were exposed to a larger daily elevation in T_{sk} (~4.2°C) during a controlled hyperthermia protocol that matched the endogenous thermal stimulus (Regan *et al.*, 1996). Similarly, Mee *et al.* (2018) demonstrated that sauna exposure (50°C, 30% RH) immediately before short-term heat acclimation elicited larger reductions in resting T_{re} and

peak T_{re} during exercise-heat-stress compared to short-term exercise heat acclimation alone. Together, our data and previous findings support the contention that a dual thermal stimulus is important for optimising short-term heat acclimation adaptation.

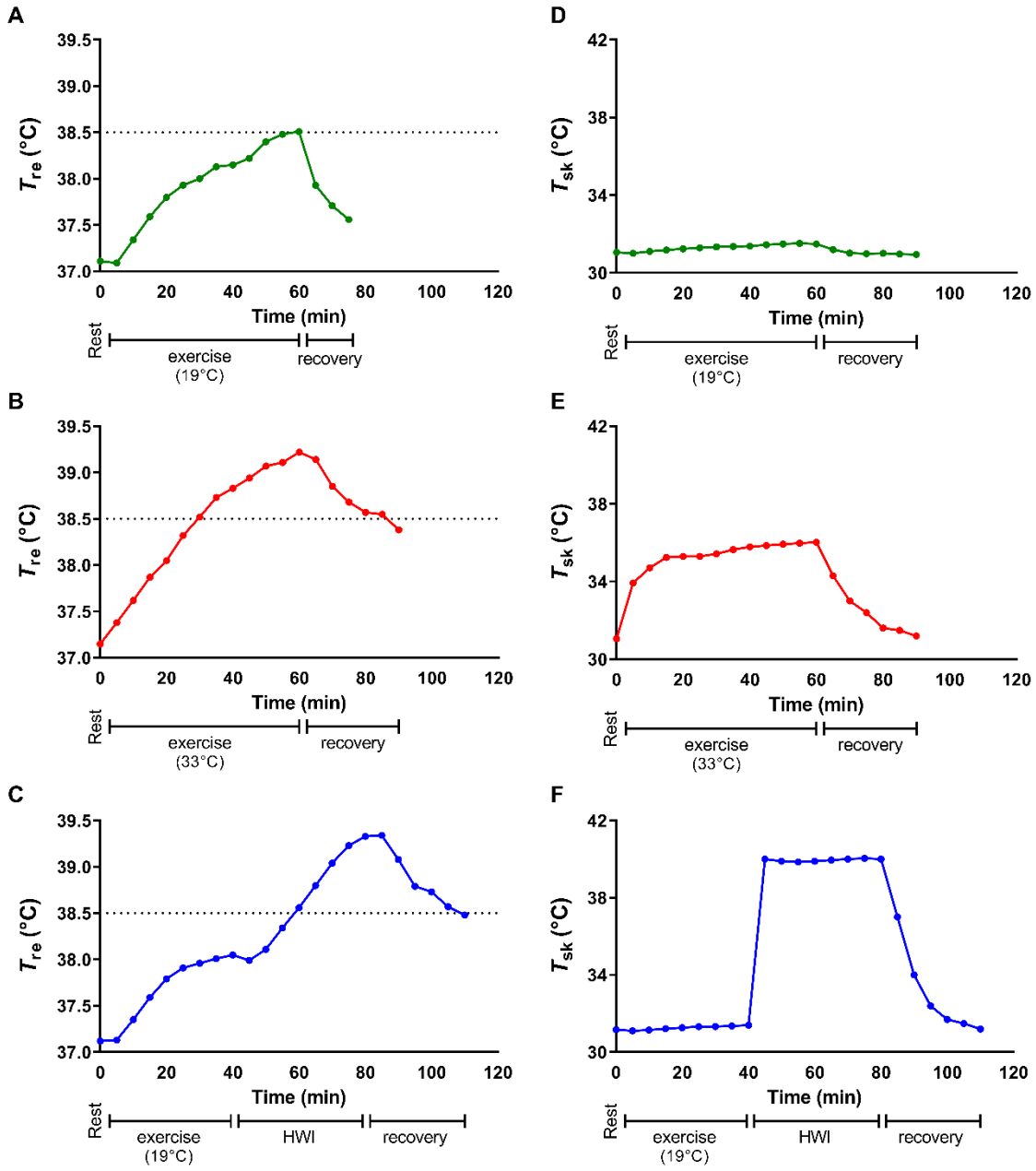

Figure 7.2. Typical change in rectal core temperature (T_{re}) and mean skin temperature (T_{sk}; estimated) during the sixth day of thermoneutral exercise (A, D), exercise heat acclimation (B, E), or post-exercise hot water immersion (HWI; C, F).

7.3 The timeframe of post-exercise hot water immersion adaptation

7.3.1 Post-exercise hot water immersion elicits rapid heat acclimation adaptations

Although short-term exercise heat acclimation is purported to be sufficient to induce adaptations in core temperature and heart rate, reductions in thermal strain are typically small and inconsistent (−0.17 ± 0.12°C, n = 144; Tyler *et al*., 2016). Recommendations state that long-term protocols are required to achieve a more complete state of heat acclimation (Périard *et al.*, 2015; Tyler *et al.*, 2016; Saunders *et al.*, 2019). In contrast, we observed that post-exercise hot water immersion elicited rapid heat acclimation adaptations after only 3 days, including reductions in core temperature at rest (−0.35°C) and following exercise in the heat after only 3 days (−0.32°C). Figure 7.3 summarises the timeframe of post-exercise hot water immersion heat acclimation adaptations. Further, the reductions in thermal strain initiated by rapid post-exercise hot water immersion accounted for 57% and 50% of the reductions observed after 6 and 12 days, respectively. Interestingly, the contribution of the reduction in resting T_{re} to the reduction in end-exercise T_{re} was greater after 3 days than after 6 and 12 days of post-exercise hot water immersion (Figure 7.4). It appears that, like exercise heat acclimation, adaptations that contribute to the reduction in change in T_{re} during exercise appear to take longer to manifest. These findings again highlight the importance of the reduction in resting T_{re} for the improvement in thermotolerance after post-exercise hot water immersion.

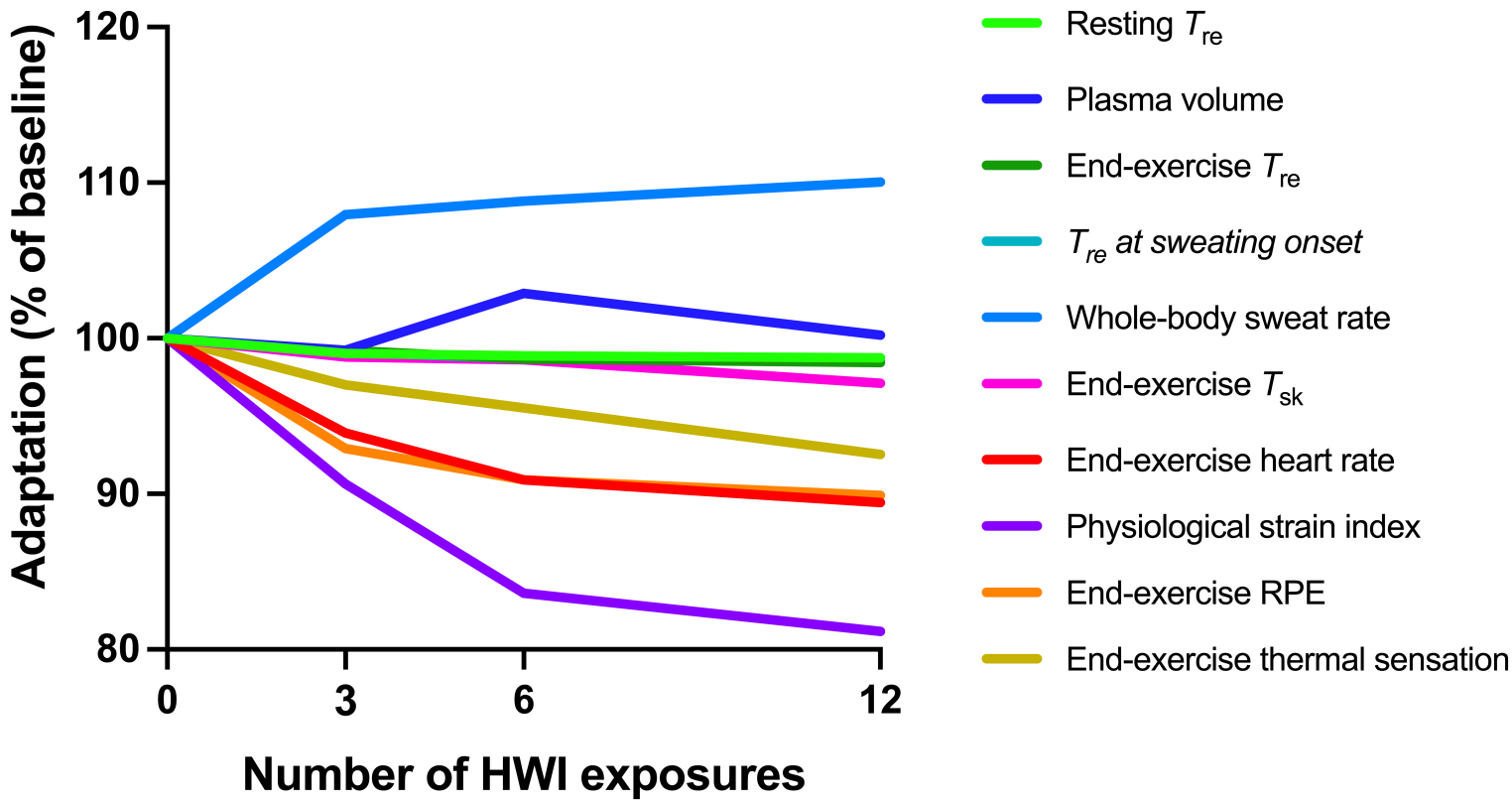

Figure 7.3. Timeframe of post-exercise hot water immersion (HWI) heat acclimation adaptations in the seven participants who completed the 12-day protocol (**Chapter 6**). T_{re}, rectal core temperature; T_{sk}, mean skin temperature; RPE, rating of perceived exertion.

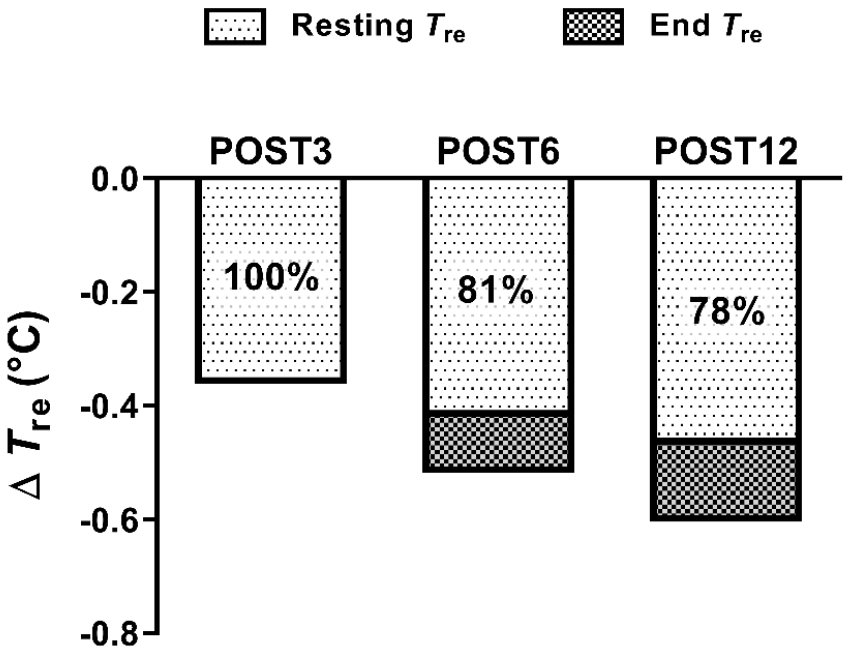

Figure 7.4. Influence of 3 (POST3), 6 (POST6) and 12 (POST12) days of post-exercise hot water immersion on resting rectal core temperature (T_{re}) and end-exercise T_{re} following a 40-min treadmill run at 65% $\dot{V}O_{2peak}$ in the heat (33°C, 40% RH). The contribution of resting T_{re} to the reduction in end-exercise T_{re} is shown as a percentage.

7.3.2 Extending the 6-day post-exercise hot water immersion intervention

A key finding from **Chapter 6** was that extending the post-exercise hot water immersion intervention from 6 to 12 days provided no further thermal benefits. For example, no difference was observed in the reduction in end-exercise T_{re} after 6 days (−0.56°C) compared to 12 days (−0.64°C; Table 6.3) of post-exercise hot water immersion. These data align with previous literature demonstrating little or no difference in thermal adaptation between short- and medium-term exercise heat acclimation (Daanen *et al.*, 2011; Gibson *et al.*, 2015b; Karlsen *et al.*, 2015). In contrast, some studies have demonstrated enhanced benefits after a greater number of heat acclimation exposures (Aoyagi *et al.*, 1995; Moss *et al.*, 2020). Aoyagi *et al.* (1995) reported that the reduction in end-exercise T_{re} following an exercise-heat-stress test whilst wearing protective clothing doubled from 6 to 12 days of heat acclimation. However, the reduction in end-exercise T_{re} after 6 days was modest compared to that observed in **Chapter 6** (~−0.2°C vs −0.56°C), suggesting that the mode of heat acclimation used was less effective.

In addition, a recent study by Moss *et al.* (2020) found that RPE and TS during exercise-heat-stress tests were lower after medium-term compared to short-term heat acclimation, whereas we observed no further reduction in perceptual strain. A possible explanation of this disparity is that participants in the Moss *et al.* (2020) study became more habituated to the mode of exercise-heat stress since the heat acclimation and exercise-heat-stress test procedures were the same. Evidently, whether further benefits can be attained from prolonged heat acclimation interventions is dependent on the mode of heat acclimation being employed.

The data presented in **Chapter 4** (Figures 4.3 and 4.3) demonstrate the interindividual variability in response to heat acclimation. This variability is likely due, at least partly, to differences in participants' fitness levels as previous post-exercise hot water immersion research has shown that lesser fit individuals acquired a larger, albeit nonsignificant, benefit compared to more highly trained individuals (Zurawlew *et al.*, 2018b). Differences in initial heat tolerance presents another possible explanation for the variation in responsiveness to the intervention. Although all participants were unacclimated before commencing the protocol, the range of the first immersion duration (~25–40 min) suggests that some participants were able to tolerate the passive heat stress more than others. Consequently, some participants were exposed to a greater thermal stimulus than others and, in turn, achieved a greater magnitude of adaptation. The variation in responsiveness highlights that some individuals will achieve further thermal benefits by extending post-exercise hot water immersion protocols whilst others will not.

Despite observing no further thermal benefit from 6 to 12 days of post-exercise hot water immersion or exercise heat acclimation, we recognise that extending the interventions may have conferred enhanced cellular thermotolerance, characterised by increased Hsp72, Hsp90α (Yamada *et al.*, 2008; Magalhaes *et al.*, 2010a; Amorim *et al.*, 2011; Gibson *et al.*, 2016), and decreased markers of intestinal permeability (LPS and I-FABP) and inflammatory markers (TNF-α, IL-6 and IL-1β; Barberio *et al*., 2015); however, these were not measured. Indeed, such adaptations may only be attained after prolonged heat acclimation protocols (Horowitz, 2016). The benefit of these cellular adaptations for improving physical performance or reducing the incidence of exertional heat illness is unclear but heat shock proteins have been shown to protect against cell death via proteasomal maintenance in cell models (Beckham *et al.*, 2008). Further, high levels of LPS and cytokines are associated with exertional heat stroke (Bouchama *et al.*, 1991; Leon & Helwig, 2010).

7.4 The mechanism for the reduction in resting core temperature after heat acclimation

7.4.1 The role of thyroid hormone changes

The reduction in resting T_{re} accounts for most of the reduction in thermal strain during exercise-heat stress after post-exercise hot water immersion heat acclimation. In **Chapter 5** we tested and subsequently rejected the hypothesis that the reduction in resting T_{re} after short-term heat acclimation was associated with changes in plasma thyroid hormone concentrations. **Chapter 6** furthered this research by examining changes in plasma thyroid hormones after 3 and 12 days. Despite there being a large reduction in resting T_{re} after 3 (−0.35°C) and 6 days (−0.41°C) of post-exercise HWI, no changes in plasma thyroid hormone concentrations were detected until after 12 days. Further, similar thermal adaptations occurred after both post-exercise hot water immersion and exercise heat acclimation, however, no changes in thyroid hormones were observed after medium-term exercise heat acclimation. Together, these findings indicate that changes in thyroid hormones are unlikely the mechanism for short- and medium-term heat acclimation adaptation. Nevertheless, decreased circulating thyroid hormone concentrations have been associated with an upregulation of BDNF (Li *et al.*, 2019). Therefore, the reduction in free T3 after 12 days of post-exercise hot water immersion may affect nerve growth and cause long-term alterations to the hypothalamic neural network that coordinates changes in thermogenesis and metabolism (Iwen *et al.*, 2018). This neurometabolic cascade offers a potential mechanism for the retention of post-exercise hot water immersion heat acclimation (Zurawlew *et al.*, 2019), however, further research is required to confirm this.

7.4.2 A shift in the thermoregulatory balance point as a possible alternative mechanism

An alternative mechanism for the reduction in T_{re} after short- and medium-term heat acclimation is a leftward shift in the thermoregulatory balance point (Romanovsky, 2007) via a reduction in the core body temperature thresholds for heat gain mechanisms (Figure 7.4). While further research is required to confirm (or reject) this supposition, a recent study by Barry *et al.* (2020) demonstrated that 7 days of hot water immersion heat acclimation reduced the activation thresholds for cutaneous vasodilation and sweat production. It is unlikely that this heat acclimation adaptation is isolated to only the heat defence effectors, but that it also reduces the activation threshold of cold defence effectors. Such a shift in thermoeffector activation threshold would inevitably result in a lower resting core temperature (Figure 7.4). While this mechanism may be partly responsible for improved thermotolerance during exercise

in the heat, there is the potential ramification for post-exercise hot water immersion (and heat acclimation in general) to impair cold tolerance.

Although this thesis does not offer a conclusive answer to the question of how post-exercise hot water immersion reduces resting T_{re}, our findings broaden the understanding of heat acclimation adaptation by providing preliminary evidence that the mechanism for the reduction in core temperature after short-term protocols appears to be different from that of long-term heat acclimation. This contention is congruent with the biphasic heat acclimation model proposed by Horowitz (2014).

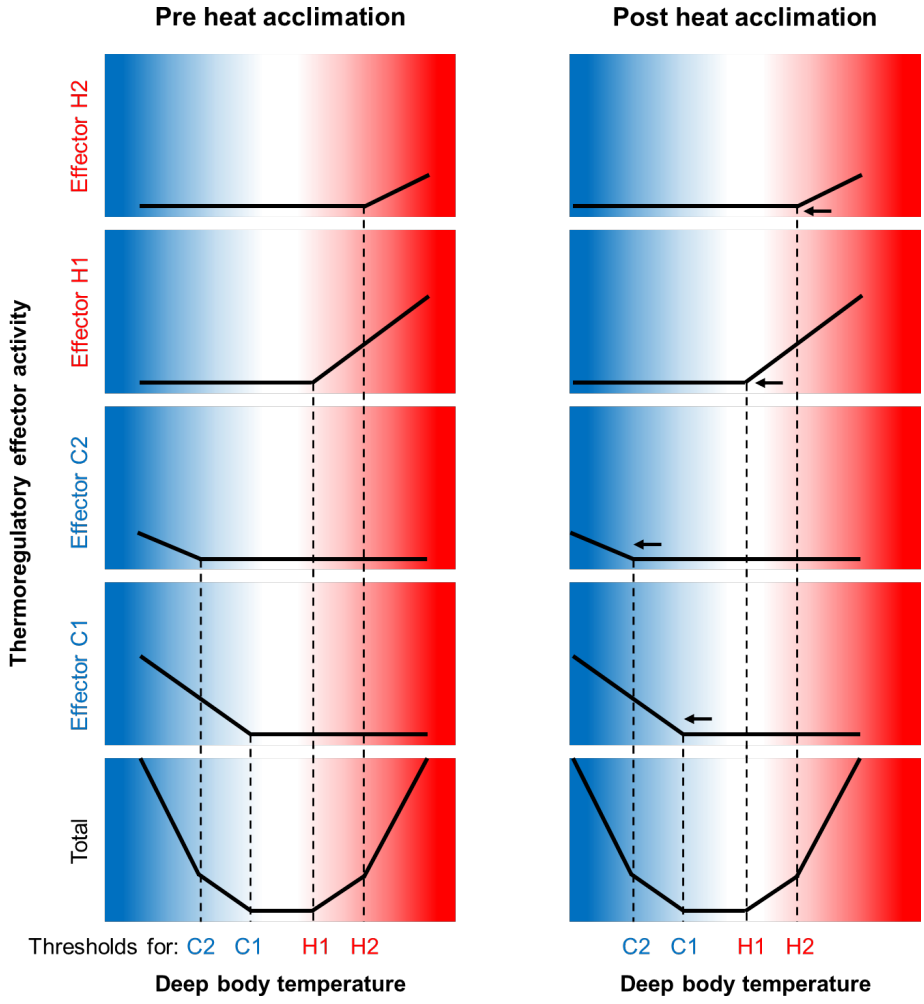

Figure 7.5. Coordinated recruitment of independent thermoeffectors to control body temperature and the hypothetical effect of heat acclimation. The top eight panels show the dependence of the activity of four effectors (two cold defences, C1 and C2, and two heat defences, H1 and H2) on body temperature. Each effector has its own threshold body temperature (dashed line). The bottom panel shows the cumulative effector activity. Developed after Romanovsky (2018).

7.5 Limitations

Although all effort was made to ensure that the investigations that comprise this thesis were conducted with the utmost rigour, we recognise our findings are not without limitation. For example, findings on the effectiveness of the interventions compared in **Chapter 4 and 6** for improving TTE should be considered with caution owing to the small sample sizes. Nevertheless, the data does suggest that improvements in endurance capacity in the heat may be more readily observed after short-term post-exercise hot water immersion compared with exercise heat acclimation, which has practical implications for applied practitioners and coaches. This contention is supported by the larger magnitude of thermal adaptation elicited by 6 days of post-exercise hot water immersion, which is associated with greater performance improvements. In addition, the findings of **Chapters 4 and 6** are limited to a recreationally active population. Although post-exercise hot water immersion heat acclimation has been shown to be effective in both endurance trained and recreationally active individuals (Zurawlew *et al.*, 2018b), less fit participants achieve a greater magnitude of adaptation. As such, the temporal patterning and magnitude of adaptation of the post-exercise hot water immersion intervention compared to exercise heat acclimation may differ in an endurance trained population. Similarly, as within many research studies in sports science, the influence of post-exercise hot water immersion heat acclimation on physiological, perceptual and performance adaptations in female, paediatric, and elderly populations was not investigated and is an important future research avenue. However, with regards to the latter, recent research has demonstrated that both exercise heat acclimation and post-exercise hot water immersion are effective strategies to prepare the elderly for heat stress (Waldock *et al.*, 2021).

7.6 Practical implications

Current recommendations to maximise heat acclimation adaptations state that individuals should exercise in the heat for at least 60 min for a minimum of 15 days (Périard *et al.*, 2015; Saunders *et al.*, 2019). However, long-term protocols can be costly, impractical, and the physical demands of daily exercise-heat-stress can disrupt training and lead to fatigue (Schmit *et al.*, 2018; Saunders *et al.*, 2019). Short-term heat acclimation offers a more practical solution, although previous research (Tyler *et al.*, 2016) and data presented in this thesis highlight that exercise-based protocols provide inconsistent thermal adaptations that are of minimal benefit beyond exercise in temperate conditions. It is perhaps for these reasons that athlete engagement

with heat acclimation is poor, with only 15% of athletes attempting to adapt to the heat before the 2015 IAAF World Athletics Championships in Beijing (Périard *et al.*, 2017).

Taking a hot bath submerged to the neck, for up to 40 min, following habitual training in temperate conditions, presents a practical and economical heat acclimation intervention — eliminating the requirement for an increased training load, access to an environmental chamber or relocation to a hot climate. This strategy can be more easily incorporated into the normal training, for example, hot water immersion as part of a post-exercise washing routine. We have demonstrated that short-term post-exercise hot water immersion provides larger reductions in thermal strain during exercise in the heat compared to exercise heat acclimation. Further, we have shown that adaptations from post-exercise hot water immersion develop rapidly, observable after only 3 days. Taken together, post-exercise hot water immersion presents a favourable heat acclimation strategy for athletes and military personnel who reside in temperate climates, especially when the time to acclimate is limited. Although we observed no statistical difference in thermal strain after 6 and 12 days of post-exercise hot water immersion or exercise heat acclimation, it is likely that some individuals will benefit from extended heat acclimation protocols. Hence, a 3-day post-exercise hot water immersion intervention is recommended as a starting point for exercise-heat stress preparations, but this should be supplemented by additional sessions on an individual basis to optimise thermal adaptation, i.e., in low responders. Initial thermal tolerance during the first and second hot water immersions may be useful for predicting how many sessions will be required heat acclimation. Pooled data from the current thesis and previous studies (Zurawlew *et al.*, 2016; Zurawlew *et al.*, 2018a, b, 2019) show that the reduction in resting T_{re} after 6 days of post-exercise hot water immersion is associated with the end-intervention T_{re} ($r = -0.55$, $P < 0.001$) and hot water immersion duration ($r = -0.42$, $P = 0.008$) on the second intervention day. These relationships suggest that individuals who can tolerate higher core temperatures and longer immersion durations may achieve a higher magnitude of adaptation.

Consensus recommendations promote the maintenance of an elevated core temperature during heat acclimation sessions (Armstrong & Maresh, 1991; Taylor, 2014). Data from this thesis highlight the importance of also inducing an elevated skin temperature to facilitate a large dual thermal stimulus. While post-exercise hot water immersion provides this dual thermal stimulus, it is apparent that this is not typically the case for exercise-based protocols (Tyler *et al.*, 2016). Our findings suggest that maintaining an elevated skin temperature during exercise heat acclimation may enhance adaptation. However, higher skin temperatures during exercise are

likely to increase perceptual strain that could in turn limit tolerance time (Périard & Bergeron, 2014).

Although this thesis supports post-exercise hot water immersion as a practical and effective alternative to conventional exercise heat acclimation, like any intervention, there are limitations. Firstly, post-exercise hot water immersion heat acclimation achieves thermoregulatory benefits without excessive physical stress; however, the repeated thermal challenge likely induces some fatigue. Thus, adequate recovery time prior to subsequent exercise-heat stress should be incorporated into training to ensure adaptations are fully realised (Saunders *et al.*, 2019). Zurawlew *et al.* (2019) has previously demonstrated that short-term post-exercise hot water immersion adaptations are retained for at least 2 weeks, providing some time to reduce training load and facilitate recovery prior to competition/military operations. If a longer taper period is required, athletes and military personnel should use "top-up" sessions in the week before competition/military operations to maintain heat acclimation (Saunders *et al.*, 2019). Secondly, the duration of post-exercise hot water immersion sessions (~75 min) are arguably longer than some previously employed exercise heat acclimation strategies, which are typically 60–90 min (Tyler *et al.*, 2016). However, this disparity can be partially negated by incorporating the hot water immersion protocol into a post-exercise washing routine. Thirdly, when directly compared, medium-term post-exercise hot water immersion provides only modest benefits over exercise heat acclimation. Therefore, it is more a matter of individual preference and the available facilities that might determine which heat acclimation method is used. However, we observed fewer incidences of lower limb discomfort during the 12-day post-exercise hot water immersion intervention than exercise heat acclimation, suggesting that the former may increase the proportion of athletes and military personnel able to complete heat acclimation, and reduce the medical cost and financial burden of acclimating individuals to the heat. Finally, post-exercise hot water immersion does not provide the same familiarisation to exercise-heat stress as conventional exercise heat acclimation. In turn, athletes may not develop appropriate pacing and psychological strategies to cope with exercise in in hot conditions (Coudevylle *et al.*, 2019). A possible solution to this shortcoming would be using a combination of conventional exercise heat acclimation and post-exercise passive heating strategies, as suggested by Guy *et al.* (2015). For example, Stephenson *et al.* (2019) demonstrated that a combination of active and passive heat acclimation was effective at stimulating thermoregulatory adaptations in Paralympic and able-bodied triathletes.

7.7 Future directions

The laboratory studies that constitute this thesis demonstrate that post-exercise hot water immersion elicits larger reductions in thermal strain compared to conventional exercise heat acclimation, and that these adaptations occur rapidly. These findings provide preliminary evidence that supports the use of post-exercise hot water as an effective and practical heat acclimation strategy; however, this should be confirmed in athletes and military personnel during field trials. Future research should assess whether the reduction in thermal strain after post-exercise hot water immersion translates to 'real-world' performance improvements and impacts the incidence of exertional heat illness.

In **Chapters 5 and 6**, we highlighted the temporal and intervention disconnects between changes in thyroid hormones and thermal adaptations, eliminating thyroid hormone changes as a possible mechanism for the reduction in resting T_{re} after heat acclimation. The potential for heat acclimation to result in a leftward shift in the thermoregulatory balance point warrants further investigation. As such, future research should assess the effect of short- and medium-term heat acclimation on the activation threshold of BAT thermogenesis. Such work will develop a broader understanding of the underlying mechanisms of heat acclimation adaptation.

Females are largely underrepresented in heat acclimation literature. This is highlighted in the comprehensive meta-analysis by Tyler *et al.* (2016), which reviewed 96 articles involving a total of 1056 participants. Notably, only 76 (7%) of the participants were female. Unfortunately, this lack of representation appears to be a recurring theme as in the more recent review on heat acclimation decay and re-induction by Daanen *et al.* (2018) only 3 of the 20 studies included females. This gap in the literature is concerning as there is convincing evidence that demonstrates sex differences in thermoregulatory and physiological adaptation. Males and females differ in their thermoregulatory response to exercise-heat-stress largely owing to females having a lower sudomotor function at high demands for heat loss (Gagnon & Kenny, 2011). In turn, females have a reduced maximal evaporative capacity, which results in an increase in physiological strain (Moran *et al.*, 1999). Additionally, previous research suggests that there are sex differences in the training-induced upregulation of Hsps. Morton *et al.* (2009) found that, in males, 6 weeks of continuous or interval training increased Hsp70 by 38 ± 41% and 23 ± 36%, respectively. In contrast, females displayed no significant increases (continuous, 3 ± 37%; interval, 4 ± 14%). Work by Mee *et al.* (2016) suggests that this disparity in Hsp response is likely a result of inhibited protein translation in females, potentially owing

to the influence of oestrogen. It follows that the temporal patterning of heat acclimation adaptation may also be different between sexes. Mee and colleagues (2015) assessed sex differences in thermoregulatory and physiological adaptation to five and 10 exercise-heat exposures. The authors confirmed that both males and females responded to short-term isothermic heat acclimation but that females required longer protocols to establish thermoregulatory and cardiovascular stability.

These findings indicate that heat acclimation protocols should be designed to target sex differences for maximum effectiveness. For example, a recent study by Mee *et al.* (2018) demonstrated that combining both active and passive heat acclimation strategies can accelerate thermal adaptations in females. The authors showed that 20 min of sauna exposure prior to each 90-min bout of controlled hyperthermia on five consecutive days resulted in enhanced reductions in T_{re}, T_{sk}, heart rate, and sweating onset compared with exercise heat acclimation alone. Evidently, more work is required to better understand sex differences in heat acclimation adaptation; as such, future research should investigate the temporal patterning of post-exercise hot water immersion heat acclimation in females. Our findings in males suggest that the large dual thermal stimulus from 6 days of post-exercise hot water immersion should be sufficient to initiate heat acclimation adaptation in females.

7.8 Main conclusions

The findings of this thesis include:

1. Post-exercise hot water immersion elicits rapid heat acclimation adaptations (observable after only 3 days) but extending the 6-day intervention to 12 days provides no further thermal adaptations or improvement in endurance capacity.

2. Short- and medium-term post-exercise hot water immersion confer more complete heat acclimation than conventional exercise heat acclimation and thermoneutral exercise.

3. The heat acclimation benefits of exercise in the heat beyond work-matched exercise in thermoneutral conditions appear modest.

4. Circulating thyroid hormone changes are unlikely the mechanism for short- or medium-term heat acclimation adaptation.

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Appendix A

Example informed consent

Title of study:

A laboratory study to refine the post-exercise hot water immersion heat acclimation intervention

MoDREC reference: **829/MODREC/17**

Please Initial Boxes

- The nature, aims, and risks of the research have been explained to me. I have read and understood the Information for Participants and understand what is expected of me. All my questions have been answered fully to my satisfaction. ☐
- I understand that if I decide at any time during the research that I no longer wish to participate in this project, I can notify the researchers involved and be withdrawn from it immediately without having to give a reason. I also understand that I may be withdrawn from it at any time, and that in neither case will this be held against me in subsequent dealings with the Ministry of Defence or Bangor University. ☐
- I understand that the screening process to decide if I am suitable to be selected as a participant may include completing a medical screening questionnaire and/or a physical examination by the independent medical officer and I consent to this. ☐
- I consent to any incidental medical findings being reported to my GP after attaining my permission. ☐
- I consent to the processing of my personal information for the purposes of this research study. I understand that such information will be treated as strictly confidential and handled in accordance with the provisions of the Data Protection Act 1998. ☐
- I agree to volunteer as a participant for the study described in the information sheet and give full consent. ☐
- This consent is specific to the particular study described in the Information for Participants attached and shall not be taken to imply my consent to participate in any subsequent study or deviation from that detailed here. ☐
- I understand that in the event of my sustaining injury, illness or death as a direct result of participating as a volunteer in Ministry of Defence research, I or my dependants may enter a claim with the Ministry of Defence for compensation under the provisions of the no-fault compensation scheme, details of which are attached. ☐
- I understand the compensation arrangements that have been provided. ☐

Participant's statement:

I …… agree that the research project named above has been explained to me, to my satisfaction and I agree to take part in the study. I have read the participant information sheet about the project and understand what the research study involves.

Signed: Date:

Witness' Name: Signature: Date:

Investigator's statement:

I **……** confirm that I have carefully explained the nature, demands and any foreseeable risks (where applicable) of the proposed research to the Participant.

Signed: Date:

Authorising Signatures

The information supplied above is to the best of my knowledge and belief accurate. I clearly understand my obligations and the rights of research participants, particularly concerning recruitment of participants and obtaining valid consent.

Signature of Chief Investigator Date:

Name and contact details of independent volunteer advocate

Dr Anthony Blanchfield

Email: a.blanchfield@bangor.ac.uk

Name and contact details of chief investigator:

Prof Neil Walsh (Chief Investigator)

Email: n.walsh@bangor.ac.uk

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Email: pep409@bangor.ac.uk

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Email: r.d.mcintyre@bangor.ac.uk

When completed provide a copy to the participant and retain one copy for records.

Appendix B

Physiology informed consent and medical questionnaire

Date:
Participant number:
Date of Birth:
Age:
Place of Birth:
Nationality:
Contact email:
Mobile number:

Have you recently taken part, or are you presently taking part, in a research study at Bangor University? Yes / No
Please list the studies and dates (if known)

Do you feel well now? *Yes / No*
Have you felt well for the last 7 days? *Yes / No*
Have you had a fever in the last 7 days? *Yes / No*
(Please provide details)

Are you currently attending your GP for any condition or consulted your GP in the last three months? *Yes / No*
(If yes, please provide details)

Is your body mass steady? *Yes / No*

Have you suffered from a serious illness or injury/ accident? *Yes / No*
(If yes, please provide details)

Have you suffered from a heat illness / intolerance? *Yes / No*

(If yes, please provide details)

Do you have any allergies? *Yes / No*

(If yes, please provide details)

Do you consider yourself to have a colour vision deficiency? *Yes / No*

(If yes, please provide details)

Do you suffer, have a history of, or currently receiving medical treatment for any conditions related to:

(Please tick as appropriate, if yes, please provide details)

	YES	NO	If yes, please provide details.
Blood Borne Diseases (e.g. Hepatitis, HIV)			
Blood Pressure (e.g. high (elevated systemic) or low)			
Cancer			
Cardiovascular (e.g. prior recognition of a heart murmur)			
Chronic Kidney Disease			
Chronic Obstructive Pulmonary Disease (COPD)			
Diabetes			
Dizziness or Fainting (e.g. Unexplained (near) syncope)			
Exertional or unexplained dyspnea/fatigue, following exercise.			
Exertional chest pain / discomfort			
Epilepsy			
Eyes			
ENT (ears, nose throat)			
Gastrointestinal			
Musculoskeletal (e.g. Arthritis, Tendinitis)			
Neurological			
Respiratory (e.g. Asthma, Bronchitis)			
Skin (e.g. Eczema, Psoriasis)			
Stroke or Transient Ischaemic Attack (TIA)			
Thyroid Disease (e.g. hyper, hypo)			
Other			

Please list your current medications (including over the counter (OTC) and herbal)

Please list any supplements (including quantities) you regularly consume

Please list any immunisations you have received in the previous year

Please list all foreign travel, including dates, in the previous year

Do you regularly expose yourself to hot baths, saunas or sun beds? *Yes / No*
(If yes, please provide details)

What is your average daily caffeine intake?

What is your average weekly alcohol intake?

Do you smoke? *Yes / No*
(If yes, please provide daily consumption)

How often do you exercise?

< Once per month		Once per week		4-5 times per week	
Once per month		2-3 times per week		> 5 times per week	

Approximately, how long is each exercise bout?

What approximate intensity level are these exercise bouts?

Vigorous		Moderate		Low intensity	

Family Medical History

Father:

☐ Alive Current age

My father's general health is: Excellent Good Fair Poor

Reason for poor health:

☐ Deceased Age at death

Was this a premature death (sudden or unexpected)?

Cause of death:

☐ I am not in contact with my father

Mother:

☐ Alive Current age

My mother's general health is: Excellent Good Fair Poor

Reason for poor health:

☐ Deceased Age at death

Was this a premature death (sudden or unexpected)?

Cause of death:

☐ I am not in contact with my mother

Siblings:

Number of brothers / sisters

Age range

Please list any known health problems with any of you siblings.

Other close family members:

Are you aware of any close family member who has a disability from a heart disease?

Are you aware of any of the following cardiac conditions in any close family members?

- Hypertrophic or dilated cardiomyopathy,
- Long-QT syndrome or other ion channelopathies,
- Marfan syndrome,
- Clinically important arrhythmias.

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 ……..…..

Record participants:

Height	cm
Weight	kg
Resting heart rate	beats.min^{-1}
Resting blood pressure	mmHg

The following section is to be completed by the assigned Independent Medical Officer

12-lead ECG observations

Summary

Is this volunteer fit to take part in the laboratory study to refine the post-exercise hot water immersion heat acclimation intervention (829/MoDREC/17) *Yes / No*

Recommendations

Signed **Date**

Name of Physician

GMC No

Appendix C

Profile of Mood States questionnaire

Participant ID:	Date:

Trial:	**PRE**	**POST3**	**POST6**	**POST12**

Below is a list of words that describe feelings people have. Please **CIRCLE THE NUMBER THAT BEST DESCRIBES HOW YOU FEEL RIGHT NOW.**

	Not at all	A little	Moderately	Quite a lot	Extremely
Tense	0	1	2	3	4
Angry	0	1	2	3	4
Worn out	0	1	2	3	4
Unhappy	0	1	2	3	4
Proud	0	1	2	3	4
Lively	0	1	2	3	4
Confused	0	1	2	3	4
Sad	0	1	2	3	4
Active	0	1	2	3	4
On-edge	0	1	2	3	4
Grouchy	0	1	2	3	4
Ashamed	0	1	2	3	4
Energetic	0	1	2	3	4
Hopeless	0	1	2	3	4
Uneasy	0	1	2	3	4
Restless	0	1	2	3	4
Unable to concentrate	0	1	2	3	4
Fatigued	0	1	2	3	4
Competent	0	1	2	3	4
Annoyed	0	1	2	3	4
Discouraged	0	1	2	3	4
Resentful	0	1	2	3	4
Nervous	0	1	2	3	4
Miserable	0	1	2	3	4
Confident	0	1	2	3	4
Bitter	0	1	2	3	4
Exhausted	0	1	2	3	4
Anxious	0	1	2	3	4
Helpless	0	1	2	3	4
Weary	0	1	2	3	4
Satisfied	0	1	2	3	4
Bewildered	0	1	2	3	4
Furious	0	1	2	3	4
Full of pep	0	1	2	3	4
Worthless	0	1	2	3	4
Forgetful	0	1	2	3	4
Vigorous	0	1	2	3	4
Uncertain about things	0	1	2	3	4
Bushed	0	1	2	3	4
Embarrassed	0	1	2	3	4