# PHYSIOLOGICAL ADAPTATIONS TO HEAT ACCLIMATION; REPERCUSSIONS ON CYCLING PERFORMANCE

David Stephen Nichols

A thesis submitted in partial fulfillments of the requirements of

Liverpool John Moores University

for the degree of

Doctor of Philosophy

This research programme was carried out in collaboration with

ASPETAR, Orthopaedic and Sports Medicine Hospital, Doha, Qatar

August 2018

# ACKNOWLEDGEMENTS

First and foremost, I would like to thank Dr Sebastien Racinais for the opportunity he has granted me to study and learn under his guidance for the past 4 years. The continual support he has offered, both within the realms of my PhD and research opportunities outside of it, has been invaluable to my professional and career development, and the world class research facility he has led at ASPETAR sports medicine hospital has made it possible to carry out research unavailable to the majority of researchers working in this field. My gratitude extends to the many colleges and friends within ASPETAR who supported and assisted me throughout my PhD.

Secondly I would like to thanks Professor Greg Whyte for his invaluable contribution and for overseeing this project over the past 4 years.

I owe a vast debt to all the participants who, by volunteering to participate, made the completion of this thesis possible.

To my family, for their enduring support and encouragement throughout.

Above all, I would like to thank my wife, Catiane, for her love, support and unwavering belief in me. I couldn't have completed this without you.

# CONTENTS

# ABSTRACT

The aims of this thesis were to 1) investigate the haematological adaptations to a novel long-term, progressive, work-matched heart rate clamp protocol, 2) to ascertain the neuromuscular adaptations to intermittent sprint training during heat acclimation and the effect on intermittent sprint performance and 3) to examine the phenomena of cross- acclimation where the stressors of one environment (heat), might convey a performance benefit in alternative environments (cool and hypoxia). Results from chapter 4 and 5 characterised the haematological adaptations of heat acclimation, and provided evidence of alterations in the red blood cell compartment of the total blood volume. Whilst modification of the plasma volume compartment is well researched, little is known regarding the red cell compartment, with no study previously measuring haemoglobin mass during heat acclimation. While training in a temperate environment led to a stable haemoglobin mass, training in a warm environment led to a transient drop in haemoglobin mass within 4 days of heat exposures, although this response was generally reversed within one week following the heat acclimation procedure. In chapter 6, all out intermittent sprint performance was shown to be not different between temperate and warm environments, and heat acclimation had no additional performance benefits over the same training when completed in cool environments. As such no differences were observed in electromyographic activity or tissue oxygen saturation either between environments or throughout acclimation. Chapter 7 revealed that heat acclimation led to enhancement of cycle time trial performance in hot, cool and hypoxic environments, where completing work matched training in a cool environment had no effect on exercise performance in any of the tested environments. Given that we only observed an increase in maximal aerobic capacity in the heat after

heat acclimation, but improvements in maximal aerobic power and time trial performance in all three environments, it is possible that heat acclimation either led to enhancement of anaerobic energy supply, or increased sub-maximal cycling efficiency, beyond that which cool training alone achieved.

# LIST OF TABLES

# LIST OF FIGURES

6.5: Starting and minimum tissue saturation index for each sprint during the temperate and exercise heat acclimation training interventions.

7.1: Schematic of the performance trials.

7.2: Mean and individual responses in maximal oxygen uptake, maximal aerobic power and time trial performance before and after the temperate and exercise heat acclimation training interventions.

7.3: Heart rate response during the ramp test before and after the temperate and exercise heat acclimation training interventions.

7.4: Sweat rate before and after the temperate and exercise heat acclimation training interventions.

7.5: Resting and post exercise concentrations of extracellular and intracellular HSP70, pre- and post-intervention for each testing environment.

7.6: Resting and post exercise concentrations of extracellular and intracellular HSP90, pre- and post-intervention for each testing environment.

# LIST OF ABBREVIATIONS

[Hb]: Haemoglobin concentration
BV: Blood volume
CON: Control experimental trial
EHA: Exercise heat acclimation training intervention
EMG: Electromyography
EPO: Erythropoietin concentration
Fe: Ferritin
HBmass: haemoglobin mass
Hct: Haematocrit
HIF1-$\alpha$ Hypoxia-inducible factor 1-$\alpha$
HOT: Hot experimental trial
HR: Heart rate
HSP: Heat shock protein
HSP70e: Extracellular heat shock protein 70
HSP70i: Intracellular heat shock protein 70
HSP90e: Extracellular heat shock protein 90
HSP90i: Intracellular heat shock protein 90
HSR: Heat shock response
HYP: hypoxic experimental trial
OBLA: Onset blood lactic acid
PV: Plasma Volume
RBC: Red blood cell
RBCV: Red blood cell volume
RMS: Root mean squared
RPE: Rating of perceived exertion
SD: Standard deviation
$T_{body}$: Whole body temperature
TC: Thermal comfort
$T_{core}$: Core rectal temperature
TEMP: Temperate training intervention
TS: Thermal sensation
$T_{skin}$: Skin temperature
TT: Time trial
VL: Vastus lateralis
$\dot{V}O_{2max}$ : Maximal oxygen uptake
$W_{max}$: Maximal aerobic power

# CHAPTER 1

# INTRODUCTION

Competitive cycling events are frequently held throughout the summer season, often in hot and/or humid conditions. It has been reported in both laboratory (Galloway and Maughan 1997) and field (Racinais et al. 2015b) studies that hot and/or humid environments reduce cycling performance compared to cool ambient environments. Heat acclimation has been demonstrated to be the primary strategy to attenuate this decrement in performance (Racinais et al. 2015a).

It is generally agreed that most adaptations to heat acclimation develop rapidly within the first 4-7 days, and further smaller changes occur within 2 weeks to fully optimise cardiovascular and sudomotor function to support aerobic exercise performance in hot ambient conditions (Périard et al. 2015). Modification of blood volume, primarily associated with the expansion of the plasma volume, is widely viewed as the primary haematological adaptation supporting exercise in the heat by providing greater maintenance of stroke volume and cardiac output, and reduced heart rate at any given workload (Senay 1972; Bonner and Edwards 1976; Sawka and Coyle 1999; Frank et al. 2001). It is sometimes (Bass et al. 1955; Wyndham et al. 1968; Shapiro et al. 1981), but not always (Patterson et al. 2014), reported that plasma volume returns to pre-treatment baseline levels as adaptation progresses, despite individuals retaining improved thermoregulatory function and exercise capacity. This apparently transient effect may be an experimental artefact of constant work-rate protocols, as the physiological overload continually declines with progressive adaption to heat (Périard et al. 2015). The use of clamped hyperthermia protocols, where core temperature is

maintained throughout the acclimation period (e.g. 38.5°C), may facilitate a continual adaptive stimulus and maintain plasma volume expansion for a long as 22 days (Patterson et al. 2014). However, clamped hyperthermia models are practically unsuitable outside of laboratories, and even within a laboratory setting participants may spend large portions of time at rest (i.e. without training stimulus) to maintain their target core temperature (Gibson et al. 2015a). A more efficacious method may be to enforce a target heart rate, allowing power to fluctuate and gradually increase as adaptation occurs (Périard et al. 2015). This HR clamp model might better maintain the thermal stimulus and facilitate continual and progressive adaptations over a long-term acclimation protocol, which can easily be transferred to field based practice. Therefore, the first aim of the present thesis was to characterise the physiological adaptations to a novel HR clamp heat acclimation protocol.

A sustained increase in plasma volume (haemodilution) may have implications for the regulation of blood volume, and of the red blood cell (RBC) mass due to homeostatic pressures. The kidney closely regulates the relative volumes of these two blood components, through the production of erythropoietin and retention of sodium and fluid (Dunn et al. 2007). A marked and sustained decrease in haematocrit induced by heat acclimation might have two opposite consequences; firstly, an increase in RBC mass to return haematocrit to pre-treatment levels suggesting master regulation of haematocrit; or secondly a decrease in RBC mass due to finite space in the vascular compartment by master regulation of total blood volume. This concept broadens the scope of blood volume regulation during heat acclimation to include the modulation of RBC mass as well as the plasma volume. As such, the second aim of the present thesis was to characterise the haemoglobin mass (HBmass) response to heat acclimation.

Heat acclimation research has typically employed low intensity, long duration exercise to study the cardiovascular adaptations to heat exposures. Research suggests that athletes tend to train with a polarized training distribution (Seiler and Kjerland 2006), combining long duration low intensity training with high intensity training. Heat exposure during sprint exercise exacerbates the rise in core temperature which can lead to an acute decrease in voluntary activation, muscle force production, and therefore performance (Morrison et al. 2004; Thomas et al. 2005). There is some evidence to suggest that heat acclimation might improve skeletal muscle function: in animal models heat exposure has been shown to enhance mitochondrial adaptations (Tamura et al. 2014) and increase cell proliferation potential and muscle protein content (Uehara et al. 2004). Only 2 studies have examined skeletal muscle adaptation in human participants. One study reported that 10 weeks of 8h/day of localized passive quadriceps heating increased maximum isometric force during knee extension, and increased mean cross-sectional area of the vastus lateralis and rectus femoris (Goto et al. 2011). The other reported an increased evoked peak twitch amplitude, increased maximal voluntary torque production and an improvement of the relative torque/EMG linear relationship after 11days of 1/h day whole body heating (Racinais et al. 2017b). Both of these studies, however, utilized a passive heating protocol, and explored only single, isolated muscle models. It is unclear whether such adaptations would exist during a whole body sprint exercise modality. Thus, the third aim of the present thesis was to evaluate the neuromuscular adaptations to heat acclimation, and the subsequent effect on intermittent sprint performance.

Lastly, heat acclimation has been well documented to improve $\dot{V}O_{2max}$ , and time trial performance in the heat (Sawka et al. 1985; Lorenzo et al. 2010), but the effect of heat acclimation on performance in cool environments is highly contentious, with many

studies criticized for lacking adequate control groups, or un-even training loads between hot and cool environments (Minson and Cotter 2016; Nybo and Lundby 2016). Heat acclimation has been reported to improve performance in the cool (Scoon et al. 2007; Lorenzo et al. 2010), but not always (Karlsen et al. 2015b; Keiser et al. 2015; Neal et al. 2016). The phenomena of cross acclimation, whereby adaptation to the stressors of one environment might convey benefits in another is not only limited to less stressful conditions (i.e. normothermic), but could also improve performance upon exposure to other environmental stressors (i.e. hypoxia) (Ely et al. 2014; Lee et al. 2016), provided they share a common adaptive response (Fregly et al. 2011). At a cellular level, both acute and chronic exposure to heat and/or hypoxia induce a heat shock protein (HSP) response (Morimoto 1998). Heat shock proteins are highly conserved molecular chaperones which facilitate the synthesis and folding of proteins, conveying a cytoprotective effect against subsequent thermal (Hutter et al. 1994) or ischaemic insult (Levi et al. 1993). Furthermore, interactions between the heat shock response (HSR) and hypoxia-inducible factor 1-$\alpha$ (HIF1-$\alpha$), the global regulator of cellular and systemic oxygen homeostasis, have been suggested in human models undergoing heat acclimation (Salgado et al. 2014; Lee et al. 2016). This could elicit down-stream effects on erythropoietin (EPO) expression, and consequently on HBmass, a key determinant of endurance exercise performance (Garvican et al. 2011). Accordingly, the fourth aim of the present thesis was to investigate whether heat acclimation improves cycle time trial performance in cool and normobaric hypoxic environments.

In summary, the modification of the blood volume; both red blood cell mass and plasma volume, may have positive effects on exercise performance in hot, cool, and normobaric hypoxic environments. The overall objective of the present thesis was to

characterise the haematological and neurological adaptations to a polarized, 3 week heat acclimation stimulus, and the subsequent effect on cycle time trial performance in hot, cool and normobaric hypoxic environments.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Introduction

This review firstly discusses the influence of heat stress on both endurance and sprint exercise performance, and the models of heat acclimation which may be implemented as a counter-measure. Secondly, the haematological adaptation to heat acclimation is discussed, specifically the regulation of blood volume. Finally this review examines the potential for cross acclimation, whereby heat acclimation may convey an ergogenic effect in both cool and hypoxic conditions.

## 2.2 Endurance exercise performance

The capacity to perform prolonged exercise is significantly reduced when exercise is undertaken in a warm environment. In their classic study, Galloway and Maughan (Galloway and Maughan 1997) demonstrated a significant reduction in mean time to exhaustion of cyclists working at ~70% of their maximal oxygen uptake ($\dot{V}O_{2max}$ ) as ambient temperature increased from 11 to 31°C (93.5 vs 51.6 min, respectively). The etiology of fatigue when exercise is undertaken under heat stress is multifaceted, with several integrative mechanisms responsible for accelerated fatigue when compared to normal ambient environments.

### 2.1.1 *Acute responses*

Aerobic exercise performance progressively deteriorates as ambient temperatures increase, even as ambient temperatures rise above 11°C (Galloway and Maughan

1997), suggesting a thermal constraint on exercise performance even under relatively cool conditions. It is well documented that exercise in a warm environment poses a significant thermal challenge to man and has the potential to reduce exercise performance (Febbraio et al. 1996b; González-Alonso et al. 1999; Parkin et al. 1999).

Several explanations for the progressive deterioration in performance during exercise in the heat have been proposed, including a reduction in maximal aerobic capacity ($\dot{V}O_{2max}$ ), and increased physiological demands; in particular greater cardiovascular strain to support competing demands from both oxygen delivery to the working skeletal muscle and the skin blood flow requirements for heat dissipation by the processes of conduction and convection (Sawka et al. 2011). Blood flow to the active muscle is not limited by heat stress (González-Alonso et al. 2008) Therefore, the skin remains under perfused as cardiac output is not sufficient to meet competing blood flow demands simultaneously from both the skin and muscle due to a marked reduction in stroke volume (Gonzalez-Alonso et al. 2000). At the onset of exercise, an increased vasoconstrictor activity causes cutaneous vasoconstriction in both warm and cool conditions to maintain central blood volume (Johnson and Kellogg 2010). As exercise continues and core temperature rises, an active cutaneous vasodilation facilitates increased skin blood flow but this is blunted by high skin temperatures and appears to plateau at a threshold well below the maximum skin blood flow that could be achieved at rest with the same thermal drive (González-Alonso et al. 1999). The combination of limitations on the cutaneous vasodilator function, heat production from working skeletal muscles, and reduction in the rate of heat loss from high ambient temperatures and/or humidity results in an exacerbated rise in core temperature (hyperthermia) for any given exercise intensity. Some degree of core temperature elevation during exercise is normal, with the increase proportional to the absolute

power output (Nielsen 1938), but the narrowing of the skin to ambient temperature gradient will increase the rate of heat gain at a given power output (Galloway and Maughan 1997). Hyperthermia per se can impair exercise performance (Nybo et al. 2014), and consequently decreases power output compared with temperate environments. Studies have provided evidence that hyperthermia profoundly influences brain function during exercise, resulting in altered brain activity (Nielsen et al. 2001) , reduced voluntary activation of muscle during sustained contractions (Nybo and Nielsen 2001a), and an increased perception of effort (Nybo and Nielsen 2001b). It is apparent that there is a significant central nervous system component to fatigue when exercising under a high thermal load, and that central integration of core temperature, skin temperature and other peripheral inputs also determines an individual's ability to maintain a particular workload in the heat (Nybo 2010b). Further evidence for this assertion has been provided by studies showing that a number of centrally-acting pharmacological agents are able to improve exercise capacity in hot environments, but not in more temperate conditions (Watson et al. 2005; Roelands et al. 2008). Although the exact balance between these factors remains unclear, it is clear that fatigue fundamentally occurs because of heat related mechanisms, rather than any pressure on substrate availability (Parkin et al. 1999) or the metabolic limitations that closely describe fatigue occurring during prolonged exercise in temperate conditions (Maughan et al. 2007). However, the rate of carbohydrate oxidation is increased when exercise is performed in warm conditions (Febbraio et al. 1994, 1996b). This response appears to be caused by increased rates of muscle glycogen oxidation (+25%), and is accompanied by a reduction in exogenous carbohydrate usage (Jentjens et al. 2002). While the rate of muscle glycogen use is increased during exercise undertaken in warm conditions, relatively high muscle

glycogen concentrations have been reported at the point of fatigue (Parkin et al. 1999), suggesting that substrate availability is not limiting when exercise is undertaken in the heat (Maughan et al. 2007). Moreover, there is a significant cardiovascular component to fatigue in the heat, primarily driven by the competing demands of provision of oxygen delivery to exercising muscles and vital organs and increased thermoregulatory demand of skin blood flow requirements for heat dissipation by the processes of conduction and convection to aid heat loss (Sawka et al. 2011). Dehydration during exercise in the heat further exacerbates the thermal and cardiovascular strain, and further impairs aerobic performance (Casa et al. 2010). It is evident exercise in the heat is limited by a plethora of mechanisms of cardiovascular, metabolic and central origins.

### 2.1.2 *Chronic responses*

The effects of heat acclimation on sub-maximal exercise performance can be remarkable, allowing acclimated individuals to complete a task that in an un-acclimated state could not be completed. Pandolf and Young (Pandolf and Young 1992) reported that of 24 participants, all reached voluntary fatigue before completing a 100-min walk in 49°C and 20% RH on the first day of exposure. By day 3, 10 were able to complete the 100-min, and after 5 days 19 had completed it, all but one were successful by the seventh acclimation day. A recent meta-analysis by Tyler and colleagues (Tyler et al. 2016) identified fifty two articles examining the effect of heat acclimation or acclimatization on exercise capacity (n = 26) or performance (n = 26). Of note, heat acclimation was shown to have a moderately beneficial effect on exercise capacity and performance, irrespective of the regimen employed (i.e. constant work-rate, clamped hypothermia). While the most common approach in the literature is to utilize 7-14 days of heat exposure, longer regimens were more effective

than shorter ones and it is suggested that adaptations to heat acclimation are greatest when regiments exceeding 14 days are employed. Nevertheless, the average improvement in exercise performance in the heat identified in the meta-analysis was ~7%. The ergogenic effect of heat acclimation on subsequent performance in the heat is unequivocal. Cycle time-trial performance has consistently been shown to be improved in the heat after heat acclimation in both laboratory (Lorenzo et al. 2010; Keiser et al. 2015; Rendell et al. 2017) and field settings (Racinais et al. 2014b). In the latter study, Racinais and colleagues showed that cycling time trials (43 km) undertaken in hot outdoor (i.e. field setting) conditions (~37ºC) were initiated at a similar power output to that of a time trial conduced in cold conditions (~8ºC). Subsequently however, a marked decrease in power output occurred in the heat, which was partly recovered after one week of heat acclimatisation and almost fully restored after two weeks. The fact that after 2 weeks of heat acclimatisation, cycle time trial performance in the heat can be restored to similar levels as that observed in cool environments is testament to the remarkable adaptive capacity of the human body, and the extraordinary ability to adapt to environmental challenges.

## 2.2 **Sprint performance in the heat**

Whilst there is a large body of research examining the role of heat stress during endurance training, there remains comparatively little research on the effects of hot ambient environments on sprint or intermittent sprint performance.

### 2.2.1 *Acute responses*

It is generally regarded that power output in a single sprint is enhanced when muscle temperature is elevated by either passive local muscle heating (Sargeant 1987; Gray et al. 2006a), active warm ups (Yaicharoen et al. 2012a), passive heating to elevate core temperature 1°C (Linnane et al. 2004) or prior exposure to hot ambient conditions

including a warm-up (Falk et al. 1998; Ball et al. 1999). It has been reported there is a 4%/°C improvement in vertical jump performance as muscle temperature increased (Bergh and Ekblom 1979). After 45 minutes of leg immersion in a water bath set to 44 °C, which induced muscle temperatures of 39.3 °C, Sargeant and colleagues (Sargeant 1987) reported an ~11% increase maximal peak force and power output during a 20s all out sprint cycling effort. Conversely, cooling the legs in 18 and 12 °C water baths, which resulted in muscle temperatures of 29.0 and 31.9°C resulted in reductions in power output of 12% and 21%, respectively. The magnitude of the temperature effect was dependent on pedal cadence; the optimum cadence to maximize power output increased proportionally with muscle temperature. At 54 and 140 revolutions per minute, there was a 2% and a 10% improvement in power output for every 1 °C increase in muscle temperature at 3 cm depth, respectively (Sargeant 1987). Further, recent research has investigated the use of passive heating protocols to maintain muscle temperature prior to competition and improve sprint cycling performance (Faulkner et al. 2013). The maintenance of post warm-up muscle temperature with heated garments improved peak and average power during a single 30s Wingate task by 9.6% and 9.1% respectively. Furthermore, Ball and colleagues (Ball et al. 1999) found that power output during 2 bouts of all out 30s sprints, separated by 4 minute recovery, was improved when participants completed the task in a hot (30°C) compared to thermo-neutral (19°C) environments. However, heating is not always shown to enhance single sprint performance (Dotan and Bar-Or 1980; Backx et al. 2000; Bishop and Maxwell 2009). The exact nature of these discrepancies is unclear, but may relate to factors such as; active or passive warming, heat maintenance strategies, testing environment – temperature and humidity, time of day,

hydration, and nutritional status, which might influence the magnitude of heat- related effects on sprint performance.

Several mechanisms have been proposed to explain the ergogenic effects of increasing temperature on sprint performance, although the precise mechanisms by which temperature elevations may improve sprint performance are not fully understood. Increased ATP production from a more rapid utilization of phosphocreatine in fast twitch $II_A$ muscle fibers has been suggested as an explanation for enhanced sprint performance (Gray et al. 2006). Similarly there have been observations of increased activity of glycolytic enzymes to facilitate ATP production after heating, and adenine nucleotide degradation at high muscle temperature (Stienen et al. 1996; Febbraio et al. 1996) However, Sargeant and Rademaker (Sargeant and Rademaker, 1996), suggested that the contribution of type II fibres was unchanged after heating, and instead observed a preferential increase in power output via type 1 fibres. It has also been suggested that muscle fibre conduction velocity is increased in the heat, due to increased activation of individual sarcomeres at higher temperatures (Farina et al. 2005; Gray et al. 2006). To determine the effect of temperature on human compound action potentials, Bolton and colleagues (Bolton et al. 1981) cooled and heated the upper limbs. As skin temperature increased from 22°C to 31°C, compound muscle action potential of the motor and sensory propagated at a faster conduction velocity (e.g., 1.64–2.31 m/s/°C). The biochemical and contractile adaptations of increasing temperature have the global effect of an increased rate of cross-bridge cycling within the muscle tissue (Karatzaferi et al. 2004), which is likely to facilitate increased pedal cadence and thus greater power output (Ball et al. 1999). Power output could similarly be improved by increased torque at a similar pedal cadence. However, while passive skin cooling was shown to impair peak torque during

isokinetic contractions of the knee extensors, no significant differences in peak torque were observed after heating (Cheung and Sleivert 2004), although it should be noted participants were pre-heated and performed all trials in a hyperthermic state. Whether power output is increased during whole body sprint exercise at a clamped pedal cadence (i.e. isokinetic) is unclear.

While heat can modulate one off sprinting, multiple sprints are often undertaken in training and competition in sporting environments. Sprint performance can be further sub-divided into repeated sprint and intermittent sprint. Subtleties exist between the two definitions. Repeated sprints are typically characterized by the short sprint bout with brief recovery periods (<60s), and marked decrements in performance, while intermittent sprint protocols utilize larger recovery periods (60-300s) to facilitate more complete restoration of power output (Girard et al. 2015). This review examines only the effect of heat on intermittent sprint performance, before exploring the potential of heat acclimation to improve intermittent sprint performance.

Intermittent sprint performance maybe enhanced in individuals exercising in warm environmental temperatures provided there is only a mild increase in body core temperature. During a series of five 15s cycling sprints, interspersed with 30s of active recovery, average power output was 8% higher in 35 °C versus the same task performed in 22 °C. Importantly it was reported that core body temperature, oxygen uptake, heart rate and blood lactate concentration were similar between conditions during the 60 minute post-exercise recovery period (Falk et al. 1998). Also, after 10 x 6s all out cycling sprints, voluntary activation and resting twitch amplitude of the knee extensors were similar between hot (35°C) and temperate environments (24°C), in the presence of only marginally increased core temperature in the heat versus control (38.1 °C vs 37.7 °C, respectively). However heat exposure led to a ~3% improvement

in repeated sprint performance (Girard et al. 2013). Therefore, the performance benefits associated with heat exposures may depend upon the level of concomitant core body hyperthermia. While single bout sprint performance and muscle function are improved with heating, intermittent sprint tasks performed in the heat have been shown to result in reduced performance towards the end of prolonged exercise tasks (Morris et al. 1998; Morris et al. 2000). Drust and colleagues (Drust et al. 2005) reported that repeated sprint performance after an intermittent protocol (5 x 15s sprints interspersed by 15s rest period) designed to elevate both core and muscle temperature was impaired in hyperthermic compared to normothermic participants, where exercise was undertaken in hot (40°C), and cool (20°C) conditions. Similarly, participants ran almost twice as far during a prolonged intermittent shuttle running test in 17°C than 33°C, and exhibited a greater decline in average 15m sprint performance in the heat (Morris et al. 2005). There was a strong correlation (r= 0.90) between the rate of rise and core temperature and the reduction in intermittent sprint performance with the authors suggesting that the earlier onset of exhaustion during prolonged intermittent shuttle running in the heat is associated with the magnitude of hyperthermia. Supporting this assertion, during intermittent sprint protocols in the heat but where the rise in core temperature is small, there appears to be no effect on intermittent performance. Indeed during 35minutes of intermittent cycling comprising of 8 maximal 6s sprints, interspersed by 5minute of passive and active recovery in 24°C and 40°C, heat exposure did not alter either peak power output or related muscle activation and neuromuscular efficiency in the absence of hyperthermia (Almudehki et al. 2012). Average core temperature in the cool and hot condition reach only ~37.6°C, and 37.7°C, respectively. Similarly, intermittent sprints consisting of 80mins of 4s sprint, 100s active recovery and 10s passive recovery, only increased core

temperature to 38.6°C in 36°C heat, which was insufficient to impair performance vs that of a cool environment (23°C) (Yaicharoen et al. 2012b). It is therefore posilbe that interventions (i.e. heat acclimation) to attenuate the rise in core temperature may enhance intermittent sprint performance.

### 2.2.2 *Chronic responses*

There is extremely limited research on the effect of heat acclimation on sprint or intermittent sprint performance. No differences were observed in sprinting speed or the decline in repeated 15m sprint performance after four 30-45min exposures to 30°C sessions (Sunderland et al. 2008). Whilst the efficacy and adaptive response to a relatively short heat acclimation regimen is questionable, it was reported that distance run was increased, thermal comfort improved and rectal temperature decreased, suggesting at least some level of heat acclimation was present. However Castle and colleagues (Castle et al. 2011) observed a 2% increase in peak power output during a 40min intermittent sprint protocol in the heat after 10days of heat acclimation. Such discrepancies are expected in such limited literature owing to different heat acclimation regimens, and mode of exercise test, and no clear consensus exists on the effect of heat acclimation on intermittent sprint performance.

Nevertheless, there is some evidence to suggest a mechanistic hypothesis for an augmented effect of heat acclimation on intermittent sprint performance. In animal models heat exposure has been shown to enhance mitochondrial adaptations (Tamura et al. 2014) and increase cell proliferation potential and muscle protein content (Uehara et al. 2004). Only 2 studies have examined this in human subjects. One study reported that 10-weeks of 8h/day localized passive quadriceps heating increased maximum isometric force during knee extension, and increased mean cross-sectional area of the vastus lateralis and rectus femoris (Goto et al. 2011). The

other reported increased evoked peak twitch amplitude, increased maximal voluntary torque production and an improvement of the relative torque/EMG linear relationship after 11days of 1/h day whole body heating (Racinais et al. 2017b). However, both these studies utilized a passive heating protocol, and explored only single, isolated muscle models. It is unclear whether such adaptations would be prevalent during whole body exercise.

## 2.3 **Heat acclimation strategies to combat heat stress**

While there are many acute strategies to attenuate the negative effects of heat stress on performance (*i.e.* pre-cooling), acclimatizing to the heat in the weeks prior to competition is regarded the primary strategy in preparation for competition in the heat. It is well regarded that natural heat acclimatization in the environment the athlete will compete in is the most effective means for heat adaptation (Racinais et al. 2015a), although heat acclimation, artificial replication of the environment, sufficiently reproduces the physiological stress and thus induces adaptation to convey a benefit. Despite this, the optimal approach to induce these adaptation is unclear. There are 2 main approaches to heat acclimation in the literature: constant work-rate, and controlled hyperthermia.

Constant work rate protocols are the most commonly reported method to induce heat acclimation. Participants typically exercise at a set power output (i.e. during cycling), or velocity (i.e. during running), for a specified time period each day. However, since heat storage is reduced as adaptation develops, body temperatures, and overall thermal load decrease as acclimation progresses, constraining the potential adaptation. Controlled hyperthermia (sometimes called isothermal), methods clamp core temperature above the sweating threshold, using intermittent work/rest periods. Whilst this approach was first reported in the 1960's (Fox et al. 1967), it has gained

popularity in heat acclimation research more recently (Patterson et al. 2004, 2014). The premise of this approach is that it better maintains the thermal stimulus, as the workload increases at adaptation progresses to maintain a set core temperature (~38.5°C). The use of controlled hyperthermia protocols, where core temperature is maintained throughout the acclimation period (e.g. 38.5°C), may facilitate a continual adaptive stimulus and maintain plasma volume expansion for a long as 22days (Patterson et al. 2014). Clamped hyperthermia models are practically unsuitable outside of laboratories, and even within a laboratory setting participants tend to spend large portions of time at rest to maintain their target core temperature (Gibson et al. 2015a).

## 2.4 **Haematological adaptations to heat acclimation**

Expansion of blood volume (hypervolaemia) has been well documented as an adaptive response to endurance exercise training (Coyle et al. 1990). Expansion of the plasma volume compartment accounts almost exclusively for hypervolaemia within the first 4 weeks of endurance training; after this time blood volume expansion may be attributed equally between plasma and red cell volumes (Convertino 1991a). The addition of a thermal stimulus to exercise increases the magnitude of the plasma volume response, such that plasma volume expansion is one of the primary adaptations when repeated exercise is undertaken in warm environments (Périard et al. 2015). Expansion of the plasma volume has historically been suggested as a critical factor in heat acclimation (Senay et al. 1976; Nielsen et al. 1997). Indeed, plasma volume expansion has clear benefits in thermally challenging environments, including greater vascular filling and stroke volume to support cardiac output thereby minimizing the trade-off between skin and muscle blood flow, and increased specific heat of the blood to marginally reduce the skin blood flow requirement (Sawka et al. 2011).

### 2.4.1 *Regulation of blood volume*

The original documentation of blood compartments may be attributed to Hippocrates (c.400BC) and the application of the four humors to medicine. It was suggested that the body contains four fluids; blood, phlegm, yellow bile and black bile, and that health was primarily determined by the correct proportion of each. Fahraeus (Fahraeus 1921) suggested this primitive observation related to the erythrocyte sedimentation rate of clotting blood in a test tube, separating into serum (yellow bile), the clot (phlegm), oxygenated red blood cells (blood) and deoxygenated red blood cells (black bile). Even historically, tight regulation of blood volume compartments was recognized as critical to human health. Blood volume is a tightly controlled, homeostatic process primarily regulated by the kidney.

Plasma volume is modulated by alterations in blood pressure and vascular filling pressure. During disturbances to plasma volume, afferent signals from multi-site baroreceptors which are integrated in the central nervous system generate efferent signals to restore homeostatic blood volume balance, controlled by renal sodium and water excretion via hormonal modulations (Dunn et al. 2007). Sodium transport is regulated by angiotensin II, which increases sodium transport and thereby increasing sodium retention along the proximal tubules, thick ascending limb of the loop of Henle, distal and collecting tubules. At the site of the collecting tubules, aldosterone acts by stimulating sodium transport from the tubular fluid into the interstitium. Both angiotensin II and aldosterone increase sodium retention and consequently fluid volume in the body. Antidiuretic hormone (vasopressin), increases fluid permeability in the late distal tubules and collecting tubules enabling water to diffuse from the tubular fluid into the hypertonic interstitium. This reduces urine volume and therefore water loss, resulting in increased blood volume.

Blood volume also compromises of the red cell compartment. Red blood cell mass is highly responsive to hypoxia and tightly regulated to ensure adequate tissue oxygenation. The kidney detects small changes in blood oxygen tension, affected by the concentration of haemoglobin, arterial oxygen tension, haemoglobin-oxygen affinity and renal blood flow (Jelkmann 1992), and synthesizes or inhibits erythropoietin production. Erythropoietin is produced within the kidney by epithelial-like cells lining the peritubular capillaries (Lacombe et al. 1988; Koury and Bondurant 1988). Circulating erythropoietin exerts an effect on target cells in the bone marrow by interacting with specific receptors on the surface of these cells (Jones et al. 1990). This stimulates the release of marrow reticulocytes and mobilization of marrow progenitor cells. Iron bound to transferrin is taken up by the developing erythroid cell where it is split off and used for haemoglobin synthesis.

Together, the plasma volume and red blood cell components are coordinated in the process of regulating haematocrit. The kinetic of blood volume restoration after blood donation provides a key insight into the chronic nature of blood volume regulation. Alterations in plasma volume are a transient phenomenon and compensation from blood loss can occur very rapidly within 24-48 hours (Tølløfsrud et al. 1995, 1998). Mobilization of interstitial fluids from organs such as skeletal muscle, and skin with large tissue mass and fluid reservoirs primarily contributes to restoration of plasma volume (Länne and Lundvall 1992). In contrast, complete recovery of the red blood cell volume or HBmass is much slower, with a mean recovery of 36 days after a standard 550mL donation of whole blood (Pottgiesser et al. 2008). The interaction of recovery of plasma volume and red blood cell mass to achieve optimal haematocrit is uncertain, and is not well understood. The need to regulate haematocrit is primarily recognised in the relation of oxygen delivery to haematocrit. At low haematocrits,

oxygen carrying capacity of the blood is impaired, while high haematocrits impair oxygen delivery, due to increased viscosity of the blood, increased resistance and hence decreased flow (Fan et al. 1980). Therefore haematocrit is best regulated around an inflection point yielding optimal oxygen supply. It has been suggested plasma volume homeostasis is due to the regulation of blood vessel radius and endothelia response to changes in shear rate and blood viscosity (Birchard 1997). Others have suggested an intrinsic sensing and effector mechanism function directly of the kidney, coordinating the relative plasma volume and red blood cell volume mediated by the tissue oxygen tension (Dunn et al. 2007).

### 2.4.2 *Measurement of blood volume*

The accurate measurement of blood volume has been postulated in medical science since Harvey's discovery of the circulation of the blood in 1628. The earliest attempts to measure blood volume involved exsanguination and washing out the circulatory system of two criminals to weigh and quantity the blood obtained (Welker 1854). It was established then, that one thirteenth of body weight was blood. The failure to make secondary measures using such a technique renders it largely impractical. Currently, a plethora of techniques are available for determination of plasma volume. These can be categorised into two approaches; direct measurement by determination of the concentration of a plasma marker, or indirectly by deriving plasma volume from red cell volume and haematocrit. However, the accuracy of estimating plasma volume from the latter is uncertain and it has been suggested both should be directly and independently measured for greater accuracy (Gómez Perales 2015).

All known methods for blood and plasma volume determination to date are based on dilution techniques. Early dilution techniques involved introducing a known quantity of water, saline, serum or blood into the intravascular space and determining the blood

volume from the change in specific gravity of the blood, or the concentration of plasma solids, haemoglobin or red cells (Meek and Gasser 1918). However, the rapid dilution of water and salt solutions out of the blood make such a technique inaccurate, and new techniques were developed based on dilution molecules that do not leave the bloodstream so rapidly. Plasma markers with a high affinity for plasma proteins were the most common dye dilutions, with T-1824 (Evans Blue) the most frequently utilized. The basic premise for dilution techniques remains the same today and calculated from the relationship between a known volume and concentration of a plasma markers and the subsequent concentration of the marker in a blood sample. Plasma volume can be estimated from the following formula:

*PV = Volume injected x concentration of marker/concentration of maker in blood sample*

Evans Blue is considered a good label for the plasma protein albumin and as strong indicator for intravascular space (Gibson and Evans 1937), although its reproducibility has been debated (Henschen et al. 1993; el-Sayed et al. 1995). Other dye dilutions using a similar principle include; Vital Red (Meek and Gasser 1918), Congo Red (Bazett et al. 1940) and Indocyanine Green (Bradley and Barr 1968), although the latter is not particularly stable (Henschen et al. 1993), but does provide comparable plasma volumes to albumin labelled with Evans Blue (Bradley and Barr 1968) or radioactive iodine (Haneda and Horiuchi 1986). The use of radioactive tracers as albumin markers began approximately 60 years ago. Radioactive isotope methods are considered the gold standard for determination of plasma volume, specifically radioiodine marking of human serum albumin as the plasma label (International Committee for Standardization in Hematology, 1980). Labelling albumin with $^{131}$iodine, and yields good agreement with Evans Blue (Schultz et al. 1953). However, the

inherent danger of radioactive isotope administration *in vivo* limits the application of this technique in research and athletic populations. An alternative approach to labelling albumin is the use of polysaccharides, originating with acacia gum (Meek and Gasser 1918). More recently hydroxyethyl starch, a synthetic carbohydrate polymer, has been employed as a plasma marker (Tschaikowsky et al. 1997). The technique gives good agreement with the conventional carbon monoxide technique, although this was observed on a group of hemodynamically stable and anesthetized patients which cannot account for the effect of fluid shifts on plasma volume measures in an exercising populations. Furthermore, 10% hydroxethyl starch is hyperoncotic, and thus expands the plasma volume whilst simultaneously measuring it.

Plasma volume can be calculated indirectly by the relationship of the red blood cell volume and haematocrit by the following formulas:

$$BV\ (ml) = (Hb\ mass\ (g)/Hb\ (g \cdot dl^{-1})) \times 100$$

$$RBCV\ (ml) = BV\ (ml) \times (Hct/100)$$

$$PV\ (ml) = BV - RBCV$$

Pentavelant chromium ($^{51}Cr$) is a standard radioactive labelling (International Committee for Standardization in Hematology, 1980). $^{51}Cr$ enters red cells and trivalent chromium binds to β chains of haemoglobin to form a suitable labelling complex. Other radioactive techniques include administration of technetium sestamibi ($^{99m}Tc$), indium-111 ($^{111}In$) and indium-113m ($^{113m}In$). Whereas $^{51}Cr$ is stable and has a long half-life (27.7 days) meaning red cells can be tracked over a series of weeks, $^{99m}Tc$, $^{111}In$ $^{113m}In$ have lower radioactivity and their short half-lives allows for repeated measures to be made in the same subject at short intervals, although a correction

factor must be applied for the radioactive decay between the counting of one sample and another. While radioactive isotopes provide valid and reliable determination of the blood volume (Gómez Perales 2015), their routine use is limited.

Dilution of inhaled carbon monoxide (CO) to determine red blood cell volume has been employed for over a century (Haldane and Smith 1900; Douglas et al. 1912). The principle of the CO-rebreathing technique is to derive HBmass from the difference in carboxyhaemoglobin concentration in the blood before and after rebreathing a known volume CO, and the binding capacity of haemoglobin for CO (1.39ml $g^{-1}$). The technique has undergone multiple refinements and is now frequently utilized in clinical practice and sport medicine for routine determination of HBmass and blood volume (Barker 1998). The traditional CO-rebreathing technique requires a relatively long time of inspiration (10 – 15 minutes) of a mixture of $O_2$ and CO, and multiple venous blood samples, making it an impractical tool for routine use in sport performance. The optimised CO-rebreathing technique as proposed by Schmidt and Prommer (Schmidt and Prommer 2005) requires minimally invasive capillary samples and 2 minutes of rebreathing a small (1.2g/kg body mass) bolus of CO. This technique attains both reliable and valid results for HBmass compared to the traditional CO-rebreathing technique (Thomsen et al. 1991) and the gold standard technique of $^{51}Cr$ radioactive labelling (Schmidt and Prommer 2005).To date the optimised CO-rebreathing technique remains the most viable and practical tool for repeated blood volume measures.

### 2.4.3 *Heat acclimation and plasma volume expansion*

Plasma volume expansion has received much attention for its importance in conveying a thermoregulatory advantage during a thermal challenge. Exercise in the heat provides a profound challenge to the human thermoregulatory system. From the onset

of exercise in the heat, there is a progressive haemoconcentration caused by a decrease in plasma volume as fluid shifts from the intravascular to interstitial and intracellular compartments of contracting skeletal muscle (Van Beaumont 1973; van Beaumont et al. 1981). Retention of the plasma volume is a function of filtration and absorption acting on the capillary beds (Harrison 1985). During exercise, a combination of increased arterial pressure and intramuscular compression on venules in the microcirculation boosts capillary hydrostatic pressure, forcing plasma ultrafiltrate into the extravascular compartment (Levick 1991). This effect is exacerbated with heat stress as greater skin blood flow facilitates opening of more capillary channels and greater hydrostatic pressure in the capillary beds (Rendell et al. 1993). Thus fluid shifts into the extravascular space and results in a more pronounced haemoconcentration (Harrison et al. 1983). Additionally exercise in the heat induces a marked increase in muscle lactate accumulation (Febbraio et al. 1994) generating an osmotic gradient which favours movement of plasma ultrafiltrate into myocytes and the interstitial compartment. Finally, insensible fluid losses from both perspiration and respiration further augment the reduction in plasma volume. With repeated heat exposures, the body undergoes several haematological adaptations to counteract this haemoconcentration.

Haematological alterations from repeated heat exposures were first reported nearly a century ago by Barcroft and colleagues (Barcroft et al. 1922). Plasma volume expansion is one of the primary adaptations and occurs rapidly within the first few days of heat acclimation (Senay 1972; Bonner and Edwards 1976; Sawka and Coyle 1999), causing a haemodilution as haemoglobin concentration and haematocrit falls. The processes by which plasma volume expands has been a widely contentious area of research. Historically, plasma volume expansion was viewed as a selective vascular

compartment enlargement at the expense of the interstitial fluid, an effect determined by collective actions of elevated plasma colloid and crystalloid pressures (Senay et al. 1976; Harrison et al. 1981). An increase in crystalloid pressure was suggested as the result of increased retention of sodium chloride facilitated by higher circulating aldosterone and vasopressin. Equally an influx of protein into the intravascular compartment by reduced capillary leakage (Harrison et al. 1981) and increase delivery of protein from the cutaneous interstitial space via the lymphatic system can account for greater colloid pressure (Senay et al. 1976). These pressure changes alter the Starling forces, favoring absorption and result in an intravascular fluid influx from the interstitial compartment (Levick 1991). However, recent research in which 6 fluid compartments were concurrently measured throughout a 17 day heat acclimation protocol found plasma volume was not selectively expanded, with an increase in total body water retention supporting an intravascular, extracellular and interstitial fluid compartment expansion (Patterson et al. 2014). Indeed, no fluid compartment is favorably expanded at expense of another, and heat acclimation results in an increase in total body water. The magnitude of expansion varies greatly between individuals with reported adaptations of between 3 - 27% (Senay 1975; Patterson et al. 2014). However, it has frequently been reported that plasma volume returns to pre-treatment baseline levels as adaptation progresses, despite individuals retaining improved thermoregulatory function and exercise capacity (Bass et al. 1955; Wyndham et al. 1968; Shapiro et al. 1981). This apparently transient effect may be an experimental artefact of constant work-rate protocols, as the physiological overload continually declines with progressive adaption to heat. The use of clamped hyperthermia protocols, where core temperature is maintained throughout the acclimation period (e.g. 38.5°C), may facilitate a continual adaption stimulus and maintain heightened

plasma volume expansion (Patterson et al. 2014). Heat acclimation unequivocally improves subsequent exercise performance in the heat (Lorenzo et al. 2010; Racinais et al. 2014b) mediated by improved thermoregulation and cardiovascular stability. Plasma volume expansion is frequently reported as the primary mechanism to support these adaptations, by maintaining fluid balance in the vascular space (Convertino 1987), restoring central venous pressure and thus improving skin blood flow and heat loss through sweating (Ekblom et al. 1976; Fortney et al. 1981a). Furthermore augmented plasma volume increases the specific heat of the blood to marginally reduce the skin blood flow requirement (Sawka et al. 2011). However it has also been suggested in animal models that alterations in the mechanical contractility of the cardiac muscle may improve ventricular function and explain a cardiovascular ergogenic effect of heat acclimation independently of plasma volume expansion (Horowitz 2002). Furthermore, in a series of experiments, (Bass et al. 1955; Bass et al. 1958) suggested that the magnitude of the plasma volume expansion was not related to improved heat loss and was not required for heat acclimation. Firstly, subjects lived continuously for 2 weeks in the heat (49°C, 20%rh day; 38°C, 20%rh night), with well controlled diet, exercise and hydration. Plasma volume expanded ~27% by day 5 and ~14% by day 14, and the rise in core temperature with exercise was attenuated throughout the 2 weeks. Subsequently, their second experiment followed nearly identical procedures, except that subjects were exposed to 150min of daily heat exposure. Plasma volume did not increase, but core temperature response was similar to the original experiment (Bass et al. 1958). Additionally, Mitchell and colleagues (Mitchell et al. 1976) showed attenuation of the rise in core temperature to occur from day 5 through to 10 of heat acclimation, and therefore almost all of the plasma volume expansion occurred before any change in heat loss. Corroborating

this, plasma volume has been reported to return to pre-treatment levels after 1 week withdrawal of heat exposure despite maintenance of cardiovascular stability and incremental ramp exercise performance (Garrett et al. 2009), indicating it is not clear they are causally related.

### 2.4.4 *Plasma volume expansion, cardiovascular stability and thermoregulation*

A principal feature of plasma volume expansion is that it may support cardiovascular stability, indicated by a raised stroke volume and lowering of heart rate. Cardiovascular stability is impaired when exercise is performed in the heat. Stroke volume is significantly reduced with an increased heart rate to meet the greater cardiac output required to sustain competing demands of skeletal muscle and cutaneous blood flow. The reduction in stroke volume is described by a downward shift in the Frank-Starling curve, due primarily to a reduced pre-load; a function of blood volume redistribution, venous return, and systemic vascular conductance. Heat stress induces a cutaneous vasodilation to increase skin-blood flow, facilitating evaporative cooling. Cutaneous vascular volume is increased (Deschamps and Magder 1990) at the expense of central reservoirs as blood translocates out of the splanchnic and renal pools (Crandall et al. 2008). Any decrease in central venous pressure will reduce left ventricular filling pressure, left-ventricular end diastolic volume, and therefore pre-load and stroke volume (Wilson et al. 2009; Wilson and Crandall 2011). Thus heart rate is increased to meet the blood flow requirements of cardiac output. Furthermore, during prolonged exercise in warm environments heart rate responses are observed to progressively increase over time (Rowell 1974). This cardiac drift has been associated with falling pulmonary artery pressure and stroke volume (Rowell 1974). The enhancement of cardiovascular stability which occurs with heat acclimation, manifested by a lowered heart rate for any given power output, is widely attributed to plasma volume expansion

(Senay et al. 1976; Shapiro et al. 1981; Harrison 1985; Sawka et al. 2000). Indeed, artificial plasma volume expansion has frequently been reported to lower the heart rate response to exercise in the heat (Fortney et al. 1981a; Nose et al. 1990; Deschamps and Magder 1990; Keiser et al. 2015), but not always (Watt et al. 2000). The later of these studies is the only one to have employed a time-trial performance measure, which may contribute to the disparate findings. At sub-maximal exercise intensities in the heat, both skeletal muscle and skin blood flow are perfused, with limited competition between the two. Thus increased plasma volume facilitates higher venous return, stroke volume and lowered heart rate responses. Furthermore, the core-to-skin temperature gradient is relatively large and so the skin blood flow requirement is correspondingly lower. As exercise intensity increases, competition for blood between the skeletal muscle and skin is increased. Any increase in plasma volume allows for greater skin perfusion by better maintenance of cardiac output and heart rate to meet the increased skin blood flow demands. While plasma volume expansion is widely accepted as the mediator of the cardiovascular improvement observed with exercise training (Fellmann 1992) and early during heat acclimation (Nielsen et al. 1993), it may merely be a supportive adaptation to enable lower heart rate responses to sub-maximal exercise, but may not mediate cardiovascular stability during maximal performance exercise.

It is frequently proposed that plasma volume expansion may convey a thermoregulatory advantage, although it is not clear that plasma volume expansion *per se* and improved thermoregulation are causally related. Several studies have shown that IV plasma volume expansion has no effect on core temperature, skin temperature, or sweat rates (Fortney et al. 1981a; Hubbard et al. 1984; Watt et al. 2000), during exercise tasks in the heat. Only one study has observed a significant

universal attenuation of core temperature with IV plasma volume expansion (Fortney et al. 1981a). However, skin temperatures in both active and inactive muscles and whole body sweat rates were similar to the placebo condition. Furthermore, plasma volume expansion had no effect on the core temperature threshold for sweating or the average slope of the sweating rate-to-core temperature relationship at all sites. The authors concluded that plasma volume expansion attenuated the rise in core temperature mediated by greater cutaneous perfusion, although skin blood flow was not measured. The exact mechanisms responsible for a lower core temperature response are unclear. Another study observed a slight but significantly attenuated core temperature with plasma volume expansion but only after 45min of exercise (Nose et al. 1990). The reason for such a finding is unclear, although differences in study design may explain these disparate results. In this study, saline was infused at a constant rate throughout the 50minute exercise bout, affording greater yet un-physiological plasma volume retention during exercise. Whilst this better represents a drinking strategy which athletes might adopt it fails to account for the delayed transit and absorption of fluid within the gastro-intestinal tract. This study also reported small increases in skin blood flow with IV plasma volume expansion. However, the heat flux due to radiation and convection in the heat is extremely low compared with that due to evaporation, and small differences in skin blood flow would have only minor effects on heat transfer as sweat rate was not affected by volume expansion.

## 2.5 **Cross acclimation**

While heat acclimation has been well documented to improve $\dot{V}O_{2max}$ , and time trial performance in the heat in both a laboratory (Sawka et al. 1985) and field (Racinais et al. 2015b) settings, the effect of heat acclimation on performance in alternative

environments (i.e. cool, hypoxic) is highly contentious (Minson and Cotter 2016; Nybo and Lundby 2016) and no clear consensus exists on the efficacy of this approach.

### 2.5.1 *From heat to cool*

Since endurance performance peaks in relatively cool environments (10-14°C) (Ely et al. 2007), and deteriorates even with modest increases in temperature (Galloway and Maughan 1997; Ely et al. 2007), it is evident a thermal limitation can attenuate performance even in apparently thermo-neutral environments, For example, in cool environments (WBGT 13°C) competitive runners reached a peak core temperature of ~39.8°C during an 8km time trial (Ely et al. 2009). Many physiological mechanisms have been proposed to explain the potential benefit heat acclimation may convey on performance in cool environments; reduced oxygen uptake and a given power output (Sawka et al. 1983b; Young et al. 1985), increased lactate threshold (Young et al. 1985; Lorenzo et al. 2010, 2011), muscle glycogen sparing (Young et al. 1985; Febbraio et al. 1994), skeletal muscle force generation (Kodesh and Horowitz 2010), plasma volume expansion (Bass et al. 1958; Senay et al. 1976), improved myocardial efficiency (Horowitz et al. 1993) and increased ventricular compliance (Horowitz et al. 1986b). However, there are also potential ergolytic effects of heat acclimation which should be considered. Plasma volume expansion, or hypervolemia will induce significant weight gain, in line with the magnitude of expansion; a 10% increase in plasma volume would theoretically induce a 300mL increase in plasma volume for a typical 70kg male with 3L of plasma. Although small, this could be significant in weight bearing sports, for example hill climbing in cycling. Another interesting caveat is the induction of increase sweat response with heat acclimation. Whilst increased sweat rates increase the potential of evaporative heat loss, this also promotes accelerated dehydration, where body mass losses are not adequately replaced. Furthermore, in

tropical environments where evaporative sweat loss is already limited, dehydration occurs with minimal heat loss.

Heat acclimation has been reported to either improve or have no effect on exercise performance in cool environments (Table 2.1). Despite the limited available literature, the assertion that heat acclimation can improve performance in more temperate environments is not a recent contention. Indeed, more than three decades ago, Sawka and colleagues (Sawka et al. 1985) showed that nine days of 120min daily treadmill walking in 49°C, 20% RH increased $\dot{V}O_{2max}$ by ~4% in both hot and cool environments and maximal power output 4 and 2%, respectively. However, given that there was no control intervention, a training effect cannot be excluded. A lack of control, or adequate control group is a common feature in much of the available literature since this discovery. Indeed of the 7 studies that have found an ergogenic effect of heat acclimation on temperate exercise performance, only two included a control group (Scoon et al. 2007; Lorenzo et al. 2010). Without similar control training in a cool environment, the effect of training alone cannot be ruled out in providing at least some of the performance benefits observed. However, Scoon and colleagues (Scoon et al. 2007) used a crossover study design and observed a ~29% increase in running time-to-exhaustion after 3 weeks of sauna bathing immediately following each training session, versus controlled training. Of note, heat acclimation was passive so the ergogenic effect could not be attributed to an additional training effect. In two of these studies, the heat acclimation stimulus was combined with sleeping at altitude (Buchheit et al. 2013; Racinais et al. 2014a), and no differences were observed between groups. It is currently unclear whether the effects of heat and altitude stimulus combined are additive or conflicting with each other, and so this methodological approach may confound the results. It should be considered that the effect of heat

acclimation on temperate exercise performance was not the primary goal of these studies. An interesting finding of Hue and colleagues (Hue et al. 2007) was that 400m swim time was improved after 30days of an 8 day tropical heat acclimation stimulus, despite no effects after 10 days. This is surprising given that the benefits of heat acclimation are short-lived and transient, and generally decay within 1 month (Daanen et al. 2018). However, Lorenzo et al (2010) showed that 10 days of heat acclimation improved $\dot{V}O_{2max}$ and time trial performance in cool environments in trained participants, by 5 and 6%, respectively. Whist this study did employ a control intervention, the heat training was conducted at albeit a marginally relatively higher intensity than the control training, it was not a cross-over design, and some of the individual improvements after 10days of heat acclimation $\dot{V}O_{2max}$ (~10%) and TT (~15%) performance in the cool are remarkable, in already trained cyclists. These findings have generally not been replicated in more recent studies, with robust methodological designs (adequate control group, adequate wash-out). Karlsen and colleagues (Karlsen et al. 2015b) observed no improvement in peak aerobic power or a field based time-trial in highly trained cyclists. Similarly, Keiser and colleagues (Keiser et al. 2015) reported no improvement in maximal aerobic power, $\dot{V}O_{2max}$ or a 30min time trial under laboratory conditions after 10 days of heat acclimation. However, Rendell and colleagues (Rendell et al. 2017) found an increase in maximal aerobic power, lactate threshold and work done in a 30min time trial undertaken in cool conditions after 10 days of exercise heat acclimation. However, while the trial was a cross-over design, all training was undertaken in the heat, with an additional hypoxic sleeping intervention. Thus whilst performance was improved similarly in both interventions, a training effect along cannot be discounted.

There remains no clear consensus on the efficacy of heat acclimation on exercise performance in temperate environments. Studies are required with adequate control groups and matched training between hot and cool environments. Furthermore, since the differences in performance are likely to be small, and that endurance training promotes some phenotypical characteristics of heat acclimation (i.e. hypervolaemia), it is crucial that trained individuals with stable performance are tested. It should also be considered that it is not possible to blind participants to whether they are training in warm or cool environments. Therefore participants should be naive to the aims of the study.

**Table 2.1:** Human studies reporting effect of heat acclimation on subsequent exercise performance in temperate environments. Modified and updated from Corbett and colleagues (Corbett et al. 2014).

| Study | Sample | Heat Acclimation protocol | Control group | Performance Test | Effect |
|---|---|---|---|---|---|
| Sawka et al. 1985 | 13 soldiers | 9 days, 120min/day (49°C, 20% RH with exercise (40-50% $\dot{V}O_{2max}$ ) | None | Ramp exercise test (21°C, 30% RH) | 4% increase in $\dot{V}O_{2max}$ ($p < 0.01$). 4% increase in ramp test peak power ($p < 0.05$) |
| Morrison et al. 2002 | 9 highly trained cyclists | 7 days, 90min/day (37°C, 50% RH) with exercise (days 1 and 7 at 45% maximum power from $\dot{V}O_{2max}$ test, days 2-6 self-paced) | Randomized controlled trial with 2-week washout: 7days 90min/day (20°C, 50% RH) at matched absolute work rate (days 1 and 7), or RPE days 2-6 | 40-km cycling time-trial (20°C, 50% RH) | 0.4% faster relative to control. (35/54/11 % beneficial/trivial/harmful) |
| Hue et al. 2007 | 6 trained swimmers | 8 days (~30°C, 80% RH) 14 swim training sessions (30°C water) | None | 400m maximal swim test 19 and 30 days post heat exposure (27.1°C water) | 10% faster 30 days post heat exposure ( $p < 0.03$) |
| Scoon et al. 2007 | 6 trained distance runners and triathletes | 3 week normal running training with ~7 sauna exposures (90°C), ~31mins immediately post training | Randomized controlled trial with 3 weeks washout: 3 weeks usual training, no sauna | Run time to exhaustion at current best 5km speed (ambient temperature not reported) | 32% increase in run time to exhaustion ('almost certain >99% improvement') |
| Lorenzo et al. 2010 | 12 trained cyclists | 10 days, 100min/day (40°C, 30% RH with exercise (2 x 45min at 50% $\dot{V}O_{2max}$ , 10min recovery) plus usual training | 8 trained cyclists (4 undertook both experimental conditions: washout not reported) 10 days, 100min/day (13°C, 30% RH with exercise, 2 x 45min at 50% $\dot{V}O_{2max}$ , 10 min recovery) plus usual training | 60 min cycling time trial. Lactate threshold test. $\dot{V}O_{2max}$ test (13°C, 30% RH) | 6% increase in time trial work ($p$ = 0.005). 5% increase in power output at lactate threshold $p$ = 0.002). 5% increase in $\dot{V}O_{2max}$ $p$ = 0.004) |
| Buchheit et al. 2011 | 15 competitive soccer players | 7 days, 60-95min/day (~40°C, 27% RH), with exercise (50-83% heart rate max) | None | Yo-Yo intermittent recovery test level 1 (~22°C, 50% RH) | 7% increase in run distance ($p$ = 0.009) |

| | | | | | |
|---|---|---|---|---|---|
| Buchheit et al. 2013 | 17 professional Australian Rules football players | 14 days, 10 football skills training sessions (~70min, 29-33°C, 37-50% RH) plus 15h total incidental heat exposure and ~13h total additional interval/strength training (23°C, 55% RH) | None | Yo-YO intermediate recovery level 2 test (~22-23°C | 44% increase in run distance, run distance also increased 4 weeks post-intervention |
| Racinais et al. 2013 | 18 professional Australian Rules football players | 14 days, 10 football skills training sessions (~90min, 29-33°C, 37-50% RH) plus additional strength and conditioning sessions (~22°C) | None | Yo-YO intermediate recovery level 2 test (~22-23°C | 44% increase in run distance ($p < 0.001$) |
| Karlsen et al. 2015 | 18 well trained cyclists | 9 cyclists, 2 week outdoor training camp (~34°C, 18% RH), ~14h:40 per week | 9 trained cyclists completed matched training in a cool environment. 14h:17 per week | Incremental test and outdoor 43.4km cycle time trial | No change in $W_{max}$ ($p$ = 0.54). No change in $\dot{V}O_{2max}$ ($p$ = 0.55). No change in TT power ($p$ = 0.19). |
| Keiser et al. 2015 | 8 trained males | 10 days | Randomized controlled trial with 3 month washout of regular training with no heat exposures. | Incremental test and 30min cycle time trial (18°C) | No change $W_{max}$ ($p$ = 0.19). No change $\dot{V}O_{2max}$ ($p$ = 0.11). No change TT power ($p$ = 0.32). |
| Rendell et al. 2017 | 8 trained males | 11 days. Days 1, 6 and 11 heat stress tests (40°C, 50% RH) at 35% peak power for 60min. Days 2-5, 7-10 (40°C, 50% RH) isothermal (target core temperature 38.5°C) | Randomized controlled trial with 3 month washout. Heat vs heat plus altitude (8h per night). No cool training group. | Graded exercise test and 30-min cycle time trial (22°C, 50% RH) | 4% increase $W_{max}$ ($p$ = 0.011). 4 % increase in TT work done ($p$ = 0.045). No difference between interventions. |

### 2.5.2 *From heat to hypoxia*

The concept that heat acclimation might have beneficial effects in hypoxia was first proposed in the 1950's. Hiestand and colleagues (Hiestand et al. 1955) heat acclimated mice in an incubator maintained at 36-37°C for 10 to 14 days, after which the mice were subsequently drowned. Heat acclimation increased anoxic resistance to drowning by 14.5% after 10 days and by 28.9% after 14 days, and the authors proposed possible circulatory and metabolic adaptations. Further evidence for cross-tolerance between heat and hypoxia has been demonstrated in animal models by improved myocardial efficiency and enhanced cardioprotection during ichaemia and reperfusion injury in rats hearts when exposed to 30days of heat acclimation (Levi et al. 1993; Horowitz 2003). This could be explained by greater ATP preservation following heat acclimation caused by the transition from fast to slow myosin isoforms (Horowitz et al. 1986), and delayed acidiosis within the myocardium acclimation (Levi et al. 1993; Horowitz 2003). Evidence for cross-acclimation between heat and hypoxia is remarkably limited in humans with the predominant focus on the adaptation at a cellular level, particularly the heat shock response (HSR). At a cellular level, both heat and hypoxia have been shown to induce the HSR, characterized by a post-exercise increase in heat shock proteins (HSP) (Lee et al. 2014). HSPs are highly conserved molecular chaperones which facilitate the synthesis and folding of proteins, conveying a cytoprotective effect against subsequent thermal (Hutter et al. 1994) or ischaemic stressful insult (Levi et al. 1993). The cardiovascular, neurological and metabolic adaptations to heat acclimation, which previously mentioned in this review might enhance performance in the cool, may similarly apply to exercise performance in hypoxia. However, at the cellular level the heat shock response is likely to be implicated solely in exercise tolerance at altitude.

Both acute heat (Fehrenbach 2001, Lee 2014, Periard 2015) and hypoxic (Taylor 2011, Lee 2014) exposure have been shown to induce the heat shock response (Morimoto 1998), as exhibited by elevated levels of HSP72 in the hours following stressful exercise in these environments. Acclimation to heat or hypoxia is sometimes reported to increase resting levels of HSP72 (Maloyan et al. 1999; Yamada et al. 2007a; Mcclung et al. 2008; Magalhães et al. 2010), but not always (Marshall et al. 2006; Watkins et al. 2007). Enhanced resting HSP72 following heat acclimation is associated with reduced exercise-induced perturbations in the HSP response (*i.e*. acquired thermal tolerance), which may contribute to cross tolerance between heat and ischemic stressors (Maloyan et al. 1999). Further evidence for this assertion is the observation of increased resting peripheral blood mononuclear cell (PBMC) HSP72 protein (Lee et al. 2014, 2015) and HSP72 mRNA (Gibson et al. 2015b) following heat acclimation. Upon exposure to a standardized hypoxic exercise test, the post exercise induced increases in the HSP72 response was attenuated in heat-acclimated individuals (Lee et al. 2015; Gibson et al. 2015b). Together, these data support the existence of both cross-acclimation and cross-tolerance in humans.

However, the nature of the heat shock response is not fully understood, and while both heat and altitude exposure seemingly induce similar responses, the casual role of the heat shock response and common adaptive benefit remains equivocal. When an exercise heat acclimation protocol was undertaken with a heat shock response inhibitor (Quercitin supplementation 2000mg/day), both cellular (HSP70), and systemic heat adaptations (body core temperature) were attenuated compared to a non-supplemented control condition (Kuennen et al. 2011). However, adaptive responses to heat acclimation (i.e. reduced rectal temperature, reduced heart rate, increased sweat rate) has also been observed with no change in HSP72 levels

(Marshall et al. 2006; Watkins et al. 2007; Hom et al. 2012). Thus, the heat shock response in isolation may not lead to cross-acclimation per se.

More recently, researchers have suggested links between the heat shock response, and the metabolic pathways which govern HBmass (Salgado et al. 2014). Since the primary haematological outcome of altitude training is to increase HBmass, interactions between the heat shock response and those of the HBmass pathway may provide better insights into the nature of cross tolerance. In rodent models, acute heat stress has been shown to increase HIF1-$\alpha$ a, the master regulator of oxygen-regulated genes; erythropoietin receptor, erythropoietin, and vascular endothelial growth factor (Shein et al. 2005). Similar observations have been made after heat acclimation (Maloyan et al. 2005; Tetievsky et al. 2008; Assayag et al. 2012; Umschweif et al. 2013). Clinical data in human models further supports this interaction. HSP90 inhibitors have been shown to decrease bioavalibilty of HIF1-$\alpha$ in tumor cells, leading to degradation of HIF1-$\alpha$ and vascular growth (Mabjeesh et al. 2002). Interestingly, this was shown to occur independently of changes in oxygen tension. To the authors best knowledge only 2 studies have investigated the potential influence of heat acclimation on HIF1-$\alpha$ regulation (Keiser et al. 2015; Lee et al. 2016). While Keiser and colleagues were unable to detect HIF1-$\alpha$ in their samples, possible due to the timing of collection and the short half-life of HIF1-$\alpha$ in normoxia (Jewell et al. 2001), Lee and colleagues observed similar increases in HIF1-$\alpha$ after both heat and altitude acclimation interventions, strengthening support for this potential pathway.

Remarkably few studies have attempted to confirm whether cross-acclimation between heat and hypoxia leads to enhancement of exercise performance (Table 2.2). To the authors best knowledge only 3 studies have measured exercise performance,

or markers of, in hypoxia following heat acclimation (Heled et al. 2012; Lee et al. 2016; White et al. 2016) with confounding results.

To the authors best knowledge Heled and colleagues (Heled et al. 2012) were the first to examine the efficacy of a cross-tolerance model in humans. After 12 days of heat acclimation, cognitive function was improved in hypoxia, and an increase in power output at the onset of blood lactate accumulation (OBLA) was observed, despite no change in maximum aerobic capacity. However, their altitude stimulus was brief (10 min) and it should be noted that the primary aim of the study was to examine cognitive function, and a direct measure of exercise performance was not measured. In the study of Lee and colleagues (Lee et al. 2016) 21 males (7 per group) performed 10 daily, 60min training sessions at 50% $VO_{2max}$ in cool (18°C, 35% RH), hot (40°C, 25% RH), or hypoxic environments ($FiO_2$ 0.14%, 18°C, 35% RH). 16.1km time trial performance was improved equally in hypoxia after both the hot, and hypoxic training intervention, but was unchanged after control training. It was suggested that both heat and hypoxic acclimation elicited similar changes in basal HSP72, and HIF1-$\alpha$, and also provided evidence of acquired thermal tolerance, as shown by attenuation of post exercise HSP72 and HIF1-$\alpha$ responses to a controlled exercise stimulus. White and colleagues (White et al. 2016) found small and non-significant increases in maximal oxygen uptake and 16.1km cycle time trial performance at 4350m in 8 male cyclists after 10 days of exercise heat acclimation. Given that there was also no control group, it further cannot be ruled out that any change in performance was due to a training effect alone. A confounder of this study is that all participants habitually resided at mild altitude (1600m), and thus may already have benefited from some of the adaptations to support exercise performance at higher altitude, which could minimize the potential gains the heat acclimation intervention could yield. Given the limited research

available, methodological constraints (lack of control group in 2 of the 3 studies) and conflicting results, whether heat improves exercise performance during acute hypoxia remains equivocal.

**Table 2.2;** Human studies reporting effect of heat acclimation on subsequent exercise performance in hypoxic environments.

| Study | Sample | Heat Acclimation protocol | Control group | Performance Test | Effect |
|---|---|---|---|---|---|
| Heled et al. 2012 | 8 males | 12 days, 120min/day (40°C, 40% RH) with exercise at ~30% $\dot{V}O_{2max}$ | None | Incremental treadmill run (2400m) | No change in $\dot{V}O_{2max.}$ Delayed OBLA ($p$ = 0.003) |
| Lee et al. 2016 | 21 males | 7 males, 10 days, 60min/day (40°C, 25% RH) | 7 males completed matched training in cool conditions (18°C 35% RH) | 16.1km cycle time trial (3300m). | No change in $VO_{2peak}$. No change in lactate threshold. 4.8% faster cycle time trial performance ($p$ = 0.005) |
| White et al. 2016 | 8 males | 10 days, 2 x 50min, separated by 10min rest/day with exercise at 55% $\dot{V}O_{2max}$ (measured at 1600m). | None | Incremental cycling test and 16km cycle time trial (4350m) | No change in $\dot{V}O_{2max}$. No change in cycle time trial performance. |

## 2.6 **Research Aims Hypothesis**

The present thesis is comprised of a single research project, with four chapters examining the effects of long term heat acclimation on thermal, haematological and neuromuscular adaptations, and the repercussions of these on endurance cycling performance in hot, cool and hypoxic environments.

**Study One – Long term heat acclimation with a heart rate clamp**

*Aim*

1. Characterise the typical adaptations to a novel long-term, work matched, heart rate clamped heat acclimation protocol in a group of trained cyclists.

*Hypotheses*

1. Utilizing a heart rate based protocol would induce and maintain the typical adaptations to heat acclimation (i.e. expand plasma volume, increase sweat rate) over a long term (3 week) heat acclimation exposure.

**Study 2 – Haematological adaptations to heat acclimation**

*Aim*

1. Characterize the HBmass response to heat acclimation,
2. Establish the effect this may have on estimates of plasma volume.

*Hypotheses*

1. HBmass and plasma volume would both increase as a result of heat acclimation.

2. Estimates of changes in relative plasma volumes (i.e. Dill and Costill formula), would under-estimate true changes, where HBmass increases.

**Study 3 – Neuromuscular and metabolic adaptations to intermittent sprint training in warm and cool environments**

*Aim*

1. Explore the acute effect on performance of intermittent sprint training in hot and cool environments
2. Evaluate the neuromuscular and metabolic adaptations to heat acclimation, and its effect on intermittent sprint performance.

*Hypotheses*

1. Heat exposure would acutely decrease electromyography activity and impair intermittent sprint performance compared to cool environments
2. Intermittent sprint performance would be improved following heat acclimation

**Study 4 – Exercise performance and cross acclimation**

*Aim*

Examine the impact of heat acclimation on maximal oxygen uptake and cycle time trial performance in a hot, cool, and hypoxic environment.

*Hypotheses*

Heat acclimation would improve maximal oxygen uptake and cycle time trial performance in hot, cool, and hypoxic environments.

# CHAPTER 3

# GENERAL METHODS

This section provides an outline of the general methods employed transversally to the different chapter of the thesis. Any specific methods used for a study are detailed in the respective chapters.

## 3.1 Ethical Approval and Participants

### 3.1.1 *Ethical Approval*

The studies included in this thesis are part of a large scale research project approved by the scientific committee of Aspetar orthopaedic and sports medicine hospital (CMO/0000100/fj), by the external ethic committee of the Anti-Doping Laboratory Qatar (IRB: F2015000201) and by the Liverpool John Moores University ethics committee. The project was in compliance with the Declaration of Helsinki. Prior to the start of any testing, participants were fully informed of the nature, purpose, risks and discomforts of the study and, following the opportunity to ask any questions, gave their written informed consent to participate. Participants were made aware that they were free to leave the study at any time without reason or penalty.

### 3.1.2 *Participants*

Ten participants took part in the project. All participants were endurance trained male cyclists or triathletes from the local area. Their characteristics at inclusion were as follows: age 34 ± 7 yr; height 177 ± 6 cm; body mass 75.6 ± 7.5 kg; maximal oxygen uptake ($\dot{V}O_{2max}$) 4.50 ± 0.50 L/min; and maximal aerobic power 416 ± 39 W. All

participants undertook personal training regimes, and regularly competed in amateur endurance cycle races. Inclusion criteria included cycling at least 250km per week and undertaking cycle training for at least the previous 2 years. No participant had a prior history of heat illness.

Previous studies have shown clear differences in the effects of heat acclimation on exercise performance in the heat; however, the effect size is likely to be smaller when comparing the effect of heat acclimation on performance in cool or hypoxic environments. The sample size for this study was estimated based on A priori power calculations performed using G*power version 3.1.6. Assuming an effect size of 0.3 (small effect), alpha level of 0.05 and power of 0.8, 12 subjects were required to correctly reject the null hypothesis. Due to practical limitations, only 10 participants were recruited to the project.

Only male athletes were recruited to take part in the research project. Each testing block lasted for 5 weeks, crossing an entire female menstrual cycle. The menstrual cycle can influence plasma volume dynamics during exercise in the heat (Stephenson and Kolka 1988) and increased progesterone levels during the luteal phase have been shown to cause increases in both core and skin temperatures and alter the temperature at which sweating begins during exposure to both ambient and hot environments (Marsh and Jenkins 2002). Therefore females were excluded from the current project, as this may have contributed to confounding factors within our study design.

## 3.2 **General procedure**

### 3.2.1 *Project overview*

All participants completed two, 3-week training interventions consisting of exercise heat acclimation (EHA; 35.5 ± 1.8 °C, 59.0 % ± 7.7 RH) and work-matched exercise in a temperate (TEMP; 18.0 ± 0.5°C, 59.5 ± 9.9 %RH ) environment, in a counter-balanced cross-over design (Figure 3.1). The physiological adaptations to long-term (*i.e.* 3 weeks) EHA as compared to work-matched training in TEMP, utilising a novel heart rate based heat acclimation model are presented in study 1 from this thesis (Chapter 4). To determine the effect of heat acclimation on blood volumes in chapter 5, HBmass tests were performed weekly. In addition, intra- and extra-cellular markers of the biochemical pathway for haematological adaptations were investigated from venous blood samples. Once a week during the acclimation intervention, participants performed an intermittent sprint training session (5 x 30-s, with 4.5 min rest), presented in chapter 6. Muscle activation patterns (EMG) and muscle oxygenation (NIRS) measurements were obtained during each sprint to determine the neuromuscular adaptations to both EHA and TEMP training. Lastly, preceding and following each 3 week training block, participants completed a $\dot{V}O_{2max}$ test, and a 20-min time-trial (TT) in temperate (CON: 18°C, 60% RH, FiO2 20.93%), hot (HOT: 35°C, 60% RH, FiO2 20.93%) and hypoxic (HYP: 18°C, 60% RH, FiO2 15.40%) environments, each separated by 48h of rest. Each week of the training intervention included 5 laboratory controlled sessions (*i.e.* 4 sessions of low-intensity endurance training and 1 repeated sprint training), 1 monitored outdoor ride and one rest day. All training laboratory controlled sessions were performed in an environmental chamber at 35.5 ± 1.8 °C and 59.0 % ± 7.7 RH (EHA), or 18.0 ± 0.5°C and 59.5 ± 9.9 %RH (TEMP), with 4.5 m/s

fanning. The outdoor rides were performed early morning between November and April with mean outdoor temperatures of ~20– 25°C.

The environmental conditions of the performance trials and training environments were matched, since heat acclimation is specific to the environment it is performed in (Racinais et al. 2015). While heat acclimation in dry heat does provide some benefit in humid heat (Bean and Eichna 1943, Fox et al. 1967) and vice versa (Eichna et al 1945), acclimatising to humid heat evokes higher skin temperature and circulatory adaptations than dry heat, potentially increasing maximum skin wettedness and therefore the maximum rate of evaporative heat loss from the skin (Periard et al. 2015, Candas et al. 1979). Therefore, a warm humid environment was selected for the present project.

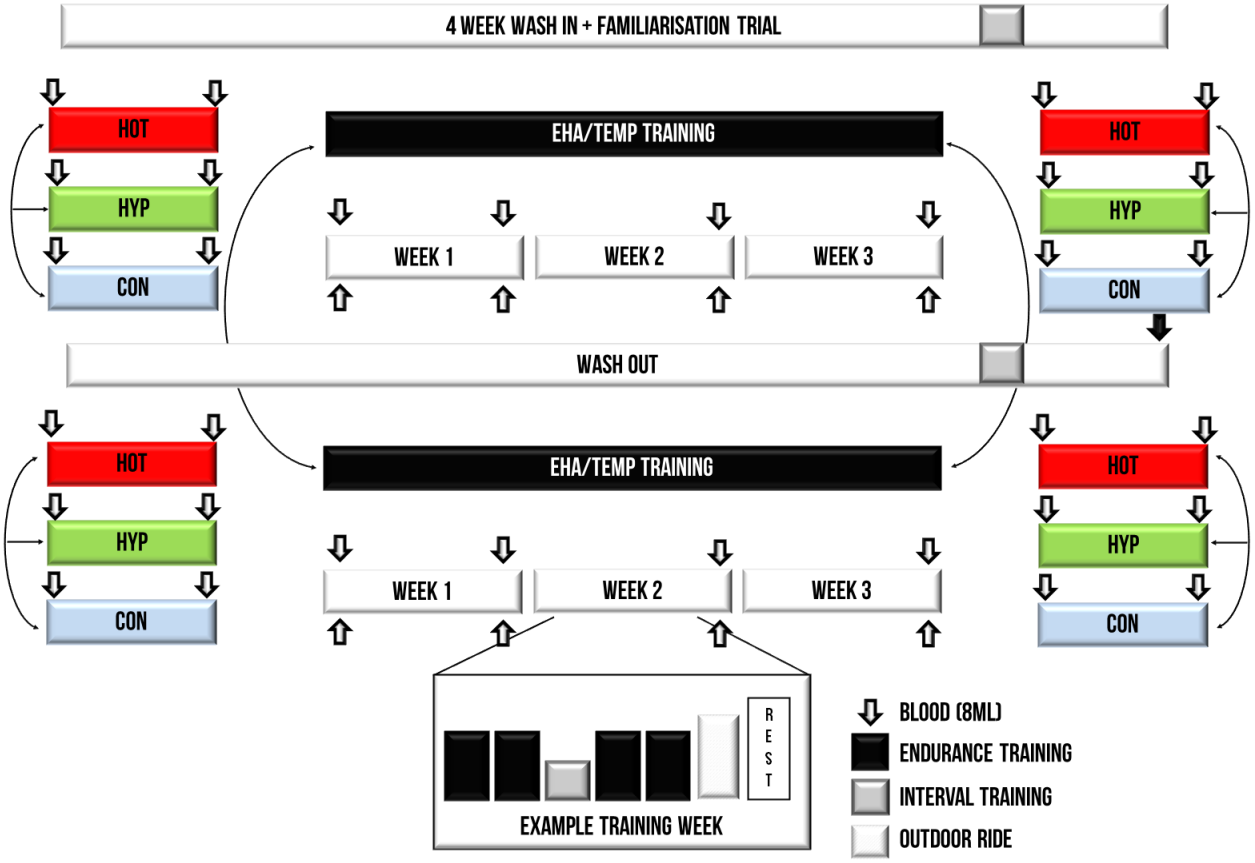


**Figure 3.1**. The counter-balanced, cross-over experimental protocol. Participants completed a $\dot{V}O_{2max}$ and 20min TT in temperate (CON: 18°C, 60% RH, $FiO_2$ 20.93%), hot (HOT: 35°C, 60% RH, $FiO_2$ 20.93%) and hypoxic (HYP: 18°C, 60% RH, $FiO_2$ 15.40%) conditions before and after each intervention. Each test was separated by 48h of rest. During each intervention, 6 different training days were completed each week: 4 low intensity endurance sessions (black box), 1 intermittent sprint training session (grey box), 1 monitored outdoor ride (hashed grey box), and 1 rest day (rest box).

### 3.2.2 *Pre-test procedures*

Prior to testing, nude body mass of all participants was measured to the nearest 0.1 kg using a set of electronic scales, and height simultaneously measured to the nearest centimetre using a SECA stadiometer (SECA, Hamburg, Germany). At least 2-weeks prior to commencement of the training protocol, participants completed an incremental cycle exercise test to volitional exhaustion on an electrically-braked cycle ergometer

(Lode - Lode Corival, Groningen, Holland). Starting at a workload of 95W, the workload increased by 35 W every 3 min thereafter until volitional exertion, with continuous measurement of oxygen uptake by an online breath-by-breath system (Oxycon Pro, CareFusion, Rolle, Switzerland) for determination of sub-maximal oxygen uptake and $\dot{V}O_{2max}$. A linear regression model was used to determine HR and power output at 65% of $\dot{V}O_{2max}$ to set the training intensity. Participants also completed a familiarisation 20 min TT on an SRM cycle ergometer (Schoberer Rad Meßtechnik, Jülich, Germany). This was undertaken to allow participants to gain additional experience of pacing the performance test, to minimise any potential learning effect and to ensure they were accustomed to the procedures employed during the investigation. At least 1-week prior to the experimental trials, participants completed a baseline intermittent sprint training session in the same environment they would train in. All participants were highly familiar with the SRM ergometer and regularly undertook personal training sessions on the ergometer.

### 3.2.3 *Standardization*

Participants completed a minimum wash-out period of at least 4 weeks prior to commencing the second intervention block. During this time, participants continued to perform their usual training but no training was undertaken in a warm environment. This was done to ensure a reversal of the manifested adaptations from heat acclimation, and that each block was commenced at a similar baseline fitness level. With short-term heat acclimation, it appears that adaptations persist for 1 week, but not 2 (Garrett et al. 2009). Whilst there is limited literature on the decay from long term heat acclimation (>2 weeks), there is evidence to suggest that after 4 weeks without heat exposure, cardiovascular adaptations are entirely reversed (Dresoti 1935, Lind 1964, Williams et al. 1967). During the wash-in/wash-out period,

participants were asked to maintain their normal dietary and training routines and to replicate this prior to and during both training interventions. To ensure metabolic conditions were comparable before each experimental trial, participants were asked to record their dietary intake and physical activity for 24-h prior to a familiarisation trial and then instructed to follow the same diet and activities before all remaining trials. No strenuous exercise, or alcohol consumption was permitted in the 24-h before each trial. Participants were asked to attend the laboratory at the same time of day for all sessions. Upon arrival to the laboratory urine samples were obtained for measurement of urine specific gravity (USG; Pocket refractometer PAL-10S, Atago, Tokyo, Japan). Readings above 1.020 were considered to indicate poor hydration status and participants were asked to consume additional plain water prior to commencing the trial. Participants were provided guidelines on how to maintain normal fluid balance and were encouraged to remain euhydrated throughout the interventions. Participants wore standard cycling shorts, socks and shoes, and used their own clipless pedals. The upper body was unclothed for all sessions. Participants sat quietly for 10 mins prior to commencing exercise to allow resting measures of HR, $T_{core}$, $T_{skin}$, and to obtain resting blood samples.

### 3.2.4 Calibration

All equipment was calibrated prior to use according to the manufactures guidelines. Rectal probes and skin thermistors were allocated to participants to ensure the same equipment was used for every trial. Cycle ergometers and the metabolic cart was placed in the environmental chamber prior to all trials to allow it to equilibrate to ambient conditions before calibration.

### 3.2.5 *Outdoor rides*

Participants completed one monitored outdoor ride per week. Participants were free to select their training but were instructed to repeat the same training for both EHA and TEMP interventions.

### 3.2.6 *Rest days*

Participants had 1 rest day per week during the training interventions. Participants were instructed to refrain from strenuous exercise, heat exposure and alcohol consumption to facilitate recovery and rehydration.

## 3.3 **Material and Methods**

### 3.3.1 *Cycle ergometers*

Two electronically braked cycle ergometers were used during the project. All incremental, ramp tests, and low-intensity HR clamped training sessions were performed using a Lode cycle ergometer using a pre-programmed file on the Lode software (Lode Ergometer Manager v9.1). All time trials, and intermittent sprint training sessions were performed using an SRM cycle ergometer and SRM software (SRM training system V6.42.18). Different ergometers were selected to optimally suit the requirements of the testing procedure. Specific details of the testing procedure are provided in the relevant chapters.

### 3.3.2 *Temperature monitoring*

A rectal thermistor probe (MRB, Ellab A/S, Hillerød, Denmark) was self-inserted 15cm beyond the external anal sphincter in order to monitor $T_{core}$ and attached to a precision digital thermometer allowing measurement to the nearest 0.1°C (DM 852, Ellab A/S,

Hillerød, Denmark). Four skin temperature loggers (iButton$^{TM}$, Maxim Integrated Products, Sunnyvale, CA, USA) were attached to the surface of the skin at the sites of the chest, forearm, thigh and calf. Area weighted mean skin temperature ($T_{skin}$) was calculated using the following formula (Ramanathan 1964):

$$T_{skin} = 0.3T_{chest} + 0.3T_{arm} + 0.2T_{thigh} + 0.2T_{calf}$$

Environmental ambient temperature and humidity was periodically measured by a calibrated heat stress monitor (3M QuesTemp 36, US) throughout every session placed in a standardized position within the environmental chamber.

### 3.3.3 *Blood collection and storage*

Venous blood samples (~8mL) were collected from an antecubital vein into K2EDTA treated vacutainers following a 10 min seated stabilisation period prior to exercise, and immediately after exercise for each trial (CON, ALT, HOT), and on days 1, 5, 12 and 19 of the training interventions. A tourniquet was applied for less than 1 min in order to avoid localized haemoconcentration of the sample (Nikolac et al. 2017). Samples were aliquoted into 3 portions. 1mL of whole blood was immediately analysed for complete blood count (CBC) (Beckman Coulter DxH 800, Beckman Coulter, Miami, FL). All CBC's were independently measured at the Aspetar Hospital laboratory, which is accredited by the College of American Pathologists (CAP). CBC's were used to determine the concentration of: red blood cells (RBC), white blood cells (WBC) haemoglobin (Hb), mean corpuscular haemoglobin (MCHC), and haematocrit (Hct). 1mL of whole blood was diluted 1:1 in phosphate buffered saline (PBS; 0.01mol/L, pH 7.2), and subjected to 35 min density centrifugation at 400*g* using 1mL Histopaque (Sigma 1077-1, Poole, UK). Peripheral blood mononuclear cell (PMBC) pellets were resuspended in PBS medium and aliquots frozen at -80°C for future analysis. The

remaining whole blood was centrifuged at 3,000rpm for 10 min at a temperature of 4°C and plasma aliquots stored at -80°C for future analysis.

### 3.3.4 *Plasma protein analysis*

Plasma heat shock protein-70 (HSP70), heat shock protein-90 (HSP 90), EPO (Erythropoietin) and ferritin (Fe) in ethlylenediaminetetraacedtic acid (EDTA) treated plasma, was measured using a pre-prepared sandwich enzyme-linked immunosorbent assay (ELISA) technique, from commercially available kits (Cloud-Clone Corp, Houston, TX USA). Samples were thawed to room temperature and 100µL of standards and samples were added to each pre-coated well and incubated for 1 h at 37°C. Standards and samples were then aspirated and 100µL of detection reagent A was added to each well and incubated at 37°C for 1 h. After three 350 µL washes with a 1x wash buffer solution, 100 µL of detection reagent B was added to each well and incubated for 30 min at 37°C. Following a further five wash steps, 90 µL of TMB substrate was added and incubated at 37°C in the dark for 15 min before 50 µL of stop solution was added. The plate was then immediately read at 450 nm using an automatic ELISA microplate reader (Infinite® 200 PRO NanoQuant, Switzerland) and Magellan Standard software (version 7.1) were used. All samples were analysed in duplicate. Reliability data are shown in table 3.1.

**Table 3.1:** Reliability data of the plasma ELISA analysis.

| Molecule | Limit of detection | Intra-assay CV (%) | Inter-assay CV (%) |
|---|---|---|---|
| HSP90 | < 1.18 ng/mL | 4.7 | 11.6 |
| HSP70 | < 0.60 ng/mL | 4.4 | 28.0 |
| EPO | < 11.5 pg/mL | 4.0 | 7.7 |
| Fe | < 0.061 ng/mL | 8.1 | 26.1 |

The intra-assay precision was determined from duplicates of standards within the same plate and inter-assay precision determined from standards assessed across plates.

### 3.3.5 *Intra-cellular protein analysis*

Intracellular protein content of HSP70 and HSP90 was also measured using the ELISA technique, from commercially available kits (Cloud-Clone Corp, Houston, TX USA). After thawing, cells suspended in 1 mL of PBS were subject to ultrasonification until the solution clarified and was centrifuged at 1500 g for 10 minutes at 4°C to remove cellular debris. The ELISA assay was performed immediately, using an identical procedure to the plasma protein analysis.

### 3.3.6 *Heart rate*

A telemetry band (Polar, Kempele, Finland) was positioned on the chest to allow continuous HR measurement. Resting HR was determined as an average of 1 minute after a 10 min seated period.

### 3.3.7 *Sweat rate and composition*

Nude body mass was measured to the nearest 100 g prior to and following every session, using a SECA stadiometer. The difference in body mass was used to determine whole body sweat rate ($L.h^{-1}$) after correcting for fluid ingestion. Sweat sodium concentration was measured during the HOT trial and on days 1, 5, 12 and 19 of the EHA training intervention. Sweat samples were collected for the duration of the test via an absorbent pad with protective dressing (Tegaderm + Pad, 3M Health Care, Borken, Germany) positioned on the right scapula after cleaning the skin with deionized water. Samples were analysed for sodium concentration ($[Na^+]$) (Dimension Xpand Plus, Siemens, Munich, Germany).

### 3.3.8 *Perceived exertion, thermal sensation and thermal comfort*

Subjective ratings of perceived exertion (RPE, 6 to 20) (Borg 1982), thermal sensation (TS, 1 to 7) (ASHRAE 1966) and thermal comfort (TC, 1 to 7) (Bedford 1936), were recorded at set intervals throughout all sessions.

### 3.3.9 *Statistical analysis*

Data are presented as mean ± standard deviation (SD), unless otherwise stated. All data were checked for normal distribution prior to analysis and tests employing repeated measures were checked for sphericity before analysis with Mauchly's sphericity test. Where sphericity was broken, p-values were corrected using the Huynh- Feldt method. Detailed explanations of the statistical approach are presented separately for each study. All data analysis was performed using Statistical Package for the Social Sciences for Windows version 22.0 software (SPSS Inc., Chicago, IL).

# CHAPTER 4

# LONG TERM HEAT ACCLIMATION WITH A HEART RATE CLAMP

## 4.1 Introduction

Reductions in endurance performance have been widely reported in both laboratory (Galloway and Maughan 1997) and field (Racinais et al. 2015b) settings in warm compared to cool environments. Heat acclimation is generally considered as the primary strategy to attenuate this decrement (Racinais et al. 2015a). Most adaptations to heat acclimation develop quickly within the first 4-7days, and further smaller changes occur within 2 weeks to fully optimise cardiovascular and sudomotor function to support aerobic exercise performance in hot ambient conditions (Périard et al. 2015). There are limited studies which have employed a longer term heat acclimation strategy (Patterson et al. 2014), and differences in the heat acclimation strategies employed (e.g. constant work-rate, clamped hyperthermia models) limits our understanding of the efficacy and adaptations to long-term approaches (Tyler et al. 2016).

Constant work rate protocols are the most commonly reported method to induce heat acclimation (Tyler et al. 2016). Participants exercise at a set workload for a specified time period each day. However, since heat storage is reduced as adaptation develops, body temperatures, and overall thermal load decreases as acclimation progresses, constraining the potential adaptation (Taylor 2014). Controlled hyperthermia (sometimes called isothermal) methods clamp core temperature above the sweating

threshold, using intermittent work/rest periods (Taylor 2014). The premise of this approach is that it better maintains the thermal stimulus, as the workload increases as adaptation progresses to maintain the thermal load. The use of controlled hyperthermia protocols, where core temperature is maintained throughout the acclimation period (usually in the region of 38.5°C), may facilitate a continual adaptive stimulus and maintain plasma volume expansion throughout 22days of heat acclimation (Patterson et al. 2014). However, clamped hyperthermia models are practically unsuitable outside of a laboratory environment, and even within a laboratory setting participants tend to spend large portions of time at rest to maintain their target core temperature (Gibson et al. 2015a). A more efficacious method may be to use a target heart rate, allowing power to fluctuate and gradually increase as adaptation occurs (Périard et al. 2015).

An additional issue with previous heat acclimation studies has been the lack of an adequate control group or not matching relative training demands between the intervention and control (Corbett et al. 2014). A suitable approach may be to match the total work-done in both conditions while allowing training duration to fluctuate. Thus, a work-matched, HR clamp model might allow maintenance of the required thermal stimulus during heat acclimation, while also matching training demands between hot and cool environments. Furthermore, such an approach is more easily transferred to field based practice.

Lastly, the majority of heat acclimation research has focused on short- (<7days) to medium-term heat acclimation (8 – 14 days) (Chalmers et al. 2014). It is generally regarded that most adaptations (decreased heart rate, skin and core temperature, sweat rate and work capacity) develop quickly within the first week of heat acclimation, with an additional week required to fully develop these adaptations and support

aerobic performance (Racinais et al. 2015a). However, it has also been suggested that progression continues beyond these time scales and does not plateau after 2 weeks (Patterson et al. 2014).

Accordingly, the aim of the present study was to characterise the adaptations to a novel long-term, work matched, heart rate clamped heat acclimation protocol in a group of trained cyclists.

## 4.2 Material and methods

### 4.2.1 *General procedure*

10 participants completed 3 weeks of exercise heat acclimation (EHA, 35°C, 60% RH) and 3 weeks of work-matched training in temperate conditions (TEMP, 18°C, 60% RH). Detailed descriptions of the participant characteristics and project overview are presented in general methods 3.1.2, and 3.2.1, respectively.

### 4.2.2 *Pre- intervention procedures*

Two weeks prior to commencement of the training intervention, participants completed an incremental cycle exercise test to volitional exhaustion on an electrically-braked cycle ergometer (Lode - Lode Corival, Groningen, Holland). Starting at a workload of 95W, the workload increased by 35W every 3min thereafter until volitional exhaustion, with continuous measurement of oxygen uptake using an online breath-by-breath system (Oxycon Pro, CareFusion, Rolle, Switzerland) for determination of sub-maximal oxygen uptake and maximal oxygen uptake ($\dot{V}O_{2max}$). A linear regression at 35W increments between 95W and 200W was used to determine the sub-maximal HR-power relationship to set the training intensity at 65% of $\dot{V}O_{2max}$.

### 4.2.3 *Training Procedure*

The daily training workload corresponded to the work (kJ) that would be completed in 60min if maintaining the power output corresponding to a relative intensity of 65% $\dot{V}O_{2max}$. It was calculated as:

Total work (kJ) = P65% · 3600 / 1000

where, P65% is the power output at 65% $\dot{V}O_{2max}$, 3600 is the time in seconds, and 1000 is the conversion from joules to kilojoules. This load was increased to 105% in week 2 and 110% in week 3 of the initial load to respect the principle of progressivity in training. This was designed to better replicate the training habits of athletes (progressive overload) and sustain the total duration of week to week training to maintain the thermal stimulus. The same progression was applied during both training interventions.

The first 20% of each training session was performed at the power output corresponding to P65% to allow HR to increase to the desired target. Thereafter, the ergometer initiated heart rate mode and power output was automatically adjusted to maintain the target HR. The Lode ergometer HR mode samples data at 30s intervals and adjusts power output accordingly to the difference between the current HR and the target HR whereby:

HR > target HR + 15, then workload = workload – (workload/8)

HR > target HR + 1 - 14, then workload = workload – (workload/16)

HR < target HR - 5, then workload = workload

HR < target HR - 6-14, then workload = workload + (workload/16)

HR < target HR - 15, then workload = workload + (workload/8)

To account for this conservative algorithm, the target HR was set 2bpm above the desired training HR. The HR during EHA was clamped 7bpm higher than in TEMP since HR increases approximately 7bpm for every 1°C rise in body temperature (Jose et al. 1970). This was implemented to account for the increased cardiac strain during exercise in the heat to maintain the same relative training intensity between conditions (Périard and Racinais 2015). Whilst *ad libitum* drinking of plain water was allowed, participants were encouraged to drink an amount estimated from the previous days sweat rate to attenuate post-training body mass losses to <1%.

Lastly, participants performed an intermittent sprint session on day 3 of each week to respect the concept of polarised training (see chapter 6). This session was the same during both training interventions and included a ~20min warm-up followed by 5 all-out sprints of 30 s duration, interspersed with 4 min 30 s of unloaded pedalling to facilitate active recovery. This session was performed in the same environmental condition as the HR-based training. A detailed description of the intermittent sprint session can be found in chapter 6.

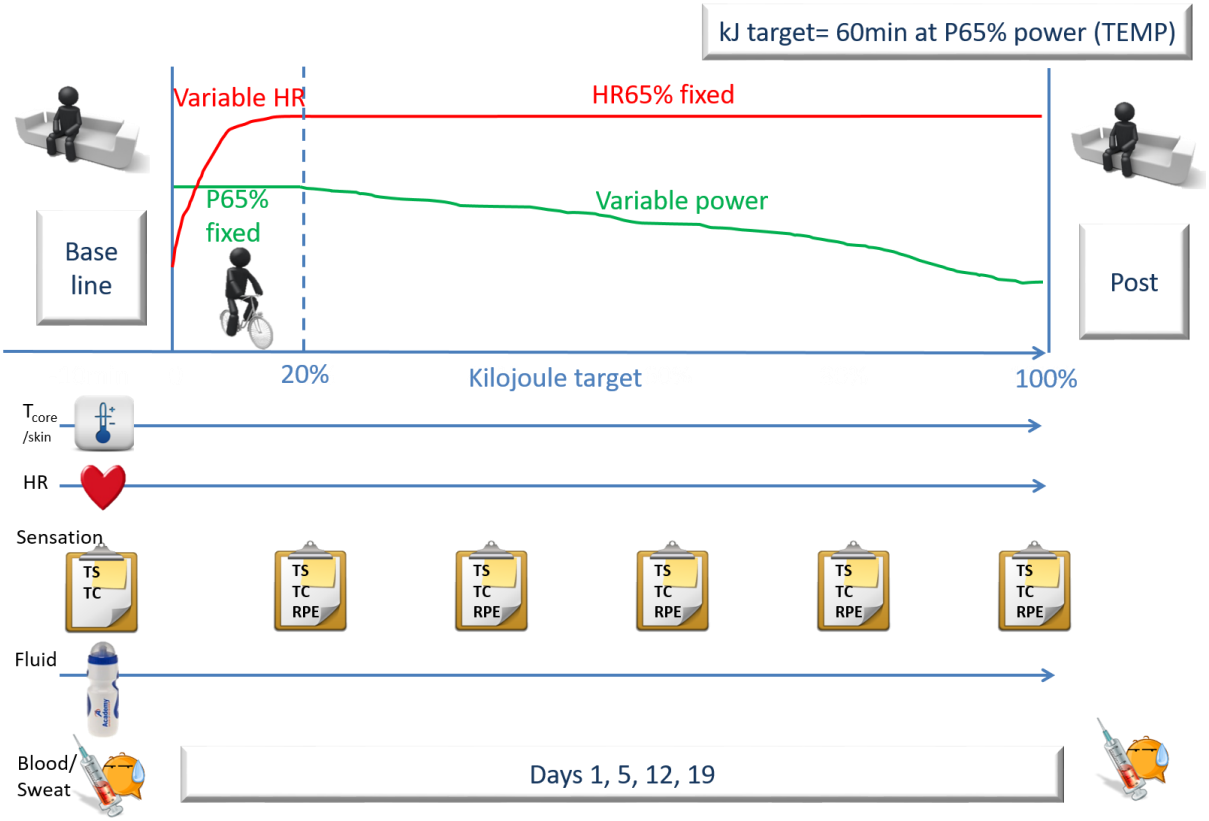


**Figure 4.1:** Schematic of the HR based endurance training session. Participants completed 20% of their calculated required work load at a fixed power output (P65%), and thereafter maintained a fixed HR (HR65%). The total amount of work required was increased by 5% in week 2, and 10% in week 3 from the week 1 workload.

### 4.2.4 *Measures and instrumentation*

Upon arrival to the laboratory for each training session, urine samples were obtained for measurement of urine specific gravity (Pocket refractometer PAL-10S, Atago, Tokyo, Japan). Readings above 1.020 were considered to indicate poor hydration status and participants were asked to consume additional plain water prior to commencing the trial. After confirmation of adequate hydration status, nude body mass was measured to the nearest 100g (SECA, Germany). A rectal thermistor probe (MRB, Ellab A/S, Hillerød, Denmark) was self-inserted 15cm beyond the external anal sphincter in order to monitor core rectal temperature ($T_{core}$) and attached to a precision digital thermometer allowing measurement to the nearest 0.1°C (DM 852, Ellab A/S,

Hillerød, Denmark). Four skin temperature ($T_{skin}$) loggers (iButton$^{TM}$, Maxim Integrated Products, Sunnyvale, CA, USA) were attached to the surface of the skin at the sites of the chest, forearm, thigh and calf. A telemetry band (Polar, Kempele, Finland) was positioned on the chest to allow continuous HR measurements.

#### 4.2.4.1 Average power

Average power was calculated as Power (w) = work done (J) / time (s) for every 10% of the work completed, and as a session average.

#### 4.2.4.2 Temperatures

$T_{core}$, $T_{skin}$, and environmental temperature was recorded at baseline and at each 10% of the total work completed, throughout all training sessions as previously described in General methodology 3.3.2. Briefly, area weighted mean skin temperature was calculated using the following formula (Ramanathan 1964):

$$T_{skin} = 0.3T_{chest} + 0.3T_{arm} + 0.2T_{thigh} + 0.2T_{calf}.$$

Whole body temperature ($T_b$) was calculated from the method of Colin (Colin et al. 1971)

$$\text{In TEMP: } T_b = 0.66T_{core} + 0.34T_{skin}$$

$$\text{In EHA: } T_b = 0.79T_{core} + 0.21T_{skin}$$

The data from each 10% of the total work done was averaged, and the highest single point (peak) was determined for $T_{core}$, $T_{skin}$ and $T_b$ for each training day.

#### 4.2.4.3 Sweat rate and composition

Sweat rate was calculated daily during EHA and TEMP. Sweat composition was recorded on days 1, 5, 12, and 19. Sweat composition was analyzed during EHA

training only as insufficient sweat volumes were collected during TEMP training for analysis. The procedure for sweat rate and composition are described in section 3.3.5 of the General methods.

#### 4.2.4.4 Perceptual responses

Rating of perceived exertion (RPE), thermal sensation (TS), and thermal comfort (TC) were recorded at every 20% of the work completed, and subsequently averaged, as previously described in General methodology 3.3.6.

#### 4.2.4.5 Plasma volume

Venous blood samples were drawn prior to exercise after a 10min seated stabilization period and immediately following completion of training on days 1, 5, 12, and 19 of each of training intervention. Samples were immediately analysed for haemoglobin concentration and haematocrit as previously described in general methods 3.3.3.

Relative changes in plasma volume throughout acclimation were calculated from the following formulas (adapted from Dill and Costill, 1974):

$$V_{D1} = BV_{Dx} (Hb_{Dx}/Hb_{D1})$$

$$CV_{Dx} = BV_{Dx} (Hct_{DX})$$

$$PV_{DX} = BV_{DX} – CV_{DX}$$

$$\Delta \text{ BV, \%} = 100 (BV_{DX} – BV_{D1}) / BV_{D1}$$

$$\Delta \text{ RBCV, \%} = 100 (RBCV_{DX} – RBCV_{D1}) / RBCV_{D1}$$

$$\Delta \text{ PV, \%} = 100 (PV_{DX} – PV_{D1}) / PV_{D1}$$

Where; BV is blood volume, HB is haemoglobin concentration, CV is red blood cell volume, Hct is haematocrit, PV is plasma volume, and subscripts D1 and DX refer to day 1 value and either day 5, 12, or 19 of the intervention

### 4.2.5 *Statistical analysis*

Data analysis was performed using Statistical Package for the Social Sciences for Windows version 22.0 software (SPSS Inc., Chicago, IL). Data are presented as mean ± standard deviation (SD), unless otherwise states. A two-way (condition x time) analysis of variance (ANOVA) with repeated measures design was used to assess differences in average power output and physiological adaptations to the interventions. Sweat [$Na^+$] was assessed by one-way ANOVA. ANOVA effect sizes are presented using partial eta squared ($\eta_p^2$). Where a significant interaction effect was observed, 95% confidence intervals (CI) and Cohen's *d* effect sizes (ES) were also determined. Pair-wise differences were evaluated using the least significance method. A Pearson product-moment correlation analysis (*r*) was used to evaluate the relationship between physiological adaptations and changes in power output. Significance was accepted at $P < 0.05$.

## 4.3 **Results**

### 4.3.1 Training Load

The average daily training time, work done and heat storage per week are presented in Table 4.1.

**Table 4.1**: Average daily training characterictics for each week during the temperate (TEMP) and exercise heat acclimation (EHA) training interventions. Data are presented as mean ± SD, n = 10.

| | TEMP | | | EHA | | |
|---|---|---|---|---|---|---|
| Training Week | 1 | 2 | 3 | 1 | 2 | 3 |
| Time (mm:ss) | 56:52 ± 2:54 | 58:43 ± 3:27 | 61:46 ± 4:06 | 67:17 ± 8:03 | 68:08 ± 7:49 | 67:45 ± 5:54 |
| Work done (kJ) | 712 ± 97 | 748 ± 102 | 784 ± 107 | 712 ± 97 | 748 ± 102 | 784 ± 107 |
| Heat storage (kJ) | 244 ± 104 | 273 ± 74 | 252 ± 137 | 545 ± 126 | 531 ± 117 | 558 ± 136 |

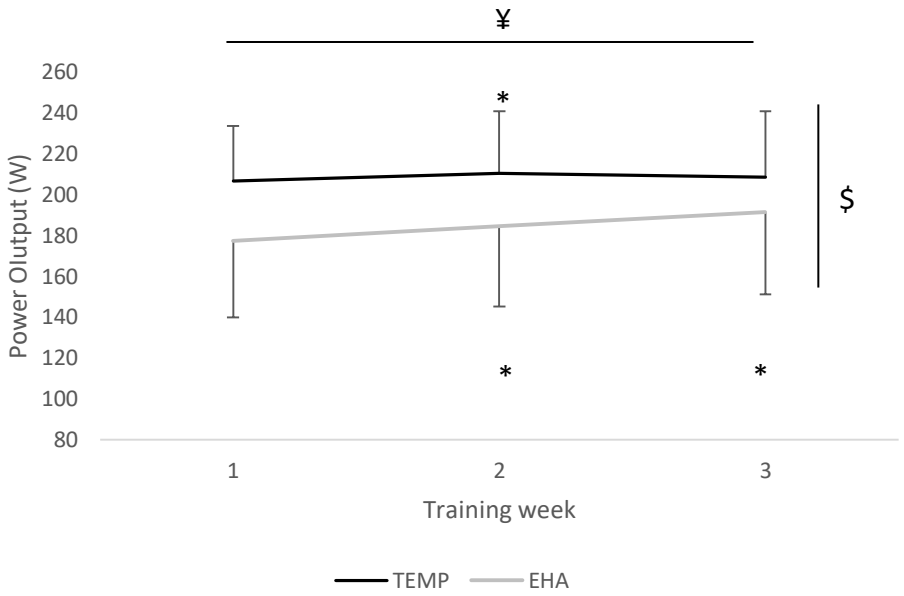


**Figure 4.2:** Weekly average power during the temperate and exercise heat acclimation training interventions. $ main effect of training condition, ¥ main effect of time, * intereaction effect significantly different to week 1, $p < 0.05$. Data are presented as mean (solid line) and SD (error bars), n = 10.

During both training interventions, the indivualised target HR was achieved and maintained in every training session, after the first 20% of work was completed at a constant power output. Average power output was lower in EHA than TEMP (Figure

4.2, $p = 0.002$, $\eta_p^2 = 0.683$). Individual kinetics of power output are presented in figure 4.8. A main effect of time was also observed ($p = 0.001$, $\eta_p^2 = 0.565$). There was a significant interaction effect (condition*time) for weekly average power ($p < 0.001$, $\eta_p^2 = 0.560$). Post hoc analyses revealed that weekly average power during TEMP was higher in week 2 than week 1, albeit the differences were very small (mean difference,[95% CI]) (+4,[1;7]W, ES: 0.14, $p = 0.036$) and was not different between week 1 and week 3 (+2[-3;7]W, ES: 0.06, $p = 0.459$). During EHA, average power was higher in week 2 than week 1 (+8[4;11]W, ES: 0.20, $p = 0.001$). Week 3 was also higher than week 1 (+14[9;20]W, ES: 0.37, $p < 0.001$) , and week 2 (+7[2;11]W, ES: 0.17, $p = 0.012$). There was a significant main effect of conditon ($p = 0.002$, $\eta_p^2 = 0.682$) and time ($p < 0.001$, $\eta_p^2 = 0.469$) for daily average power output, which was higher in TEMP than EHA, and increased across time. There was also a signficant interaction effect (conditon*time, $p < 0.001$, $\eta_p^2 = 0.423$), whereby average power output increased during EHA and remained similar during TEMP. Post-hoc analyses showed that average power was higher on day 4 compared to day 1 during EHA (+7[1;13]W, ES: 0.19, $p = 0.038$). Thereafter average power output was higher than baseline on every subsequent day ($p < 0.05$ for all). A plateau was observed on day 5, with no individual training session statistically higher. Day 19 tended to be higher than day 5 (+6[-1;12]W, ES: 0.15, $p = 0.124$) but this did not reach statistical significance.

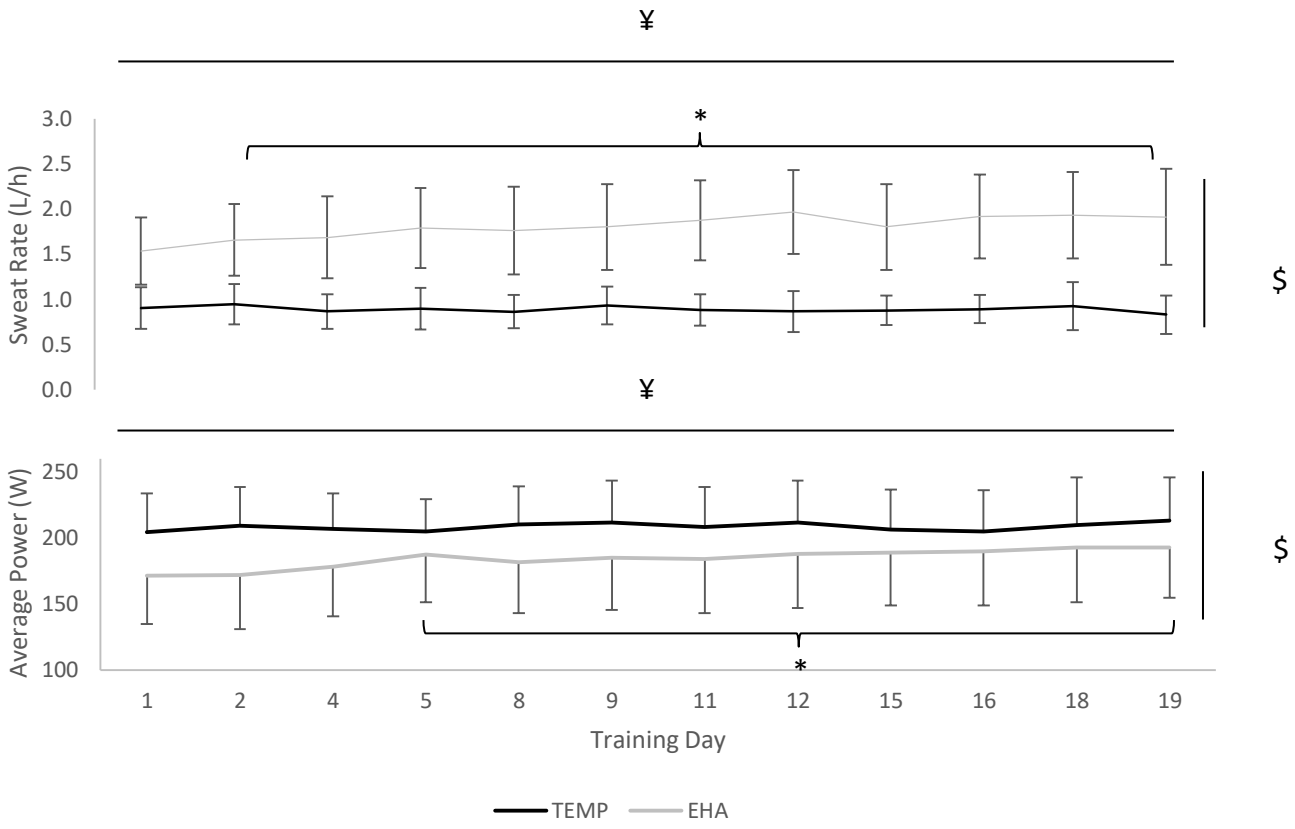


**Figure 4.3:** Power output and sweat respones during TEMP and EHA training sessions. Data are presented as mean (solid line) and SD (error bars), n = 10. *$ main effect of condition, ¥ main effect of time, * interaction effect compared to day 1, p < 0.05*

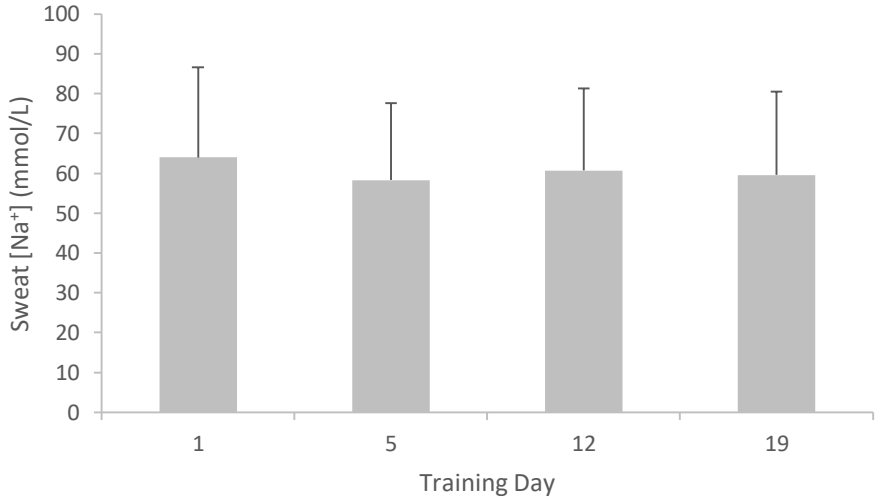


**Figure 4.4:** Sweat [$Na^+$] during EHA. Data are presented as mean (columns) and SD (error bars), n = 10.

### 4.3.2 *Thermal responses*

There was a main effect of conditon for resting core temperature which was lower during EHA than TEMP ($p = 0.016$, $\eta_p^2 = 0.491$). There was no effect of time ($p = 0.869$, $\eta_p^2 = 0.057$), or evidence of an interaction effect on resting core temperature ($p = 0.453$, $\eta_p^2 = 0.100$). There was no main effect of conditon for average core temperature ($p = 0.951$, $\eta_p^2 > 0.999$), and no main effect of time ($p = 0.944$, $\eta_p^2 = 0.045$). There was no evidence of an interaction effect ($p = 0.527$, $\eta_p^2 = 0.092$). Peak core temperature elicited in each training session was higher in EHA than TEMP ($p = 0.023$, $\eta_p^2 = 0.452$). There was no effect of time ($p = 0.985$, $\eta_p^2 = 0.032$) or an interaction effect ($p = 0.808$, $\eta_p^2 = 0.064$). Average skin temperature was higher during EHA than TEMP ($p < 0.001$, $\eta_p^2 = 0.990$). There was no effect of time ($p = 0.632$, $\eta_p^2 = 0.082$), or an interaction effect ($p = 0.609$, $\eta_p^2 = 0.085$). There was a main effect of condition for average whole body temperature which was higher during EHA than TEMP ($p < 0.001$, $\eta_p^2 = 0.986$). There was was no effect of time ($p = 0.674$, $\eta_p^2 = 0.078$) or an interaction effect ($p = 0.729$, $\eta_p^2 = 0.073$).

**Table 4.2:** Daily thermal responses during TEMP and EHA training interventions. Data are presented as mean ± SD, n = 10.

| Day | 1 | 2 | 4 | 5 | 8 | 9 | 11 | 12 | 15 | 16 | 18 | 19 | Main effect of training condition |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $T_{core}$ rest, °C | | | | | | | | | | | | | |
| TEMP | 37.2±0.3 | 37.1±0.3 | 37.2±0.4 | 37.2±0.3 | 37.0±0.3 | 37.1±0.2 | 37.1±0.2 | 37±0.3 | 37.1±0.2 | 37.1±0.4 | 37.1±0.2 | 37.1±0.3 | 0.016 |
| EHA | 37.0±0.4 | 36.9±0.3 | 36.9±0.3 | 36.9±0.4 | 36.9±0.3 | 37.0±0.3 | 36.9±0.4 | 37±0.3 | 36.9±0.2 | 36.8±0.2 | 36.9±0.2 | 36.9±0.3 | |
| $T_{core}$ avg, °C | | | | | | | | | | | | | |
| TEMP | 38.2±0.2 | 38.1±0.2 | 38.3±0.2 | 38.3±0.2 | 38.2±0.3 | 38.2±0.1 | 38.2±0.2 | 38.2±0.2 | 38.2±0.2 | 38.3±0.2 | 38.3±0.2 | 38.2±0.1 | 0.951 |
| EHA | 38.2±0.3 | 38.2±0.2 | 38.2±0.2 | 38.2±0.3 | 38.3±0.2 | 38.2±0.2 | 38.2±0.2 | 38.3±0.2 | 38.3±0.2 | 38.2±0.3 | 38.3±0.3 | 38.2±0.2 | |
| $T_{core}$ peak, °C | | | | | | | | | | | | | |
| TEMP | 38.6±0.2 | 38.5±0.2 | 38.5±0.3 | 38.6±0.3 | 38.6±0.5 | 38.5±0.2 | 38.5±0.3 | 38.5±0.2 | 38.6±0.2 | 38.6±0.3 | 38.6±0.3 | 38.5±0.1 | 0.023 |
| EHA | 38.8±0.4 | 38.9±0.4 | 38.8±0.4 | 38.8±0.4 | 38.8±0.4 | 38.8±0.3 | 38.8±0.4 | 38.8±0.4 | 38.9±0.4 | 38.8±0.4 | 38.8±0.5 | 38.8±0.5 | |
| $T_{skin}$ avg, °C | | | | | | | | | | | | | |
| TEMP | 30.8±0.7 | 30.5±1.3 | 30.2±1.4 | 30.8±1.4 | 30.9±0.9 | 31.0±0.5 | 30.4±0.8 | 30.7±0.8 | 30.6±0.8 | 30.6±1.2 | 30.9±1.3 | 30.4±1.0 | < 0.001 |
| EHA | 35.8±0.6 | 35.7±0.5 | 35.8±0.3 | 35.7±0.6 | 35.7±0.6 | 35.6±0.4 | 35.6±0.5 | 35.7±0.3 | 35.8±0.6 | 35.7±0.4 | 35.7±0.4 | 35.5±0.5 | |
| $T_b$ avg, °C | | | | | | | | | | | | | |
| TEMP | 35.7±0.3 | 35.6±0.5 | 35.5±0.6 | 35.7±0.6 | 35.7±0.4 | 35.8±0.2 | 35.6±0.3 | 35.6±0.3 | 35.6±0.3 | 35.6±0.5 | 35.7±0.5 | 35.6±0.4 | < 0.001 |
| EHA | 37.7±0.3 | 37.7±0.2 | 37.7±0.2 | 37.7±0.3 | 37.7±0.3 | 37.7±0.2 | 37.7±0.2 | 37.7±0.2 | 37.8±0.2 | 37.7±0.3 | 37.7±0.2 | 37.7±0.2 | |

* $p < 0.05$ denoting a main effect of training condition, significantly different from EHA.

### 4.3.3 *Sweat*

Sweat rate was higher in EHA than TEMP ($p < 0.001$, $\eta_p^2 = 0.878$). There was a signifcant main effect for time ($p < 0.001$, $\eta_p^2 = 0.357$) and interaction effect (condition*time) whereby sweat rate increased during EHA but remained similar throughout TEMP ($p < 0.001$, $\eta_p^2 = 0.432$). Post hoc analysis showed that sweat rate was was higher than baseline by day 2 (+120mL, 95% CI 60 – 180mL, ES: 0.31, $P = 0.003$), and plateaued at day 9, where no increase in sweat rate was observed on subsequent days ($p < 0.05$ for all). There was no change in sweat [$Na^+$] during EHA ($p = 0.412$, $\eta_p^2 = 0.099$) (Figure 4.4).

### 4.3.4 *Heart rate*

Resting heart rate was lower during EHA than TEMP ($p = 0.048$, $\eta_p^2 = 0.368$). There was no main effect of time ($p = 0.233$, $\eta_p^2 = 0.127$). There was a significant intereaction effect (time*conditon) whereby resting heart rate did not change during TEMP, but decreased during EHA ($p = 0.050$, $\eta_p^2 = 0.173$). Post hoc analysis revealed that HR was lower than baseline by day 5 in EHA (- 4[-1;-7]bpm, ES: -0.62, $p = 0.017$) and plateaud thereafter ($p > 0.05$ for all). HR during the constant load portion of the daily training protocol (*i.e.* the first 20% of total work done) was higher during EHA than TEMP ($p = 0.001$, $\eta_p^2 = 0.706$). There was also a main effect of time ($p < 0.001$, $\eta_p^2 = 0.305$), and an interaction effect, wherby HR at 20% work done decreased during EHA, but remained stable during TEMP ($p < 0.001$, $\eta_p^2 = 0.333$). Post hoc analysis revealed that HR at 20% work done was lower than baseline by day 5 of EHA (- 6[-3;-10]bpm, ES: -0.79, $p = 0.005$) , and remained lower on every training session thereafter ($p < 0.05$ for all).

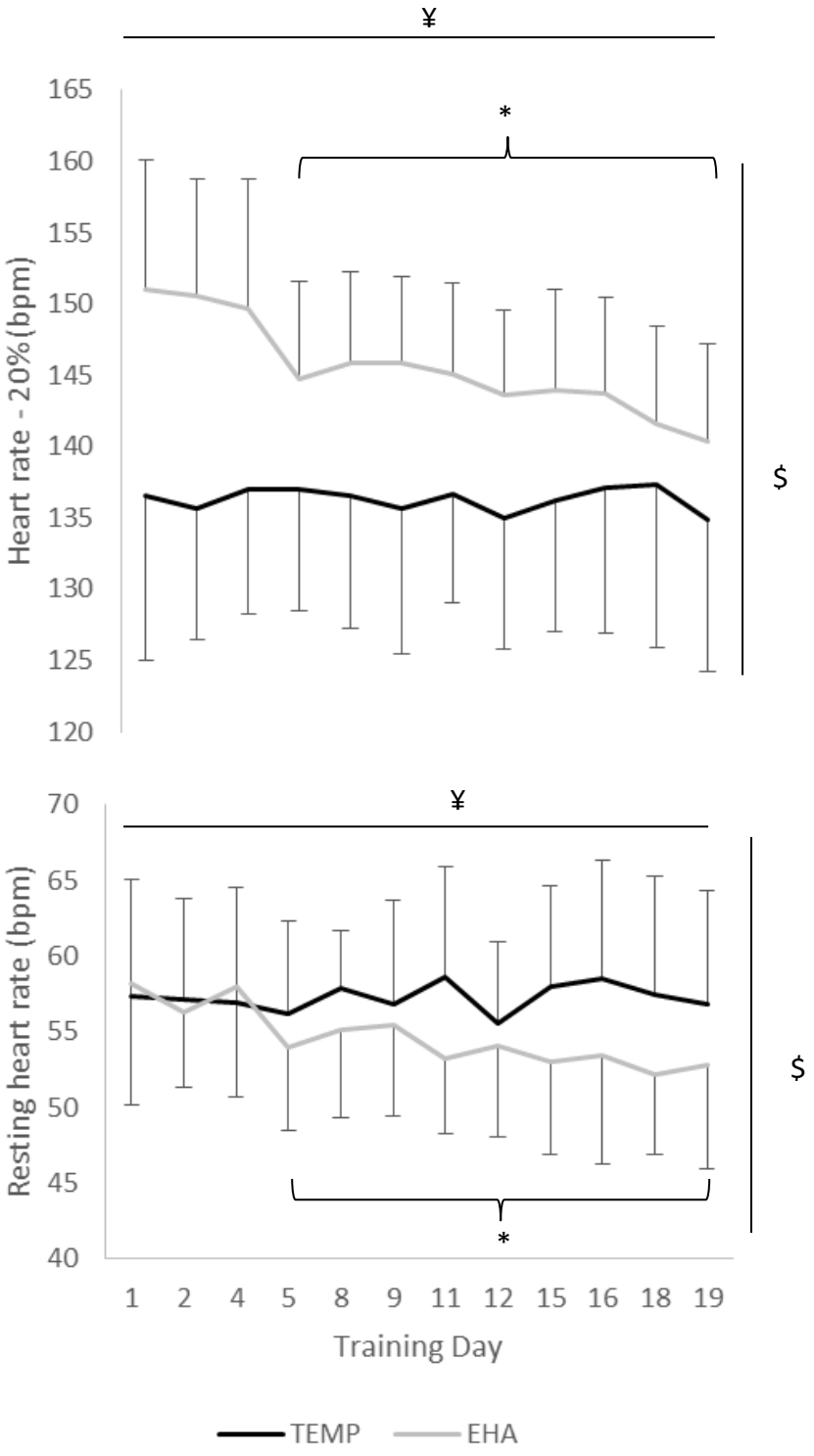


**Figure 4.5:** Resting HR and HR at the end of the constant load portion of the daily training (i.e. after 20% total work complete) during the temperate and exercise heat acclimation training interventions. Data are presented as mean (solid line) and SD (error bars), n = 10. $ main effect of condition, ¥ main effect of time, * interaction effect versus day 1, $p < 0.05$

### 4.3.5 *Perceptual responses*

Perceptual responses are reported in table 4.3. Data were analysed using a Wilcoxon Signed Rank Test for each training day between the TEMP and EHA training intervention. Average RPE was not significantly different between training interventions on any training day ($p > 0.05$ for all). TS and TC were significantly higher during EHA than TEMP on every training day ($p < 0.05$ for all).

**Table 4.3:** Perceptual measures during TEMP and EHA training. Data are presented as median(interquartile range), n = 10.

| Day | 1 | 2 | 4 | 5 | 8 | 9 | 11 | 12 | 15 | 16 | 18 | 19 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | TEMP | | | | | | |
| RPE | 12 (11.7-12.7) | 12.6 (12.1-13) | 12.2 (11.2-12.9) | 12.2 (11.6-12.8) | 11.9 (11.7-13.0) | 12.2 (11.6-12.8) | 12.5 (12.1-13.2) | 12.7 (12.1-12.8) | 12.3 (11.9-12.9) | 12.2 (11.6-12.9) | 12.5 (11.3-12.8) | 12.3 (11.4-12.7) |
| TS | 4.0 (4.0-4.6) | 4.0 (4.0-4.5) | 4.0 (4.0-4.7) | 4.0 (4.0-4.7) | 4.0 (4.0-4.8) | 4.0 (4.0-4.8) | 4.0 (4.0-4.8) | 4.0 (4.0-4.6) | 4.0 (4.0-4.8) | 4.0 (4.0-4.5) | 4.0 (4.0-4.8) | 4.0 (4.0-4.6) |
| TC | 4.0 (4.0-4.6) | 4.0 (4.0-4.5) | 4.0 (4.0-4.7) | 4.0 (4.0-4.7) | 4.0 (4.0-4.8) | 4.0 (4.0-4.8) | 4.0 (4.0-4.8) | 4.0 (4.0-4.6) | 4.0 (4.0-4.8) | 4.0 (4.0-4.5) | 4.0 (4.0-4.8) | 4.0 (4.0-4.6) |
| | | | | | | EHA | | | | | | |
| RPE | 11.9 (11.4-12.2) | 12.3 (11.7-13.1) | 11.9 (11.5-12.3) | 11.8 (11.1-12.2) | 11.6 (10.8-12.2) | 11.9 (11.1-12.2) | 11.8 (10.8-12.4) | 11.6 (10.8-12) | 11.4 (10.9-12.2) | 12.0 (11-12.6) | 12.0 (11.1-12.6) | 12.1 (11-12.8) |
| TS | 5.3 (5.1-5.5) | 5.4 (5-5.8) | 5.2 (5-5.7) | 5.1 (5-5.5) | 5.4 (5-5.8) | 5.3 (5.0-5.8) | 5.5 (5.0-5.8) | 5.1 (5.0-5.7) | 5.3 (5.0-5.7) | 5.2 (5.0-5.5) | 5.3 (5.0-5.5) | 5.2 (5.0-5.6) |
| TC | 5.1 (5.0-5.5) | 5.1 (5.0-5.8) | 5.0 (5.0-5.7) | 5.1 (5.0-5.5) | 5.1 (5.0-5.8) | 5.0 (4.9-5.8) | 5.0 (4.8-5.8) | 5.1 (5.0-5.7) | 5.0 (5.0-5.7) | 5.0 (5.0-5.5) | 5.2 (5.0-5.5) | 5.0 (5.0-5.6) |

Where RPE is rating of perceived exertion (6-20), TS is thermal sensation (1 – 7), and TC is thermal comfort (1 – 7).

### 4.3.6 *Hct, Plasma volume*

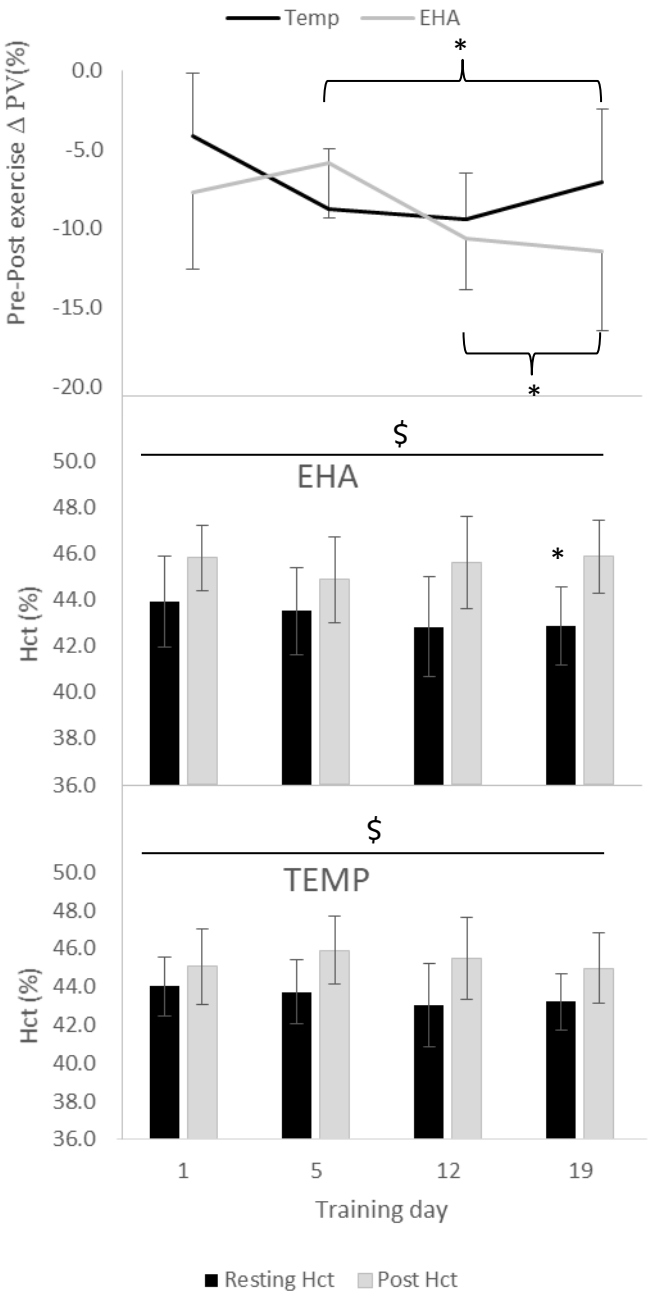


**Figure 4.6:** Resting (black) and post-exercise (grey) Hct. during TEMP and EHA training interventions. Data are presented as mean (lines/columns) and SD (error bars), n = 10. $ main effect of time for resting haematocrit, * interaction effect vs day 1, $p < 0.05$.

Resting haematocrit was similar between interventions ($p = 0.633$, $\eta_p^2 = 0.026$). There was a main effect of time as resting haematocrit decreased ($p = 0.029$, $\eta_p^2 = 0.357$). Pairwise comparisons showed that resting haematocrit tended to be lower by day 12 in EHA (-1.1[-2.2;0.0]%, ES: -0.53, $p = 0.089$), and was significantly lower by day 19 (-1.1[-1.8;-0.3]%, ES: -0.58, $P = 0.021$). During the TEMP intervention, resting

haematocrit tended to decrease by day 12 (-1.0[-1.9;-0.0]%, ES: -0.51, $p = 0.090$), and day 19 (-0.8[-1.5;-0.1]%, ES: -0.52, $p = 0.057$), but these failed to reach statistical significance. There was no interaction effect (condition*time, $p = 0.987$, $\eta_p^2 = 0.005$). The reduction in plasma volume after exercise in both EHA and TEMP was not different between conditions ($p = 0.299$, $\eta_p^2 = 0.119$). There was a significant main effect of time ($p < 0.001$, $\eta_p^2 = 0.483$), and a significant interaction effect (condition*time, $p = 0.035$, $\eta_p^2 = 0.269$). Post hoc analysis revealed a greater percentage reduction in PV on days 5, 12 and 19 compared to day 1 in TEMP (-4.6[-1.8;7.4]%, ES: -1.2, $p = 0.011$; -5.2[-2.9;-7.5]%, ES: -1.5, $p = 0.002$; -2.9[-0.8;5.1]%, ES: -0.7, $p = 0.027$, respectively). In EHA, there was no difference between days 1 and 5 (1.9[-1.4;5.1]%, ES: 0.5, $p = 0.287$). There was a larger percentage decrease in PV during day 12, and 19 compared to day 5 in EHA (-4.7[-1.9;-7.6]%, ES: -1.4, $p = 0.009$; -5.5[-2.4;-8.6]%, ES: -1.3, $p = 0.007$, respectively).

### 4.3.7 *Correlation*

There was no significant correlation between the percentage change in resting Hct and percentage change in average power output at any time point during EHA (Figure 4.6). There was a significant correlation between percentage change in post exercise Hct, and percentage change in power in EHA between days 1 and 5 ($r = -0.63$, $p = 0.049$). By day 12, and 19, the correlation was weaker and not statistically significant ($r = -0.40$, $p = 0.253$, $r = -0.27$, $p = 0.459$, respectively). There was no relationship between average body temperature and sweat rate (Figure 4.8).

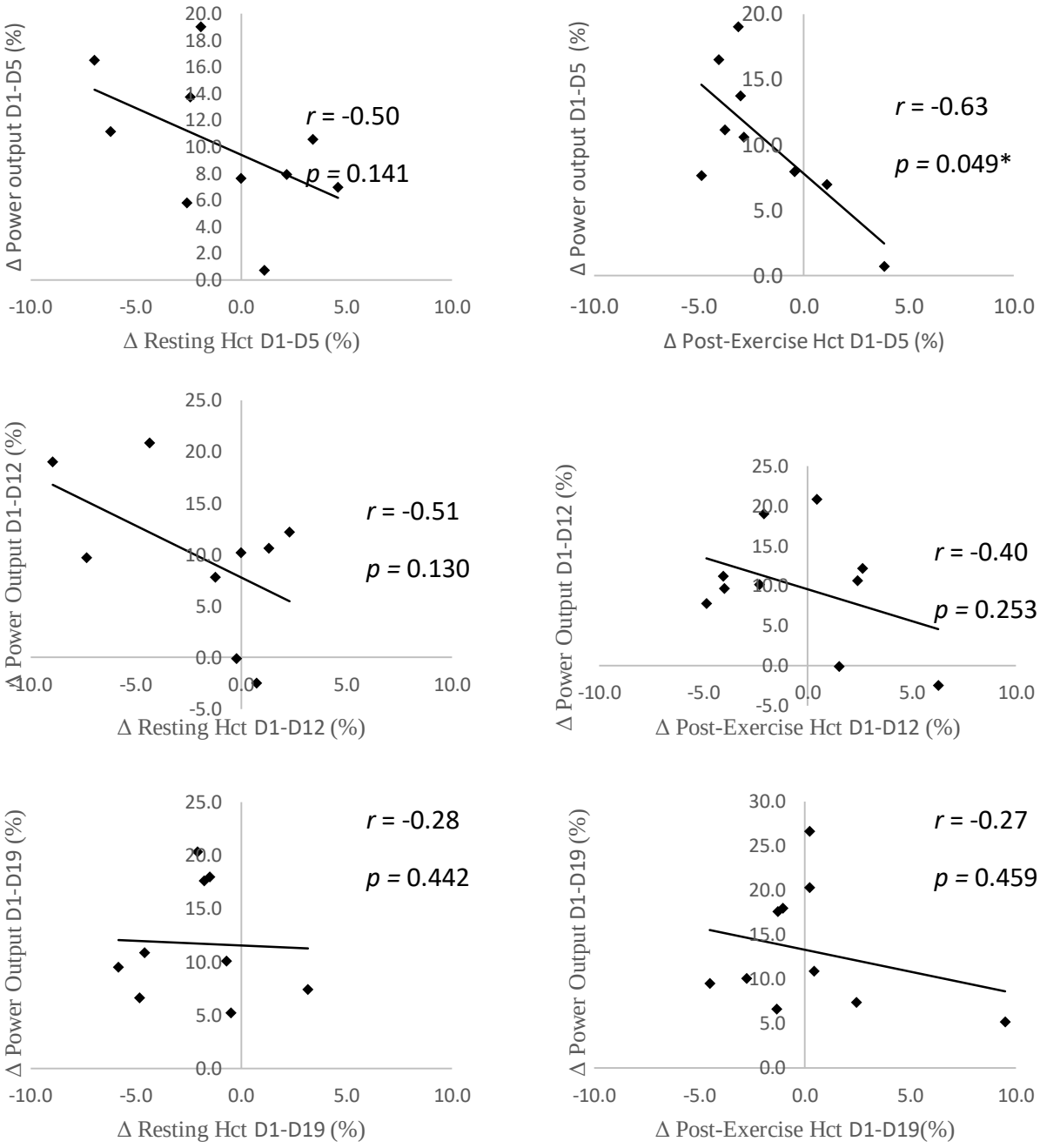


**Figure 4.7:** Correlation between relative changes in power output and the relative changes in haematocrit during daily training session. Data are presented as mean (solid line) and indiviudal points (black circle), n = 10.

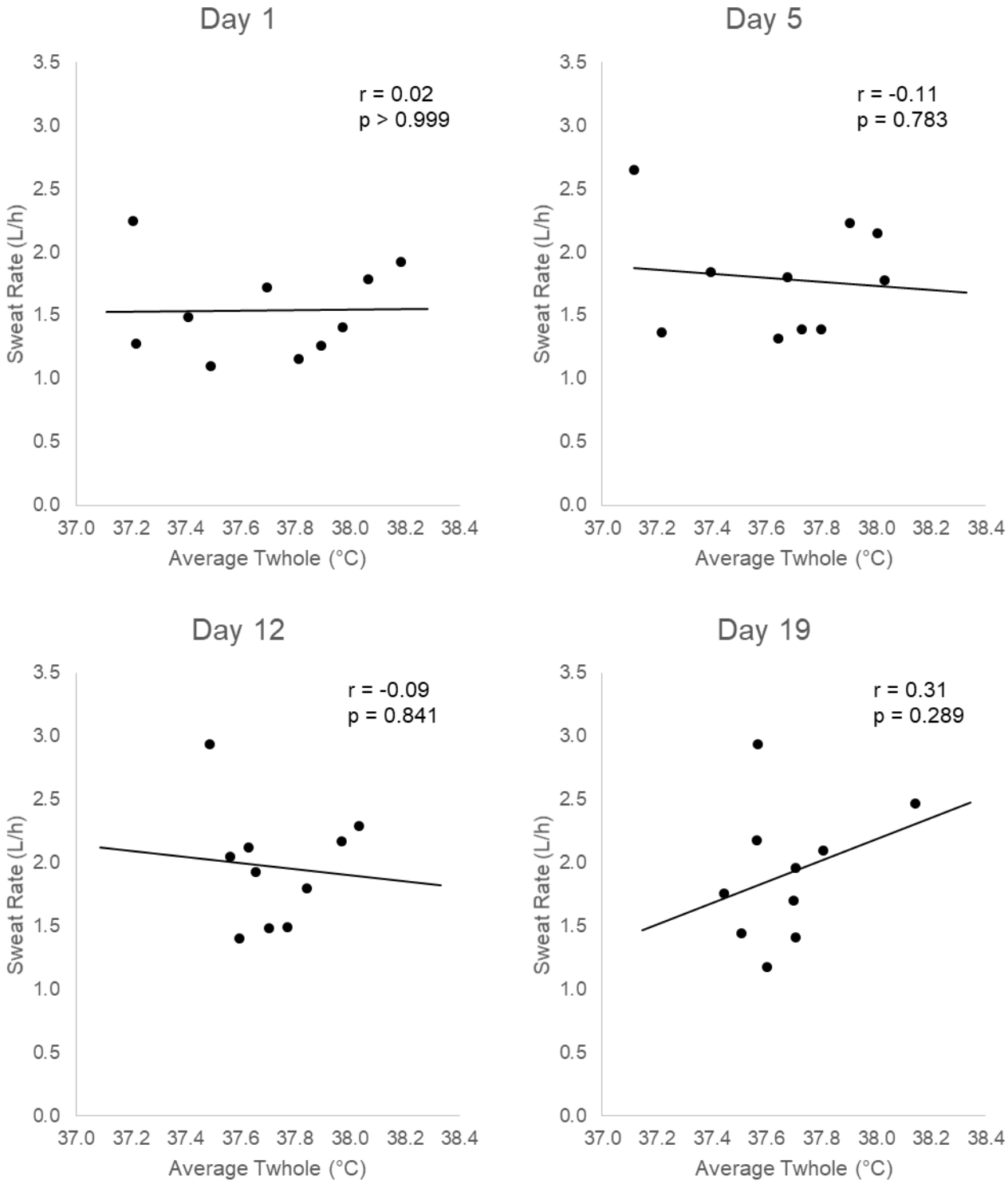


**Figure 4.8:** Correlation between average body temperature ($T_{whole}$) and sweat rate on days 1, 5, 12, and 19 of the exercise heat acclimation (EHA) training intervention. Data are presented as indiviudal points (black circle), and line of best fit. n = 10.

### 4.3.8 *Individual Variation*

Range of adaptive responses in average power, sweat sodium and rate, exercising heart rate, resting heart rate, plasma volume and core temperature are presented in figure 4.9.

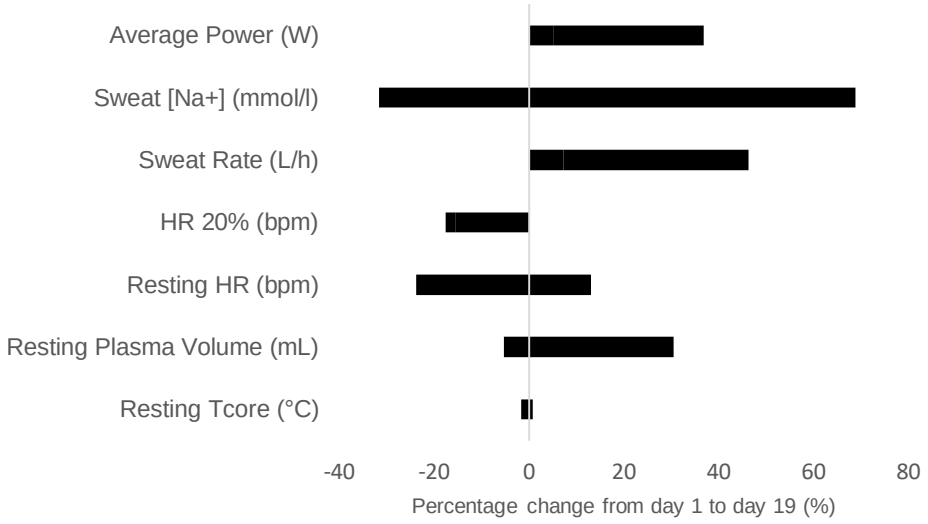


**Figure 4.9:** Range of adaptive responses during the exercise heat acclimation training intervention. Data are presented as minimum to maximum responses as a percentage change from day 1 to day 19.

## 4.4 **Discussion**

The present study outlines the time-course of adaptations to long-term heat acclimation using a novel work-matched, constant heart-rate model over a 3-week duration in a hot humid environment. Long-term heat acclimation using this model significantly increased power output at a given heart rate in the heat, which was reflected by the characteristic features of heat acclimation: increased sweat rate, decreased resting and exercise heart rate (during the constant work-rate portion of the exercise task), and increased plasma volume. Most importantly, whilst we confirmed that short-term heat acclimation (< 5 days) is effective, 3 weeks of heat acclimation provided additional benefit to sub-maximal exercise performance compared to 2 weeks, suggesting that functional adaptations to heat can not only be maintained over a 3-week period, but further enhanced.

### 4.4.1 *Heart rate-based protocol*

Participants training was work-matched to ensure that the absolute training load was similar between interventions. Heat acclimation was undertaken at a heart rate 7bpm higher than in temperate training, since HR increases approximately 7bpm for every 1°C rise in body temperature (Jose et al. 1970) and was implemented to account for the increased cardiac strain during exercise in the heat to maintain the same relative training intensity between conditions (Périard and Racinais 2015; Périard et al. 2015). Total work-done between conditions was also matched (Table 4.1), to ensure the absolute training load was similar. Many heat acclimation studies have been criticized for either lacking an adequate control group or not matching relative training demands between the intervention and control (Corbett et al. 2014). The novel approach developed in the current study provides a framework to match both relative and absolute training loads between hot and cool environments. As compared to the previously proposed core temperature-clamped protocol (Patterson et al. 2004), this further presents the advantage of maintaining an adequate training stimulus. During the core temperature-clamp protocol, participants have been required to stop exercising entirely to lower their target temperature when it exceeds the target temperature (Gibson et al. 2015a). In addition, the current HR-based training can also be easily applied in the field, for a range of sporting activities.

Furthermore, the target workload was increased by 5% per week to respect the principle of progressive training that is likely to be implemented in any training program. This also drives the thermal stimulus by sustaining the time spent training in the heat each week, as power output increased (Table 4.1). This progressive, work-matched, clamped heart-rate model successfully attenuated the decline in physiological overload with progressive adaption to heat, often observed in constant work-rate models (Périard et al. 2015), and provided additional benefit throughout the 3 week intervention.

While the heart rate clamp protocol successfully induced heat acclimation, we must consider the mechanistic constraints associated with such an approach. The demands of endurance exercise undertaken in warm environments creates completion for blood flow between the active muscle to meet the energetic demands for muscular activity, and the skin to meet the demands of temperature regulation. These combined demands for blood flow can result in a competition for the available cardiac output (Rowell 1974), which has a limit to the ability to meet the dual demands of exercise *per se* and of temperature regulation. During exercise, plasma volume falls, and heart rate increases to maintain stroke volume and therefore cardiac output. It is clear that the adaptive pressures on the cardiovascular system are primarily regulated around maintenance of cardiac output, and not heart rate. As such clamping heart rate may only provide an indirect forcing function on the cardiovascular system during heat acclimation.

### 4.4.2 *Kinetics of adaptation to long-term heat acclimation*

The adaptations from 3 weeks of heat acclimation using a heart rate based model markedly increased the power output at a set heart rate after 1 week, and exhibited additional benefit at the end of 3 weeks (+~8%). There were significant increases in average power by day 5 during EHA (~9% greater than day 1). Average power attained in the sessions was highest on day 19, and the third week of heat acclimation yielded a higher average power than weeks 1 and 2. It should also be noted that the target workload increased by 5% in weeks 2 and 3, which further strengthens these findings, as the additional 5% of work was added to the end of the session (*i.e.* 105% in week 2 and 110% in week 3), where power output was the lowest and core temperature was the highest. This is in line with current literature, suggesting that while short term (<7 days) heat acclimation is beneficial (Karlsen et al. 2015a; Racinais et al. 2015a), further adaptations are accrued after 14 days (Pandolf 1998; Racinais et al. 2015a). It should be noted that in the present study, 15 days of heat exposures were achieved

over a 19-day period. This may partly explain the observation of a ~3.1% drop in average power between days 5 and 8, where participants were permitted 2 days without a heat exposure (1 standardised outside training ride in the cool and 1 rest day). However, this decrement in average power was not observed between days 12 and 15 (+0.6%), where participants completed the same 2-day block outside of the laboratory. This suggests that while short-duration (i.e. 5 days) HA is effective in inducing adaptations, they are not well defended once the thermal stimulus is withdrawn, even after just 2 days. However, with medium to long term exposure (8-14 days), the adaptations were better defended once the heat stimulus was withdrawn. While there is limited, and conflicting, data available on the decay of heat acclimation, our results are generally in line with the current literature. The physiological adaptations after heat acclimation are relatively short-term and may reverse after only a few days or weeks after withdrawal of the heat stimulus (Armstrong and Maresh 1991; Daanen et al. 2018). Given that the primary adaptations which reverse are of cardiovascular origin (Pandolf et al. 1977; Saat et al. 2005; Garrett et al. 2009), the reduction in power output between days 5 and 8 is not surprising since we evaluated power output at a set heart rate, and so workload was directly related to the decrease in heart rate at a given exercise intensity. Furthermore, heart rate tended to be higher (+3bpm) at the end of the constant load portion of the daily training (*i.e.* after 20% of total work complete) on day 8 compared to day 5, but was the unchanged between days 12 and 15. It has been suggested that the rate of heat acclimation decay is such that for every 2 days spent without an exercise heat exposure, 1 day of acclimation is lost (Givoni and Goldman 1972). Recently, Daanen and colleagues (Daanen et al. 2018) suggested adaptations in end-exercise heart rate are decreased by 2.3% for each day of heat acclimation decay. It was also observed that lengthening the duration of heat acclimation enhanced thermal adaptions, and may speed up re-acclimation.

Data from the present study supports this hypothesis as average power on day 9 was within 1.4% of day 5, and there was no difference between day 12 and day 15.

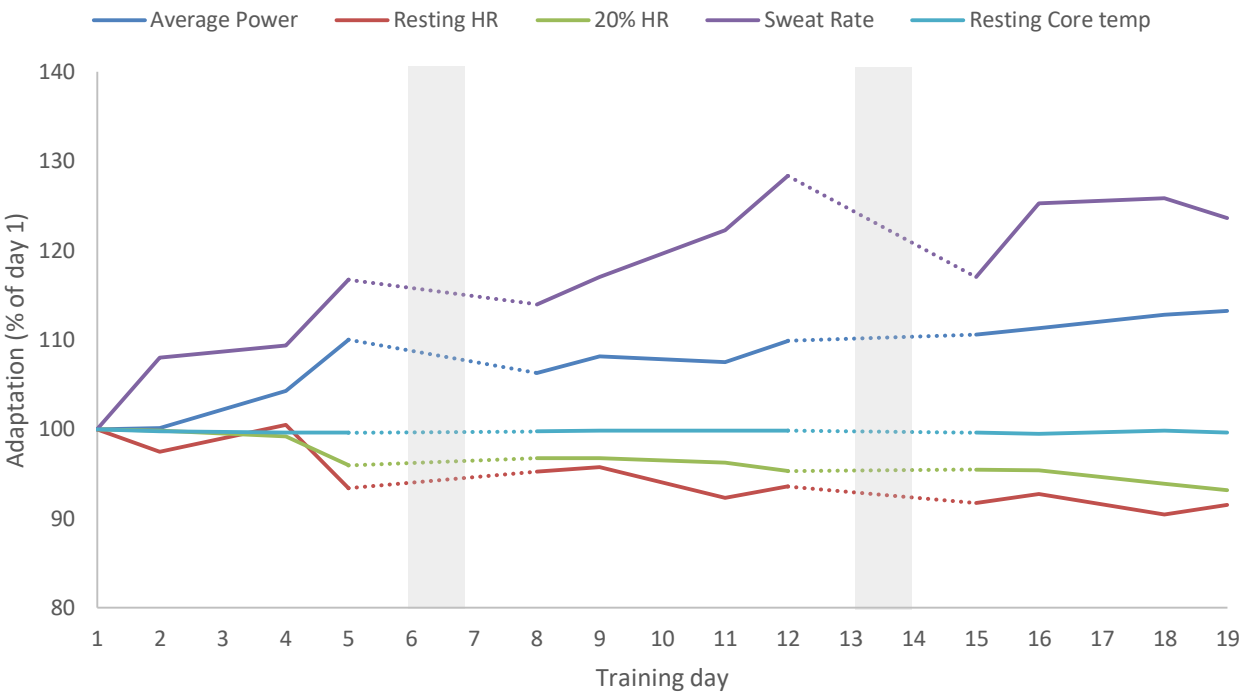


**Figure 4.10:** Kinetic of adaptation during EHA, as a percentage of day 1 (day 1 denoted as 100%). Grey boxes and dashed lines denote each 2 day period (days 6, 7 and 13, 14) where a heat exposure was not applied. Data are presented as mean only for clarity (n = 10).

The present study observed rapid sudomotor adaptations during EHA, as demonstrated by a significant increase in sweat rate even after just 1 day of acclimation. Cardiovascular adaptations were observed after ~5 days as demonstrated by reductions in resting and sub-maximal exercise heart rate. There was no observed change in resting core temperature.

Sweat rate was significantly higher by day 2 of heat acclimation, and plateaued at day 9, where no further changes were observed. Approximately 60% of the sweat rate adaptation was achieved in the first week of EHA. In the second week of heat acclimation the adaptation was complete (~40%), and no further alterations occurred in the third week. Sweat rate is generally considered to be a slow adaptation while undertaking heat acclimation, with increases occurring much later than observed in the present study, after 1 week of heat acclimation (Armstrong and Maresh 1991;

Sawka et al. 2011). Some studies have reported no change in sweat rate in trained subjects (Kobayashi et al. 1980; Houmard et al. 1990; Racinais et al. 2014b). However, Karlsen and colleagues (Karlsen et al. 2015a) similarly reported a fast and large sweat rate adaptation (~20%) in 5 days of acclimatisation in well-trained cyclists. Such a large and sudden onset of sweating is surprising, especially since there was no association between sweat rate and average whole body temperature (figure 4.8). This suggests that the daily increases in power output, and therefore metabolic heat production, coupled with the high workload and sustained relative intensity of exercise achieved utilising a heart rate based model, inducing high daily peak core temperatures, may have contributed to the rapid increase in sweat rate. Sudomotor adaptations are influenced by: the environmental conditions; the magnitude of the core temperature elevation; and the duration of heat stress (Fox et al. 1963). While sweat rates are lower in humid compared to dry heat (Frye and Kamon 1983), humid heat acclimation induces a greater elevation in sweating (Shvartz et al. 1973). Of note, sweat rate was markedly reduced immediately after the 2 days where participants received no heat exposure each week (days 9 and 15). This further supports the rapid decay of sudomotor adaptation when the heat stimulus is withdrawn. The magnitude of the decay in sweat rate was also related to the magnitude of adaptation beforehand. By day 5, sweat rate had increased ~17% from baseline, and dropped ~3% by the next heat exposure on day 8. However, sweat rate had increased ~28% by day 12, and dropped ~11% from this on day 15. Also the rate of sweating influences thermoregulatory adaptation of the eccrine glands (Buono et al. 2009). Whilst average sweat [$Na^+$] was marginally lower with heat acclimation, this was not statistically significant, and large individual responses were observed. Sweat [$Na^+$] reduced in 7 participants, but increased in 3. Sweat [$Na^+$] is widely reported to fall during heat acclimation, due to increased conservation of sodium chloride at the eccrine glands, to maintain intravascular osmotic pressure and support expansion of the plasma

volume (Nielsen 1998). This response has been observed in both trained (Kobayashi et al. 1980; Racinais et al. 2012; Karlsen et al. 2015a) and untrained participants (Kirby and Convertino 1986; Buono et al. 2007; Chinevere et al. 2008). However, sweat sodium ion reabsorption decreases linearly with increasing sweat rates, possibly due to a greater flux of sweat from the sweat ducts and insufficient capacity to retain sodium ions from the sweat duct (Inoue et al. 1998; Shamsuddin et al. 2005).

We also observed a marked cardiovascular adaptation within the first week of EHA, as evidenced by reductions in resting and sub-maximal heart rate at a given power (HR20%). Of note, the reduction during exercise was ~10bpm, with a ~5bpm reduction observed at rest. After the first week of training, cardiovascular adaptations continued to trend downwards, although the magnitude of adaptation was smaller and slower, with ~60% of sub-maximal HR adaptation achieved in the first week, and a further ~20% in week 2, and ~20% in week 3. This was concurrent with the average power output achieved for the session with ~77% of the adaption achieved within the first 5 days of heat acclimation, and the remaining 23% achieved within the second and third week.

There was no observed change in resting core temperature - a common (Buono et al. 1998; Patterson et al. 2004; Kampmann et al. 2008), but not universal finding (Shido et al. 1999; Garrett et al. 2009; Racinais et al. 2012), following heat acclimation. By design, we also observed no change in average core, skin, or whole body temperature during each daily training session. These characteristic adaptations with heat acclimation are likely the result of a constant work-rate exercise, where the forcing function declines as adaptation progresses. The increase in daily power output and therefore metabolic heat production, is likely to have masked potential reductions in core, and skin temperature during exercise. Conversely it can be concluded a greater

power output was achieved at the same core and skin temperature as adaptation progressed.

In contrast with previous literature, our data showed no change in haematocrit after 5 days of heat acclimation, but a lower and sustained reduction in the second and third week of EHA. Plasma volume expansion is considered to increase vascular filling, and therefore support cardiovascular stability. We observed reductions in resting, and sub maximal heart rate during the first 5 days of heat acclimation, which did not coincide with an increased plasma volume, suggesting factors other than plasma volume might modulate heart rate responses in the heat (Coyle and González-Alonso 2001) . The reason for this discrepancy is unclear, and the physiological adjustments that could lead to a reduction in resting and sub-maximal heart rate, independent of plasma volume expansion are uncertain. It could be speculated that this was caused by a reduced blood flow distribution to the skin in the presence of lower skin temperature, although skin blood flow was not measured in the present study. The magnitude of the plasma volume response is related to the hydration state when measured (Harrison 1985), and time of day due to alterations in circadian rhythm (Voss et al. 2011). Participants were given instructions to ensure euhydration on arrival at the laboratory and urine specific gravity was measured prior to each session to confirm hydration status (>1.022). In addition, all blood collection were performed at the same time of day to ensure blood markers were not influenced by the daily fluctuations in circadian rhythm. In contrast, resting haematocrit was significantly lower by day 12 and 19, compared to baseline. It is sometimes reported that plasma volume expansion occurs rapidly in the first few days of acclimation and follows a biphasic response to return to baseline levels after ~2 weeks (Bass et al. 1955; Wyndham et al. 1968; Shapiro et al. 1981), but not always if the stimulus is adapted (Patterson et al. 2014). Indeed, it is likely this biphasic response partly occurs as a methodological artefact of constant work-rate protocols as the physiological overload continually declines with progressive

adaption to heat (Périard et al. 2015). Patterson and colleagues (Patterson et al. 2014) reported a maintained plasma volume expansion for 22 days during a controlled hyperthermia (38.5°C) model. Similarly, the progressive heart rated clamped model utilized in the present study maintained average core temperature from week 1 to week 3, and facilitated a high peak core temperature (~38.8°C) every day. This model may better facilitate a continual adaptive stimulus to maintain plasma volume expansion throughout the acclimation period. The observation that plasma volume also increased to a similar magnitude during the TEMP intervention suggests that participants also underwent a haematoglocial adaptation during training in a cool environment. Hypervolaemia, characterized pre-dominantly by increased plasma volume, has been well documented as a consequence of endurance exercise training (Convertino 1991b). Since there were no changes in sweat rate, resting heart rate, or core temperature, it seems likely this thermal adaptation in isolation conveyed minimal thermoregulatory advantage during TEMP training. There was no evidence of a selective defence of the plasma volume compartment. Plasma volume increased at rest on days 12 and 19 compared to day 1 during EHA, whilst the post-training plasma volume did not significantly vary from the baseline (day 1) plasma volume (Figure 4.7). It is unlikely there is any homeostatic defence of the intravascular compartment with a constant cardiac strain heat acclimation model. This is in agreement with Patterson and colleagues (Patterson et al. 2014), who similarly described no preferential defence of the plasma volume after 8 and 22 days of heat acclimation with a controlled hyperthermia model.

No difference in average RPE, TS or TC was observed in the present study from days 1 to 19 in both TEMP and EHA training interventions. Similarly, there was no difference in RPE between TEMP and EHA, while TS and TC were consistently and significantly higher in EHA than TEMP. These results, combined with the maintained average core temperature and plasma volume expansion and increased power output during 3

weeks of the training intervention suggest the work-matched, heart rate based model of heat acclimation applied in the present study successfully implemented a progressive thermal strain throughout heat acclimation, through both objective physiological biomarkers and perceptual indices, conveying a benefit for up to 19 days.

### 4.4.3 *Individual Differences*

There were large individual differences in the acute response to training completed in temperate and warm environments, with average power on day 1 ranging from ~1% to 33% decrement in EHA compared with TEMP. There was also a large individual variation in the adaptive response to performance over time during EHA. After 2 weeks of heat acclimation, 3 participants exhibited negligible changes in average power output (<2%), while 5 participants increased from their week 1 power output by ~5%. 1 participant improved by ~10%. During the third week of EHA, one of the participants who exhibited minimal improvements during the first 2 weeks underwent a large 10% increase in power output, while the other 2 exhibited further minimal improvements (< 2%). 2 had improved by ~5%, and 3 improved by <10%. Having improved ~5% by week 2, one participant had a ~3% reduction in average power during week 3. This has wide implications for sporting environments where specific preparation would occur on a team, rather than individual level, since some individuals require a longer stimulus to adapt than others and 20% of the current cohort expressed minimal improvement even after 3 weeks of heat acclimation.

Phenotypical adaptions followed a similar pattern with large individual variation in plasma volume expansion (~ -5 to 31%), resting core temperature (~ -2 to 1%), resting heart rate (~ -24 to 13%), exercising heart rate at 20% work done (~ -16 to -2%), sweat rate (~7 to 39%), and sweat sodium (~32 to 69%) (figure 4.9). Whilst at a cohort level, the literature is consistent in the adaptive responses to heat acclimation, where individual data are presented considerable heterogeneity is evident, in line with our

findings. Plasma volume has been observed to increase from ~8 to 33% (Senay et al. 1976), and ~-10 to 20% (Racinais et al. 2012). Large ranges in responses to exercising heart rate (~-2 to 32 bpm) and rectal core temperature (~-0.3 to -1.2 °C) (Wyndham et al. 1976) have also been described. This has led to the notion of 'responders' and 'non-responders' to heat acclimation, although the kinetic of changes described previously suggest some individuals require an increased 'dose' of heat exposure (i.e. 3 weeks vs 2 weeks), to elicit these adaptations.

The use of a simple blood measure (Hct) to elucidate magnitude and effectiveness of adaption to heat acclimation would provide a useful tool, without the need to test athletes in controlled environments. Data from this study demonstrated that the relative improvement in power after 5 days of EHA (D1 to D5) was significantly correlated to the relative change in post-exercise Hct (Figure 4.6). This correlation may suggest that the benefit is specific to the post-exercise Hct, which incorporates both the magnitude of haemodilution at rest (*i.e.* plasma volume expansion), and the magnitude of the acute haemodynamic response to exercise (*i.e.* exercise induced haemoconcentration). This confirms, in a laboratory setting, data which has previously been observed during field acclimatization in soccer players (Racinais et al. 2012), Australian Football League players (Racinais et al. 2014a) and cyclists (Karlsen et al. 2015a). However, the correlation was much weaker by week 2, and further decreased by week 3, an observation also noted by Karlsen and colleagues (Karlsen et al. 2015a). The post-exercise haematocrit was better correlated to changes in performance than resting values. While resting haematocrit was moderately, but non-significantly, correlated to changes in average power output at day 5, this relationship was diminished by day 19. Although plasma volume expansion *per se* may not improve exercise performance (Sawka et al. 1983a; Watt et al. 2000), relative changes in post exercise haematocrit may provide a valid marker of performance changes in short (5 days), but not long term (> 10 days) heat acclimation.

### 4.4.4 *Conclusion*

In summary, 3 weeks of heat acclimation using a novel heart rate based model induced beneficial phenotypical responses in most participants after just 5 days, but these were further increased by 3 weeks of heat acclimation. This heart rate model could easily be transferred to athletes training outdoor or indoor in the heat with the use of commercially available heart rate monitors. Importantly, some athletes (20% of the present study) may require a longer exposure (< 10 days), for functional improvements (*i.e.* increased power output at a given heart rate) in sub-maximal exercise.

# CHAPTER 5

# HAEMATOLOGICAL ADAPTATIONS TO HEAT ACCLIMATION

## 5.1 Introduction

Alterations in blood volume in response to climatic changes have been reported as early as 1922 (Barcroft *et al.* 1922), yet research has almost exclusively focused only on the adaptation of plasma volume, which has been a standard observation for the past 70 years (Glaser 1949). Plasma volume expansion, is widely viewed as the primary adaptation supporting exercise in the heat, leading to a greater maintenance of stroke volume and cardiac output, and reduced heart rate at any given workload (Senay 1972; Bonner and Edwards 1976; Sawka and Coyle 1999; Frank et al. 2001). However, a sustained increase in plasma volume (haemodilution) may have further implications for the regulation of total blood volume, and of the red blood cell (RBC) volume due to homeostatic pressures. The kidney closely regulates the relative volumes of these two blood components, through the production of erythropoietin and retention of sodium and fluid (Dunn et al. 2007). Thus, it is reasonable to postulate that heat acclimation might also modulate the RBC compartment, and specifically the HBmass through the erythropoietic pathway.

Currently, the response of HBmass during heat acclimation is unknown. Erythrocyte volume (mean corpuscular volume) may acutely increase, decrease or remain constant with exercise and is dependent on plasma osmolality and blood pH (van Beaumont et al. 1981). Paterson and colleagues (Patterson et al. 2014) identified no change in erythrocyte volume after 22 days of heat acclimation, Keiser and colleagues (Keiser et al. 2015) observed no change in HBmass 4-5 days after heat acclimation. Similarly Rendell and colleagues (Rendell et al. 2017) observed no change in HBmass

regardless of adding an overnight altitude stimulus to the heat acclimation training. However, Bazett and colleagues (Bazett et al. 1940) reported an absolute increase in total circulating haemoglobin and a marked increase in reticulocytes after heat exposure. Scoon and colleagues (Scoon et al. 2007) reported an increase in RBC volume after 3 weeks of post-exercise sauna bathing, while Karlsen and colleagues (Karlsen et al. 2015b) reported a ~7% increase in HBmass 12 days after a 2-week heat acclimatization training camp. However, the findings of Karlsen and colleagues (Karlsen et al. 2015b) should be interpreted with caution, as their reports of elevated blood volume and HBmass, parameters which are strongly associated with increased maximal oxygen uptake (Schmidt and Prommer 2010), did not coincide with any change in maximal oxygen uptake. Given the contrasting results, and that HBmass was only previously measured before and after, not during heat acclimation, the kinetic of adaptation is unknown. Furthermore, any change in HBmass would influence measures of [HCT] and haemoglobin concentration, and could therefore bias any measures of relative changes in plasma volume since one of the assumptions is that HBmass is a constant (Dill and Costill 1974). Accordingly, the aim of this chapter was to characterize the HBmass response to heat acclimation, and the effect this may have on estimates of plasma volume. It was hypothesized that both HBmass and plasma volume would increase as a result of heat acclimation.

## 5.2 Material and methods

### 5.2.1 *General procedure*

10 participants completed 3 weeks of exercise heat acclimation (EHA, 35°C, 60% RH) and 3 weeks of work-matched training in temperate conditions (TEMP, 18°C, 60% RH). Detailed descriptions of the participant characteristics and project overview are presented in general methods 3.1.2, and 3.2.1, respectively. .

### 5.2.2 *Venous blood sampling*

Venous blood samples were drawn before training after a 10 min seated stabilisation period, and immediately after training on day 1, 5, 12, and 19 of each intervention, for determination of reticulocytes, plasma erythropoietin concentration [EPO], and ferritin concentration [Fe]. HBmass was determined at minimum in duplicate in the 2 weeks prior the interventions, then single measures were taken on days 4, 11, and 18 of the interventions, and finally, HBmass was measured in duplicate again post-intervention (i.e. 5 and 7 days after each training intervention).

### 5.2.3 *Haemoglobin Mass*

HBmass was measured using the optimised carbon monoxide (CO)-rebreathing technique detailed in chapter 3 (Schmidt and Prommer 2005; Prommer and Schmidt 2007). Participants fully exhaled to residual volume into a portable CO sensor (Draeger PAC7000, Draeger, Luebeck, Germany) to detect existing levels of CO within the body and baseline fingertip capillary samples (200 µL) were obtained. Participants wore a nose-clip and were connected to a closed spirometer via the mouth piece at the end of a maximal expiration. Participants were instructed to inhale deeply and hold their breath for 10-s while an individualised bolus (1.2g/kg) of 99.5% medical grade CO was administered via a 100mL syringe. Simultaneously, the valve for a 3L anaesthetic bag pre-filled with pure medical grade oxygen was opened. After 10-s participants were asked to continue normal respiration through the spirometer for 1-min 50-s. During this time, a portable CO analyser (Draeger PAC7000, Draeger, Luebeck, Germany) with parts-per-million sensitivity was held in close proximity to the mouth piece and spirometer to verify that no gas had escaped. After 2-min of re-breathing, the participants were asked to fully exhale to residual volume in the anaesthetic bag to quantify the amount of CO which was not taken up by the body. Thereafter, the spirometer was disconnected and the participants continued to sit quietly. Two minutes later, participants again fully exhaled to residual volume into a

portable CO sensor (Draeger PAC7000, Draeger, Luebeck, Germany) and was combined with the remainder of CO within the spirometer after the test to determine the quantity of CO not taken up by the body. At exactly 7-min after commencement of re-breathing, further fingertip capillary samples (200 µL) were obtained. All capillary samples were immediately analysed in triplicate for percent carboxyhaemoglobin (%HbCO) and using a spectrophotometer (ABL flex-90, Radiometer, Denmark). Total HBmass was calculated from the mean change in % HbCO as described previously (Schmidt and Prommer 2005)

### 5.2.4 *Coefficient of variation*

To determine the measurement variability of HBmass, a within-participant, between-day reliability pilot trial was conducted before implementing the study. 10 healthy active males were asked to attend the laboratory on 3 occasions. During an initial visit, participants were familiarized to the equipment and procedures involved. In each of the subsequent 2 reliability visits, HBmass was determined with at least 24h between each trial. Reliability data are shown in table 5.1.

**Table 5.1:** Reliability data of the CO-rebreathing technique to determine HBmass (n = 10)

| Trial 1 (g) | Trial 2 (g) | Average (g) | SD (g) | CV (%) |
|---|---|---|---|---|
| 957 | 964 | 960 | 14 | 1.4 |

### 5.2.5 *Blood volumes*

The HBmass measured by CO-rebreathing was used along with the [HCT] and [Hb] measured by CBC to calculate the total blood volume (BV), red blood cell volume (RBCV) and plasma volume (PV) according to the following formulas:

$$BV\ (mL) = (Hb\ mass\ (g)/Hb\ (g \cdot dl^{-1})) \times 100$$

$$RBCV\ (mL) = BV\ (ml) \times ([HCT]/100)$$

PV (mL) = BV – RBCV

BV, RBCV and PV was determined prior to and after training on days 1, 5, 12 and 19. The average HBmass measured prior to the study was used for the calculation of blood volumes on day 1 of training. During the training interventions, the HBmass was measured on days 4, 11, and 18 and this was used for the calculation of blood volumes the following day. HBmass was not measured on the same day as venous blood sampling, since inhalation of a CO bolus can induce a heat shock protein response per se (Wu et al. 1995) and thus invalidate these alternative measures. The average of the duplicate HBmass measures taken during the post-training intervention testing week (chapter 7) was utilized for all blood volume calculations during the post-test week. In order to make comparisons to existing literature, a secondary calculation for total blood volume, plasma volume, and red blood cell volume was made, using only the baseline HBmass figures and assuming that there was no change throughout the interventions. Blood volume calculations incorporating the measured HBmass data, and calculations assuming no change in HBmass are subsequently referred to as measured ($_{meas}$), and estimated ($_{est}$), respectively.

### 5.2.6 *Reticulocyte count*

In addition to the CBC, whole blood was simultaneously analysed for reticulocyte count (Beckman Coulter DxH 800, Beckman Coulter, Miami, FL). Due to technical difficulties, reticulocyte count is presented for n = 8 TEMP, and n = 7 EHA.

### 5.2.7 *Extracellular protein content*

Plasma samples collected prior to and upon completion of the performance trials were analysed using the ELISA technique for determination of extracellular [EPO] and [FE] as previously described in general methods 3.3.3.

### 5.2.8 *Statistical analysis*

Data are presented as mean ± standard deviation (SD). A two-way (condition x time) analysis of variance (ANOVA) with repeated measures design was used to asses differences in blood volumes. 95% confidence intervals (CI), and Cohen's *d* effect sizes (ES) for were also determined. Data are presented as (mean difference, [95% CI], ES, P value). Where a significant interaction effect was apparent, pair-wise differences were evaluated using the Sidak method. Data analysis was performed using Statistical Package for the Social Sciences for Windows version 22.0 software (SPSS Inc., Chicago, IL). Significance was accepted at $P < 0.05$.

## 5.3 **Results**

### 5.3.1 *Training*

Characteristics of the moderate intensity heart rate based, and intermittent sprint cycling training during each 3-week TEMP and EHA intervention are described in detail in chapter 4 and 6, respectively. Briefly, average daily training data from the 4 heart rate based training sessions, and the intermittent sprint session data for each training week are presented in table 5.2.

**Table 5.2**: Training characteristics of the TEMP and EHA training interventions. Data are presented as mean ± SD, n = 10.

| | TEMP | | | EHA | | |
|---|---|---|---|---|---|---|
| Training Week | 1 | 2 | 3 | 1 | 2 | 3 |
| HR-based training | | | | | | |
| Time (mm:ss) | 56:52 ± 02:54 | 58:43 ± 03:27 | 61:46 ± 04:06 | 67:17 ± 08:03 | 68:08 ± 07:49 | 67:45 ± 05:54 |
| Work done (kJ) | 712 ± 97 | 748 ± 102 | 784 ± 107 | 712 ± 97 | 748 ± 102 | 784 ± 107 |
| Intermittent Sprint training | | | | | | |
| Work done (kJ) | 97.7 ± 12.7 | 98.6 ± 11.8 | 98.6 ± 11.9 | 96.4 ± 15.0 | 97.5 ± 14.3 | 97.6 ± 12.6 |

The mean HBmass and haematological parameters before each training intervention are presented in Table 5.2. There were no significant differences at baseline for any parameter. As such, the changes in haematological markers are hereafter presented relatively to the baseline values.

**Table 5.3:** Baseline blood measures. Data are presented as mean ± SD, n = 10 for HBmass, [EPO] and [Fe], n = 7 for reticulocytes.

| Pre-intervention | TEMP | EHA |
|---|---|---|
| Hbmass (g) | 951 ± 113 | 948 ± 121 |
| Hbmass (g/kg) | 12.6 ± 0.9 | 12.5 ± 0.8 |
| Reticulocytes (%) | 1.0 ± 0.2 | 0.9 ± 0.2 |
| [EPO] (pg/mL) | 116.7 ± 20.4 | 122.5 ± 19.0 |
| [Fe] (ng/mL) | 32.0 ± 8.2 | 38.1 ± 11.4 |

### 5.3.2 *HBmass*

The time course of changes in HBmass observed in response to TEMP and EHA are shown in figure 5.1. There was a main effect of condition ($p < 0.001$, $\eta_p^2 = 0.770$) where HBmass was lower during EHA than TEMP. HBmass tended to decrease over time but this did not reach statistical significance ($p = 0.052$, $\eta_p^2 = 0.225$). There was a significant interaction effect ($p < 0.001$, $\eta_p^2 = 0.487$). Post-hoc analysis revealed that HBmass during TEMP remained similar to pre- at all time points ($p > 0.05$ for all). During EHA, HBmass was lower than baseline by day 4 (mean difference, [95% CI], ES, P value), (-2.5[-0.7;-4.2]%, ES: -3.37, $p = 0.005$). HBmass remained lower than baseline on day 11 (-1.9[-0.1;-3.7]%, ES: -2.42, $p = 0.043$), and day 18 (-2.6[-0.4;-4.8]%, ES: -3.70, $p = 0.020$). However, HBmass returned to baseline during the post week using the average of 2 measures on days 5 and 7 after the intervention (-0.9[-0.8;2.6]%, ES: -3.70, $p = 0.600$). Of note, in 7 participants HBmass returned to baseline (-0.1%), while 3 remained lower (-2.8%).

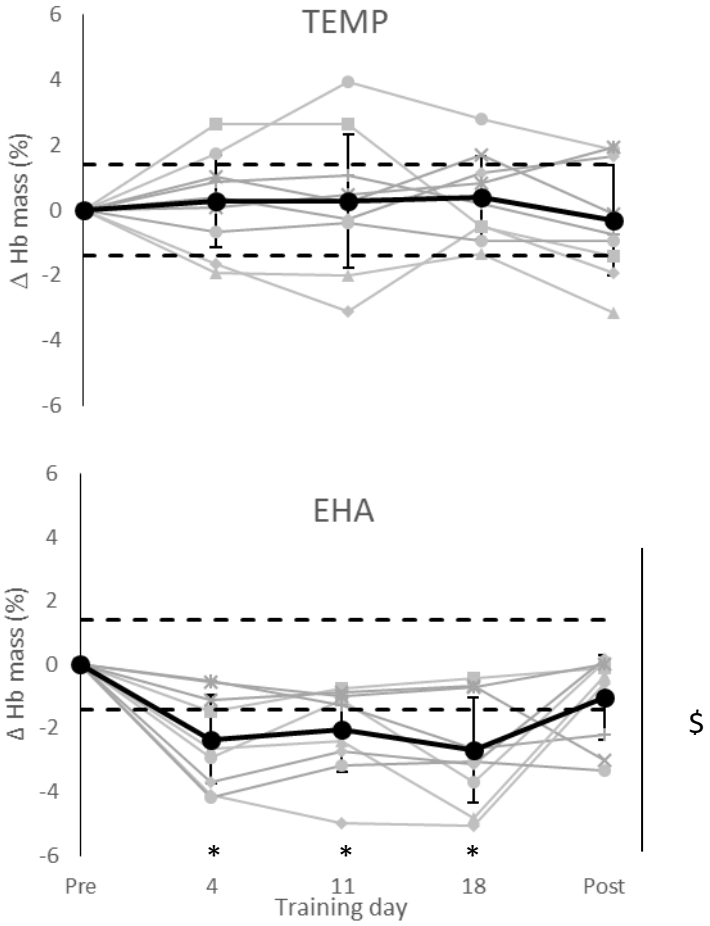


**Figure 5.1:** Time-course of HBmass during the 3-week TEMP and EHA training interventions. Data are presented as mean (solid black line), SD (error bars), and individiual responses (grey lines), n = 10. Dashed black lines represent the coefficient of variation (± 1.4%). $ main effect of condition vs TEMP, * interaction effect compared to pre, $p < 0.05$.

### 5.3.3 *Blood volumes*

All blood volume parameters are presented as calculated values incorporating the reduction in HBmass $_{(meas)}$, and estimated HBmass $_{(est)}$ assuming that there was no change in HBmass, as is conventionally reported in the literature. Blood volumes were derived from the formulae presented in section 5.2.5. Resting blood volume, plasma volume and red blood cell volume on days 1, 5, 12, and 19 of each intervention are presented in figure 5.2.

There was no effect of condition ($p$ = 0.141, $\eta_p^2$ = 0.225) or time ($p$ = 0.193, $\eta_p^2$ = 0.171) on resting $BV_{meas.}$ There was also no interaction effect ($p$ = 0.296, $\eta_p^2$ = 0.126). There was no effect of condition on $BV_{est}$ ($p$ = 0.302, $\eta_p^2$ = 0.117), but there was a significant effect of time ($p$ = 0.012, $\eta_p^2$ = 0.403), as resting $BV_{est}$ increased over time. There was no interaction effect ($p$ = 0.850, $\eta_p^2$ = 0.029). There was no effect of condition ($p$ = 0.563, $\eta_p^2$ = 0.038) on $PV_{meas}$. $PV_{meas}$ tended to increase over time but this failed to reach statistical significance ($p$ = 0.070, $\eta_p^2$ = 0.270). There was also no interaction effect ($p$ = 0.839, $\eta_p^2$ = 0.030). There was no effect of condition on $PV_{est}$ ($p$ = 0.326, $\eta_p^2$ = 0.107), but there was a significant effect of time ($p$ = 0.012, $\eta_p^2$ = 0.408), as resting $PV_{est}$ increased over time. There was no interaction effect ($p$ = 0.942, $\eta_p^2$ = 0.014). There was no effect of condition ($p$ = 0.134, $\eta_p^2$ = 0.232) or time ($p$ = 0.721, $\eta_p^2$ = 0.047) on resting $RBCV_{meas.}$ There was a significant interaction effect ($p$ = 0.001, $\eta_p^2$ = 0.430) whereby $RBCV_{meas}$ remained similar during TEMP but decreased during EHA. Post-hoc analysis revealed $RBCV_{meas}$ was lower during EHA than TEMP on day 5 (-80[-6;-155]mL, ES: -0.23, $p$ = 0.037), and day 19 (-61[-9;-113]mL, ES: -0.17, $p$ = 0.027). There was no effect of condition ($p$ = 0.818, $\eta_p^2$ = 0.006), time ($p$ = 0.208, $\eta_p^2$ = 0.152), or an interaction effect ($p$ = 0.772, $\eta_p^2$ = 0.040) on $RBCV_{est}$.

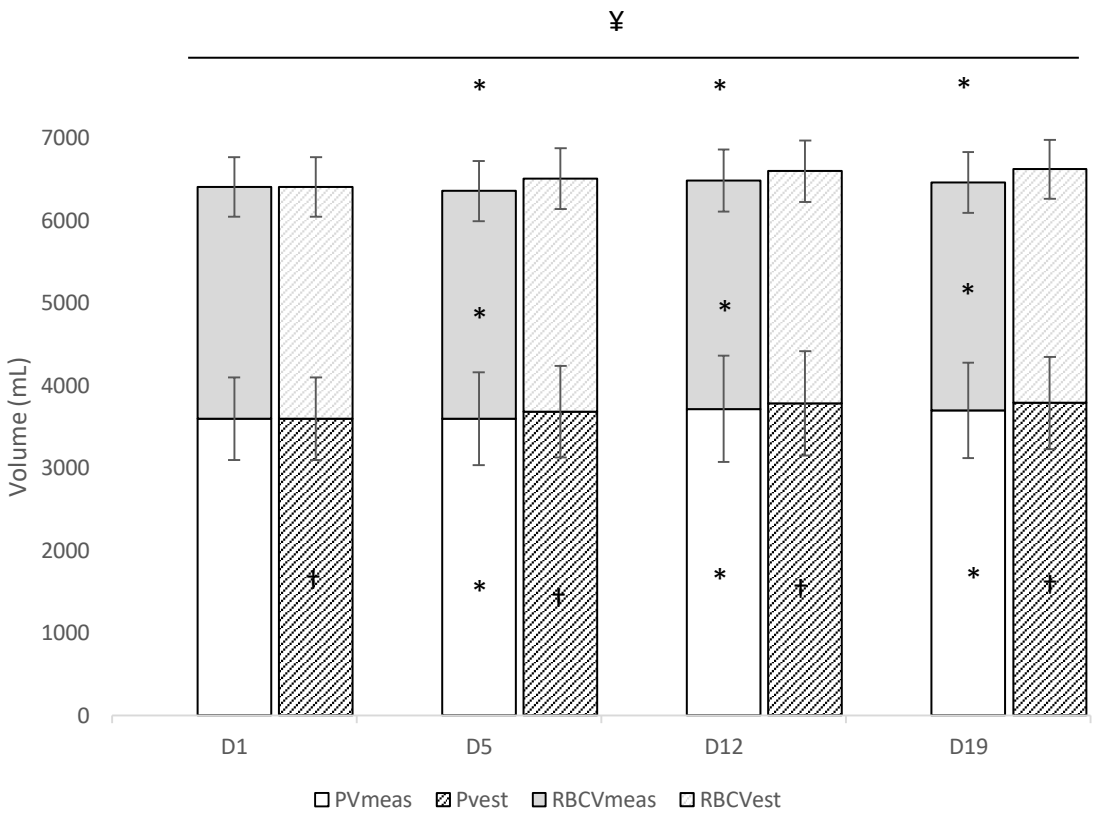


**Figure 5.2:** Measured and estimated blood volumes during the EHA intervention. Data are presented as mean (columns) and SD (error bars), n = 10. Solid white and grey boxes represent mean measured blood volumes ($BV_{meas}$). Hashed white and grey boxes represent estimated blood volumes ($BV_{est}$). * interaction effect compared to estimated ($_{est}$) values. ¥ main effect of time for $BV_{est,}$ $p < 0.05$

To identify the impact of changes in HBmass on calculated blood volumes, measured and estimated blood volumes were compared using a 2 way repeated measures ANOVA (analysis: measured vs estimated * time: day 1, 5, 12, 19) only in the EHA intervention. Measured and estimated blood volumes are presented in figure 5.2. There was a main effect of analysis on BV ($p = 0.001$, $\eta_p^2 = 0.746$) whereby $BV_{est}$ was higher than $BV_{meas}$. There was no effect of time ($p = 0.164$, $\eta_p^2 = 0.170$). There was a significant interaction effect ($p < 0.001$, $\eta_p^2 = 0.589$). Post-hoc analysis revealed $BV_{meas}$ was lower than $BV_{est}$ on days 5 (-152[-213;-90]mL, ES: -0.17, $p < 0.001$), 12 (-117[-186;-47]mL, ES: -0.12, $p = 0.004$), and 19 (-159[-240;-78]mL, ES: -0.18, $p = 0.002$). There was a main effect of analysis on PV ($p = 0.001$, $\eta_p^2 = 0.754$) whereby $PV_{est}$ was higher than $PV_{meas}$. There was no effect of time ($p = 0.098$, $\eta_p^2 = 0.205$). There was a significant interaction effect ($p < 0.001$, $\eta_p^2 = 0.582$). Post-hoc analysis

revealed $PV_{meas}$ was lower than $PV_{est}$ on days 5 (-85[-118;-51]mL, ES: -0.15, $p$ < 0.001), 12 (-66[-106;-27]mL, ES: -0.10, $p$ = 0.004), and 19 (-90[-136;-45]mL, ES: -0.16, $p$ = 0.002). There was a main effect of analysis on RBCV ($p$ = 0.001, $\eta_p^2$ = 0.733) whereby $RBCV_{est}$ was higher than $RBCV_{meas}$. There was no effect of time ($p$ = 0.536, $\eta_p^2$ = 0.076). There was a significant interaction effect ($p$ < 0.001, $\eta_p^2$ = 0.592). Post-hoc analysis revealed $RBCV_{meas}$ was lower than $RBCV_{est}$ on days 5 (-67[-94;-38]mL, ES: -0.18, $p$ < 0.001), 12 (-50[-81;-20]mL, ES: -0.13, $p$ = 0.004), and 19 (-69[-104;-33]mL, ES: -0.19, $p$ = 0.002).

### 5.3.4 *Plasma proteins*

Changes in haematological markers are presented in figure 5.3. There was no effect of condition on [EPO] ($p$ = 0.229, $\eta_p^2$ = 0.156). There was a significant effect of time ($p$ <0.001, $\eta_p^2$ = 0.675), and a significant interaction effect ($p$ = 0.019, $\eta_p^2$ = 0.390). Post-hoc analysis revealed that [EPO] remained stable during the TEMP intervention, but followed a parabolic relationship during EHA. By day 4, [EPO] was higher than baseline (+32[9;54]%, ES: 2.98, $p$ = 0.007) and remained higher than baseline on day 11 (+32[1;57], ES: 2.17, $p$ = 0.043). By day 18, [EPO] decreased and was similar to baseline (-11[-30;7]%, ES: -1.36, $p$ = 0.313). Due to technical reasons, it was not possible to measure reticulocytes in two participants of the TEMP training intervention, and three participants of the EHA training intervention. Therefore, the two different training conditions were compared as independent groups. Reticulocytes were variable during both TEMP and EHA interventions. There was no main effect of day ($p$ = 0.146), and no interaction effect ($p$ = 0.075). There was a significant effect of condition for [Fe] which was higher in TEMP than EHA ($p$ = 0.008, $\eta_p^2$ = 0.561). There was no effect of time ($p$ = 0.388, $\eta_p^2$ = 0.104). There was a significant interaction effect ($p$ = 0.013, $\eta_p^2$ = 0.323). Post-hoc analysis revealed that [Fe] was unchanged during TEMP, but tended to be lower than baseline by day 19 of EHA (-20[-41;0]%, ES: -2.11, $p$ = 0.052).

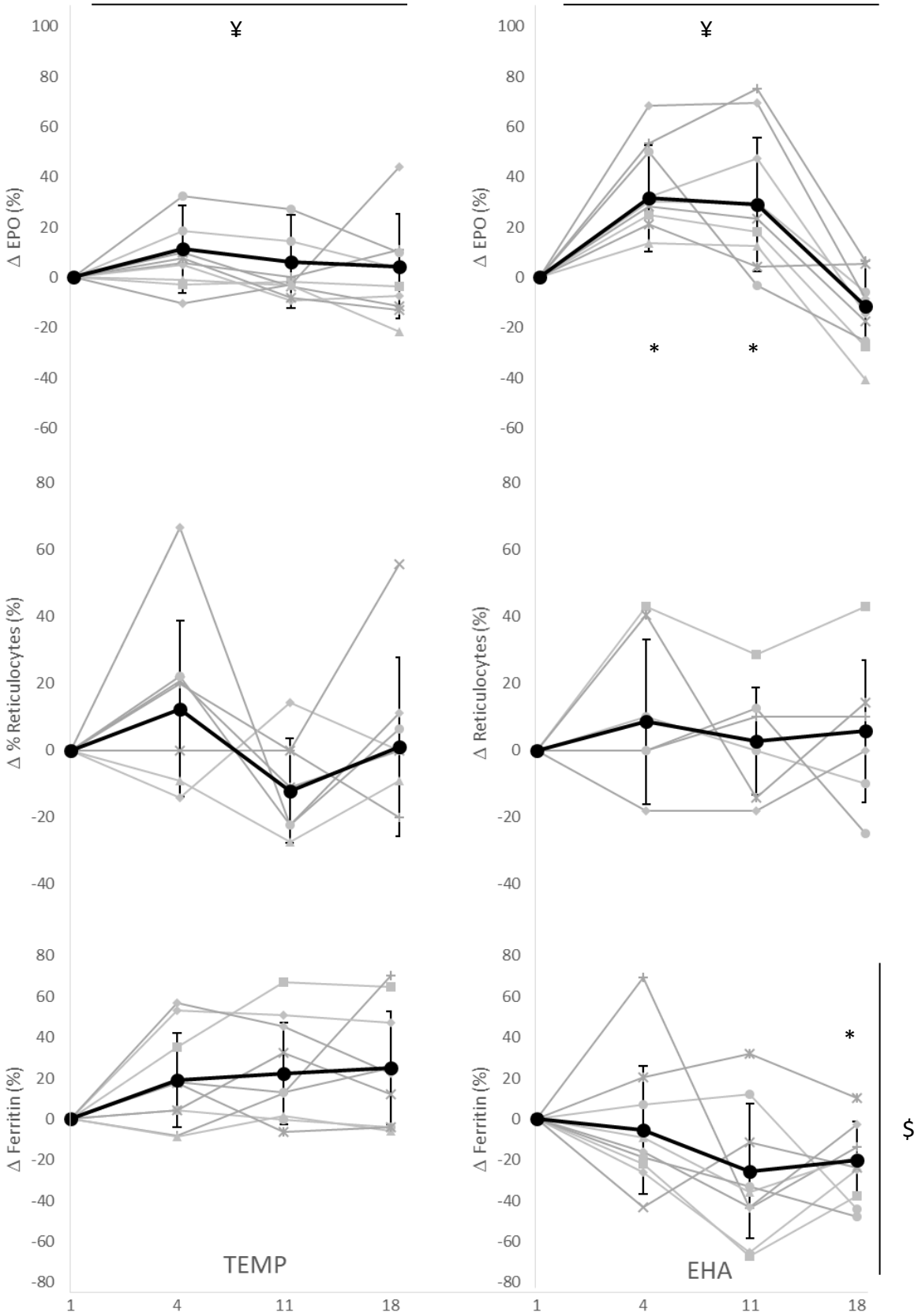


**Figure 5.3**: Changes from baseline in resting [EPO], reticulocytes and [Fe] during 3 weeks of TEMP and EHA training intervention. Data are presented as mean (solid black line), SD (error bars), and individiual responses (grey lines), n = 10 for [EPO] and [Fe], n = 7 for reticulocytes. $ main effect of condition vs TEMP, ¥ main effect of time, * interaction effect vs day 1, $p < 0.05$.

## 5.4 **Discussion**

A sustained reduction in HBmass (~2.5%) in trained cyclists was observed during a 3-week heat acclimation intervention, which returned to baseline the week following the intervention in 7 of the 10 participants, while 3 remained below baseline. Three previous studies have employed the CO-rebreathing technique: 1 and 12 days after (Rendell et al. 2017), 12 days after (Karlsen et al. 2015b) and 4-5 days after (Keiser et al. 2015) heat acclimation, however, the current study is the first to measure HBmass and its kinetic during prolonged heat acclimation. While the study of Karlsen and colleagues (Karlsen et al. 2015b) observed a 7.2% increase in HBmass, both the studies of Keiser and colleagues (Keiser et al. 2015) and Rendell and colleagues (Rendell et al. 2017) observed no change. As such our results are more closely aligned with the later, but provide a novel insight into blood regulation during heat acclimation.

### 5.4.1 *Regulation of HBmass*

The reduction in HBmass occurred within the first 4 days of heat acclimation, suggesting a more rapid modification in this blood parameter than previously thought. It is classically reported that the rate of RBC destruction is largely invariant in healthy humans and that changes in RBC production are slow acting, taking several weeks to affect HBmass (Finch et al. 1977; Sawka et al. 2000). However, fast reductions in HBmass within 1 week have been reported during rapid descents from high altitude (Rice et al. 2001; Ryan et al. 2014), space flight (Alfrey et al. 1996), and during periods of rapid weight loss in professional boxers (Reljic et al. 2013, 2016). The rapid reduction in HBmass can be explained by either a reduction in red blood cell production, or an increase in red blood cell destruction.

Although we hypothesized that HBmass would be significantly increased with long-term heat acclimation, HBmass was transiently decreased during heat acclimation. This observation contrasts with some exploratory suggestions in the existing literature

that HBmass might increase (Bazett et al. 1940; Scoon et al. 2007; Karlsen et al. 2015b), or remain unchanged (Keiser et al. 2015), as a result of heat acclimation. Given this finding, we must consider whether the decrease in HBmass could be the result of an analytical artifact related to our methods. Several factors suggest that this finding was not the result of methodological artifact. HBmass was determined at minimum in duplicate prior to and after each training intervention. Where duplicate measures were outside of our coefficient of variation of 1.4%, repeat tests were undertaken until this criterion was met. Furthermore, the magnitude of reduction in HBmass was almost 2-fold greater than our coefficient of variation. It should be noted that during each training intervention, HBmass was determined as a single measure on days 4, 11, and 19 at least 4h before (n = 2), or 4h after (n = 8) the training session, depending on each participants designated training time. Hufners constant, the CO binding capacity to haemoglobin (1.39mL CO/ g Hb), is affected by changes in blood temperature and pH (Gorelov 2004). However, Ryan and colleagues (Ryan et al. 2016) reported no significant difference in duplicate HBmass where one of the tests was completed 2h after exercise. Furthermore, in the present study participants followed the same CO-rebreathing and exercise regimen during the TEMP intervention, and HBmass remained stable throughout the intervention. We must also consider how adaptations related to heat acclimation may affect the CO-rebreathing method. Slower CO mixing kinetics or decreased extravascular loss of CO to myoglobin could cause an artificial under-estimation of HBmass (Prommer and Schmidt 2007; Garvican et al. 2010). Another consideration is that increases in muscle blood flow could reduce mixing time and augment the loss of CO to myoglobin (Garvican et al. 2010). However, we observed small, non-significant increases in blood volume with EHA and while theoretically this could marginally affect muscle blood flow at rest, this consequence would lead to an over-estimation of HBmass, and so can be rejected. Another modulating factor could be a decrease in muscle myoglobin content

that could lead to an over-estimation of CO loss from Hb to myoglobin leading to an under-estimation of HBmass (Chada and Bruce 2012). This cannot be ruled out since the intermittent sprint interval session (see chapter 6) was always performed on the day preceding the CO-rebreathing measurement. It is well documented that vigorous exercise may lead to muscle damage, often demonstrated by increased plasma creatine kinase and myoglobin content (Ebbeling and Clarkson 1989; Cipryan et al. 2017). However, given that HBmass was unchanged during TEMP training, and power output in both TEMP and EHA was similar (chapter 6), this consequence also seems unlikely. Therefore, we must consider mechanisms that would account for a rapid decrease in HBmass within 4 days of heat acclimation. Normal rates of RBC production and destruction are 0.83-1% per day (Finch et al. 1977; Franco 2009). Therefore the ~2.4% reduction in HBmass within 4 days could either be explained by either (or a mix of) an almost complete cessation in red blood cell production, or a large increase in red blood cell destruction. The latter has received increased attention from researchers examining space flight (Alfrey et al. 1996), head-down tilt bed rest (Ryan et al. 2016), and rapid descents from high altitude (Ryan et al. 2014) . Neocytolysis, the selective destruction of young red blood cell (neocytes) (Alfrey et al. 1996, 1997; Rice and Alfrey 2005), has been suggested as an explanation for such rapid haematological changes, in the presence of decreased EPO. However, the strength of evidence for neocytolysis has been questioned (Risso et al. 2014). The theoretical EPO mediated lysis of neocytes is in direct contrast to our findings, since we observed a rapid increase in EPO in the first week of heat acclimation. Evidence of an increased breakdown of RBCs also seems unlikely given the observation of a tendency for reduced plasma [Fe] during EHA. Large increases in red blood cell destruction may be associated with increased plasma [Fe] as iron contained in the destroyed RBCs is transferred to plasma iron stores (Belcher et al. 2010). However,

since we did not measure additional markers that would reflect RBC destruction (i.e. haptoglobin, bilirubin), it is not possible to confirm this outcome.

It is also possible the decrease in HBmass could reflect a decrease in red blood cell production. This is supported by the negligible changes in reticulocytes, suggesting that there was no upregulation of reticulocyte production. However, this contrasts the large increase in circulating erythropoietin we observed within 1 week of heat acclimation, which directly coincided with the decrease in HBmass. With a large upregulation of erythropoiesis, reticulocytes would be expected to increase and serum Fe would be expected to decrease, in order to produce new red blood cells. This has been observed with both altitude training (Garvican et al. 2012), and exogenous EPO utilization (Robinson et al. 2006). Accordingly, findings from the present study cannot confirm either a reduction in production or an increase in destruction of the red blood cells. An alternative explanation maybe due to a loss of erythrocytes due to bleeding. In a normal individual, any loss of erythrocytes, such as by bleeding or haemolysis, decreases delivery of oxygen to the tissues (Hsu et al. 2001). When this tissue hypoxia is sensed by cells in the kidney and liver capable of producing EPO, they produce and secrete EPO into the plasma (Thorling and Erslev 1968). While blood withdrawal can stimulate an erythropoietic response (Damsgaard et al. 2006) we deliberately withdrew only small quantities; i.e. < 8mL per sample for a total of < 64mL within in the 12days preceding the measures on day 4 of the intervention, and this is unlikely to have biased our results. Moreover there was no effect during the TEMP intervention. Alternatively, exercise induced intravascular haemolysis may develop due to an increase in red blood cell destruction induced by vigorous exercise (Yoshimura et al. 1980). This has previously been examined in mountain hiking (Martin et al. 1992), strength training (Schobersberger et al. 1990), karate (Streeton 1967) and swimming (Selby and Eichner 1986). Exercise induced intravascular haemolysis is most commonly caused by bursting of red blood cells in the circulation due to high

compressive loads (Miller et al. 1988; Telford et al. 2003), or mechanical rupture when passing through capillaries in contracting muscles and an increase in friction between red blood cells and vessel walls with increased blood flow (Broun 1922, 1923; Mairbäurl 2013). Furthermore, several studies have indicated that erythrocyte membrane compromise due to factors such as lactic acid, lysolecithin, or oxidative stress, could also be involved in hemolysis (Hiro 1982; Smith 1995; Şentürk et al. 2005). Although we do not have any direct markers to corroborate this finding, either the effects of daily training in the heat alone or the addition of an all-out intermittent sprint protocol in the heat, 1 day prior to measures of HBmass, and 2 days prior to venous blood collection, cannot be ruled out as a potential candidate for causing exercise induced intravascular haemolysis, and explanation for the decrease in HBmass. As there was no effect on HBmass in the TEMP training intervention, the mechanisms for this would be specific to heat exposures. Since hemolysis would simultaneously promote an erythropoetic response (Thorling and Erslev 1968), this would also explain the concurrent increase in erythropoietin after 1 week of heat acclimation. Indeed, Yusof and colleagues (Yusof et al. 2007) observed an intravascular haemolysis during the first 84 km of an ultramarathon running race, and concomitant increase in EPO throughout the race. The final sprint session took place on day 17 of the interventions. The post-training intervention HBmass tests took place 6 and 8 days after the final sprint session, and 4 and 6 days after the training intervention finished. We observed a rapid increase in HBmass in the week after heat acclimation in 7 of the 10 participants, such that on average, HBmass returned to baseline. Such a rapid increase in HBmass is uncommon but has been reported after four days of head-down tilt bed rest (Ryan et al. 2016). The authors suggested either a near complete cessation of red blood cell destruction, or a release of red blood cells from the spleen due to the return to orthostasis after prolonged bed rest. A previous study reported a ~3% increase in HBmass assessed 1 to 3 h after an ultra-endurance

triathlon (Gough et al. 2012), and speculated either this was due to either a sphlenic contraction, or a methodological artefact due to increased rate of CO diffusion to intramuscular myoglobin. This finding has ramifications for future research and may have consequences in the planning of training camps, especially since there has been increased interest recently in the mixing of heat and altitude stimuli (Buchheit et al. 2013), as their respective signaling pathways may be incompatible.

### 5.4.2 *Estimations of blood volume*

The reduction of HBmass has repercussions on measurement of blood volumes. Heat acclimation-mediated plasma volume expansion is often estimated as relative changes from the method described by Dill and Costill (Dill and Costill 1974). The relationship between pre- and post-exercise measures of haematocrit and haemoglobin concentration for the determination of changes in plasma volume relies on the assumption that HBmass is a constant. While this assumption may be accepted with some certainty during acute exercise, and exceptions may be made for medical incidents, our data suggests that within the context of a heat training intervention, the transient reduction in HBmass would violate this assumption. When assuming that HBmass remains the same, we observed significantly higher plasma volumes, red blood cell volumes and therefore total blood volumes than when incorporating the measured reduction in HBmass into the calculations (Figure 5.2). Importantly, this led to a statistically significant increase in the estimated plasma volume throughout the intervention and contrasting this no significant differences in our measured values, in comparison to day 1. It is therefore recommended that future research should utilize absolute measures of plasma volume, rather than relative changes during heat acclimation procedures.

## 5.5 Conclusions

The primary outcome of the present study was the transient reduction in HBmass during a 3-week heat acclimation intervention. Given that this finding was unexpected, and does not seem to be an analytical artefact, more research is indicated to confirm these findings. A further consideration of the reduction of HBmass was the effect this has on estimations of blood volumes. We observed a small increase in relative plasma volume during a during a 3-week heat acclimation camp, but this effect was negated when incorporating the measured reductions in HBmass. Furthermore, we observed significant differences in BV, PV and RBCV on days 5, 12, and 19 of the EHA intervention when the change in HBmass was considered. This is significant given the previously reported increase in relative resting plasma volumes throughout the EHA intervention in chapter 4 using the Dill and Costill equations (Dill and Costill 1974). This is not surprising given that one of the assumptions of Dill and Costill is that HBmass is constant, and as such, studies that have previously reported relative changes in plasma volume maybe biased. However, one of the major considerations of the current work is that HBmass was used to calculate blood volumes, but also that changes in blood volume might influence the measure of HBmass from the CO-rebreathing technique. Further research is required to corroborate these findings utilizing independent measures of blood volumes and HBmass during heat acclimation.

# CHAPTER 6

# NEUROMUSCULAR AND METABOLIC ADAPTATIONS TO INTERMITTENT SPRINT TRAINING IN WARM AND COOL ENVIRONMENTS

## 6.1 Introduction

Acute heat exposure can elicit an ergogenic effect on both peak and mean power output during single sprint bouts (Ball *et al.* 1999). An acute increase in muscle temperature increases muscle contractility (Close and Hoh 1968), alters the force-velocity and consequently, the power-velocity relationship such that power output increases in a dose-response manner (De Ruiter and De Haan 2000). Conversely, prolonged exposure to heat during repeated sprint tasks exacerbates the rise in core temperature which can lead to an acute decrease in voluntary activation and muscle force production (Drust et al. 2005). There is limited evidence examining the effect of heat acclimation on intermittent sprint exercise, with reports of improved (Castle et al. 2011), and unaltered (Sunderland et al. 2008) performance. There is some evidence to suggest that heat acclimation might improve skeletal muscle function, and therefore attenuate the negative consequences of hyperthermia during repeated sprint tasks. In animal models heat exposure has been shown to enhance mitochondrial adaptations (Tamura *et al.* 2014) and increase cell proliferation potential and muscle protein content (Uehara *et al.* 2004). To date, only 2 studies have examined this in human subjects. One study reported that 10-weeks of 8h/day localized passive quadriceps heating increased maximum isometric force during knee extension, and increased mean cross-sectional area of the vastus lateralis and rectus femoris (Goto *et al.* 2011). The other study reported increased evoked peak twitch amplitude, increased maximal

voluntary torque production and an improvement of the relative torque/EMG linear relationship after 11days of 1 h/day of whole body heating (Racinais *et al.* 2017b). However, both these studies utilized a passive heating protocol, and explored only single, isolated muscle models and it is unclear whether such adaptations would occur during whole body sprint exercise. Furthermore, it has recently been demonstrated that heat acclimation has a protective effect on the central nervous system, as evidenced by restoration of supraspinal capacity to maintain motor drive during sustained maximal voluntary contraction in hyperthermic participants (Racinais *et al.* 2017a).

The aim of the present study was to evaluate the neuromuscular adaptations to heat acclimation, and its effect on intermittent sprint performance.

## 6.2 **Material and methods**

### 6.2.1 *General procedure*

10 participants completed 3 weeks of exercise heat acclimation (EHA, 35°C, 60% RH) and 3 weeks of work-matched training in temperate conditions (TEMP, 18°C, 60% RH). Detailed descriptions of the participant characteristics and project overview are presented in general methods 3.1.2, and 3.2.1, respectively. At least 2 weeks prior to commencing each training intervention, participants completed a baseline intermittent sprint session (Trial 1 – T1) in the same environment they would train in. Participants completed one intermittent sprint session per week of training, on the same day each week, preceded and followed by 2 days of low intensity endurance training. Each session is hereafter denoted from trial 1 to 4 as T1, T2, T3, and T4. The intermittent sprint session included a standardised warm-up and 5 all-out cycling sprints of 30 s, separated by 4.5 min of recovery.

### 6.2.2 *Instrumentation*

Upon arrival, participants were instrumented with the below sensors after careful shaving and cleaning of the skin. A near infrared-spectroscopy (NIRS) optode (Portamon, Artinis Inc, Holland) was applied to skin surface of the right vastus lateralis, two-thirds of the distance from the anterior spina iliaca superior to the lateral side of the patella. Wireless surface EMG electrodes (Delsys Trigno, Delsys Inc., Boston, US) were fixed to the left leg in the following positions, according to the SENIAM (surface electromyography for non-invasive assessment of muscles) guidelines: the vastus lateralis, two-thirds of the distance from the anterior spina iliaca superior to the lateral side of the patella; rectus femoris, 50% on the line from the anterior spina iliaca superior to the superior part of the patella; and biceps femoris, 50% on the line between the ischial tuberosity and the lateral epicondyle of the tibia. In addition, an accelerometer was fixed to the inside of the left-hand crank, allowing time syncing of the EMG signal to the position of the limb during each pedal revolution. The positions were recorded and replicated for every trial.

### 6.2.3 *Warm-up*

Following instrumentation, participants rested in a seated position for 10 min for baseline measurements of muscle oxygenation, $T_{core}$, $T_{skin}$, and HR before commencing a standardized warm-up. A set warm-up routine was pre-programmed into the SRM. The warm-up consisted of cycling at constant load for 10 min at 100 W, 4.5 min at 150 W, 4.5 min at 200 W and followed by a 30s practise sprint effort with the SRM set to isokinetic mode at 105 rpm. Participants were instructed to maintain a cadence of 105 rpm for measurement of EMG activity and muscle deoxygenation since muscle activation varies with cadence at a given power output (Macintosh *et al.* 2000).

### 6.2.4 *Intermittent sprints*

The sprint intervals consisted of repeated all out 30 s sprint cycling on a cycle ergometer (SRM) set to isokinetic mode at 105 rpm. Participants were instructed to remain seated at all times and were verbally encouraged throughout all sprints. Participants completed five sprints per session with 4.5 min recovery between each sprint, during which time participants cycled against a light resistance (~50 W) to reduce venous pooling in the lower extremities, and minimize feelings of light-headedness or nausea. After the final sprint, participants rested in the chamber for a further 15 min. This was to ensure participants were exposed to at least 1 hour of heat exposure per day during the EHA intervention, and matched in the TEMP condition. *Ad libitum* drinking of plain water was permitted.

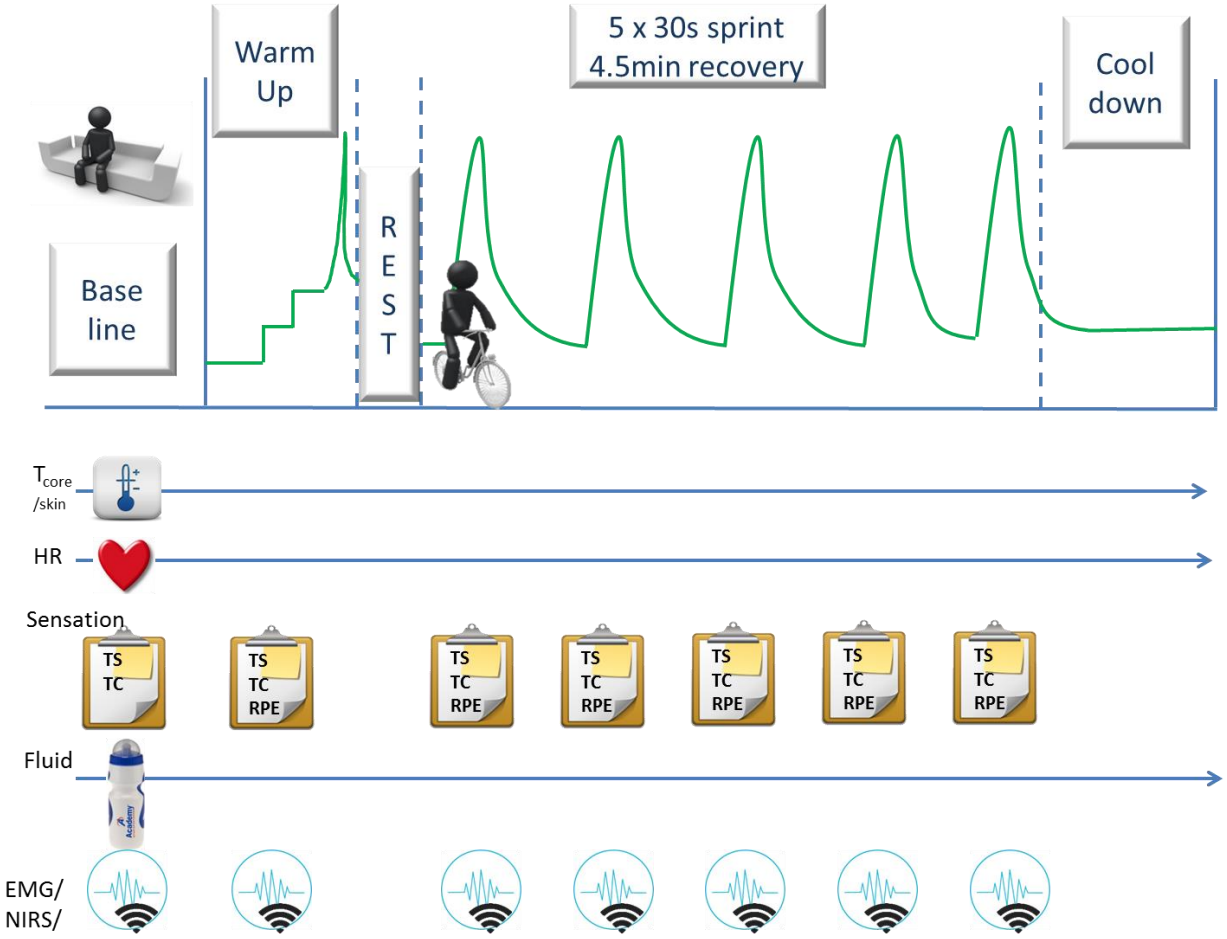


**Figure 6.1.** Schematic of the intermittent sprint training session. Participants completed a standardised warm up, followed by 5, 30 s all out isokinetic sprints at a fixed cadence of 105 rpm. $T_{core}$, $T_{skin}$, HR, muscle EMG, muscle oxygenation and power output were measured simultaneously across the warm-up and sprint efforts.

### 6.2.5 *Power output*

Power output was measured at 2 Hz. Peak power output was determined at the highest 1 s power output. Average power output was also determined for each sprint (i.e. over the 30 s of sprint).

### 6.2.6 *Surface EMG*

EMG activity was measured from the right VL, RF and BF during the intermittent sprint sessions. Surface EMG signals were recorded via wireless Delsys hardware (Delsys Trigno, Boston, USA) and software (EMGworks, Boston, USA) with a sampling frequency of 1926 Hz. The signal was analysed using a custom-made routine developed in LabVIEW (National Instruments, Austin, TX, USA). Briefly, raw electromyograms were bandpass filtered (10-500 Hz) and the RMS envelop was calculated using a 100 ms moving window. Crank revolutions were recorded simultaneously to the EMG signals by an accelerometer (Delsys Trigno, Boston, USA) located inside the left crank. The accelerometer signal was used to automatically determine each pedal stroke. The cadence was clamped at 105 rpm and all data were normalised over 100 points per pedal revolutions. The first 10 pedal strokes were averaged to create one pedal stroke representative of the peak power. The 52 pedal strokes from the entire sprint were averaged to create one pedal stroke representative of the average power. In addition, the EMG activity was also measured during the final 40 s of warm-up at 200 W (105 rpm) to verify for any changes in recording conditions from day to day.

### 6.2.7 *Tissue saturation*

Muscle tissue saturation was monitored throughout the intermittent sprints by wireless NIRS (Portamon, Artinis, Zetten, The Netherlands) and signals were analysed using a home-made routine developed in LabVIEW (National Instruments, Austin, TX, USA). This technique provides continuous, non-invasive monitoring of the relative changes

in tissue saturation (TSI). In the current study, changes in muscle saturation of the right VL muscle belly were continuously monitored at 10 Hz. The optodes were encapsulated in a single hard plastic frame to ensure a fixed and invariant 40 mm distance between the light source and the detector. The optode assembly was secured on the cleaned skin surface with tape and then covered with a black cotton tissue, thus minimizing the intrusion of extraneous light. The light emitted by the infrared probe is assumed to reach a tissue depth of 50% of the interoptode spacing (space between emitting and receiving probe) (Matsushita *et al.* 1998). A differential path-length factor (DPF) of 3.8 was used for the VL muscle (DeLorey *et al.* 2005). Changes in TSI were reported as the differences between initiation and at the end of each sprint, as an estimator of changes in tissue saturation during sprints (Racinais *et al.* 2007).

#### 6.2.8 *Temperatures*

Rectal, skin, and environmental temperatures were recorded at baseline, in the final minute of each warm-up stage, and immediately following each sprint effort, and analysed as previously described in general methods 3.3.2

#### 6.2.9 *Perceptual responses*

RPE, TS, and TC were recorded in the final minute of each warm-up stage, and immediately following each sprint effort, as previously described in general methods 3.3.3

#### 6.2.10 *Statistical analysis*

Data are presented as mean ± standard deviation (SD), unless otherwise stated. A two-way (condition (TEMP or EHA training) x trial (Trial 1 - 4) analysis of variance (ANOVA) with repeated measures design was used to asses differences in average values between trials. A three-way (condition (TEMP or EHA training) x trial (Trial 1 - 4) x sprint (Sprint 1-5) ANOVA with repeated measures design was used to assess differences in power output and physiological adaptations between sprints. Where a

significant interaction effect was apparent, pair-wise differences were evaluated using the Sidak method. Data analysis was performed using Statistical Package for the Social Sciences for Windows version 22.0 software (SPSS Inc., Chicago, IL). Significance was accepted at $P < 0.05$.

## 6.3 **Results**

### 6.3.1 *Power Output*

**Table 6.1;** Average performance outcomes of each intermittent sprint trial during the TEMP and EHA interventions. Data are presented as mean ± SD, n = 10.

| | TEMP | | | | EHA | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T1 | T2 | T3 | T4 | Main effect of trial |
| Mean session peak power (W) | 996 ± 155 | 1063 ± 158 | 1046 ± 152 | 1045 ± 146 | 967 ± 190 | 997 ± 164 | 1021 ± 155 | 1027 ± 150 | 0.001 |
| Mean session average power (W) | 637 ± 121 | 651 ± 112 | 658 ± 110 | 658 ± 117 | 631 ± 145 | 643 ± 132 | 649 ± 131 | 648 ± 126 | <0.001 |

*T1 (trial 1) represents the baseline sprint session prior to each training intervention. T2 (trial 2), T3 (trial 3), and T4 (trial 4) represent the training session performed each week of the training interventions.*

There was no main effect of condition for average session power ($p = 0.370$, $\eta_p^2 = 0.090$). There was a significant main effect of trial ($p = 0.001$, $\eta_p^2 = 0.434$), but no evidence of an interaction effect ($p = 0.998$, $\eta_p^2 = 0.001$). There was no main effect of condition for average session peak power ($p = 0.108$, $\eta_p^2 = 0.262$). There was a significant effect of trial ($p < 0.001$, $\eta_p^2 = 0.508$). There was no interaction effect ($p = 0.188$, $\eta_p^2 = 0.173$). To explore the evolution of fatigue, individual sprint efforts were analyzed by a 3 way 2 x 4 x 5 (condition*trial*sprint) repeated measures ANOVA and are presented in figure 6.2. Peak and average power had similar responses; there was no main effect of condition ($p = 0.107$, $\eta_p^2 = 0.262$, $p = 0.366$, $\eta_p^2 = 0.091$

respectively). There was a significant main effect of trial ($p$ = 0.001, $\eta_p^2$ = 0.507, $p$ = 0.001, $\eta_p^2$ = 0.433, respectively). There was a main effect of sprint ($p$ < 0.001, $\eta_p^2$ = 0.904, $p$ = 0.001, $\eta_p^2$ = 0863.), with peak and average power progressively decreasing with each subsequent sprint. There was no interaction effect ($p$ = 0.815, $\eta_p^2$ = 0.065, $p$ = 0.827, $\eta_p^2$ = 0.064, respectively).

### 6.3.2 *Thermoregulatory parameters*

Thermoregulatory and perceptual responses are presented in figure 6.3. There was no effect of condition on $T_{core}$ ($p$ = 0.179, $\eta_p^2$ = 0.191). There was a significant effect of trial ($p$ = 0.038, $\eta_p^2$ = 0.264), although post-hoc analysis was unable to identify these differences. There was a significant effect of sprint ($p$ < 0.001, $\eta_p^2$ = 0.880). There was no interaction effect ($P$ = 0.724, $\eta_p^2$ = 0.074). There was a significant main effect of condition on $T_{skin}$, whereby it was hotter in EHA than TEMP ($p$ < 0.001, $\eta_p^2$ = 0.984). There was no effect of trial ($p$ = 0.786, $\eta_p^2$ = 0.038). There was a significant effect of sprint ($p$ < 0.001, $\eta_p^2$ = 0.754). There was no interaction effect ($p$ = 0.665, $\eta_p^2$ = 0.080).

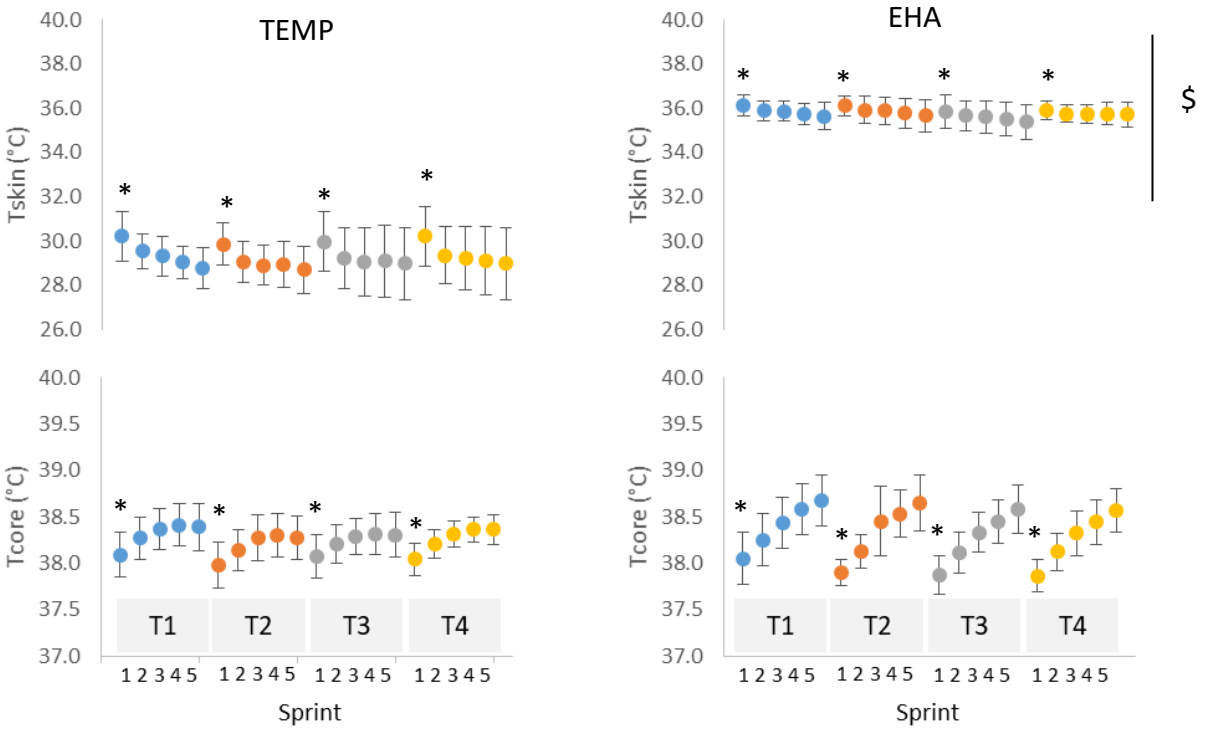


**Figure 6.3:** Thermoregulatory and responses for each sprint. Data are presented as mean (solid circles) and SD (error bars), n = 10. $ Main effect of condition, * main effect of sprint, $p$ < 0.05

### 6.3.3 *Evolution of fatigue*

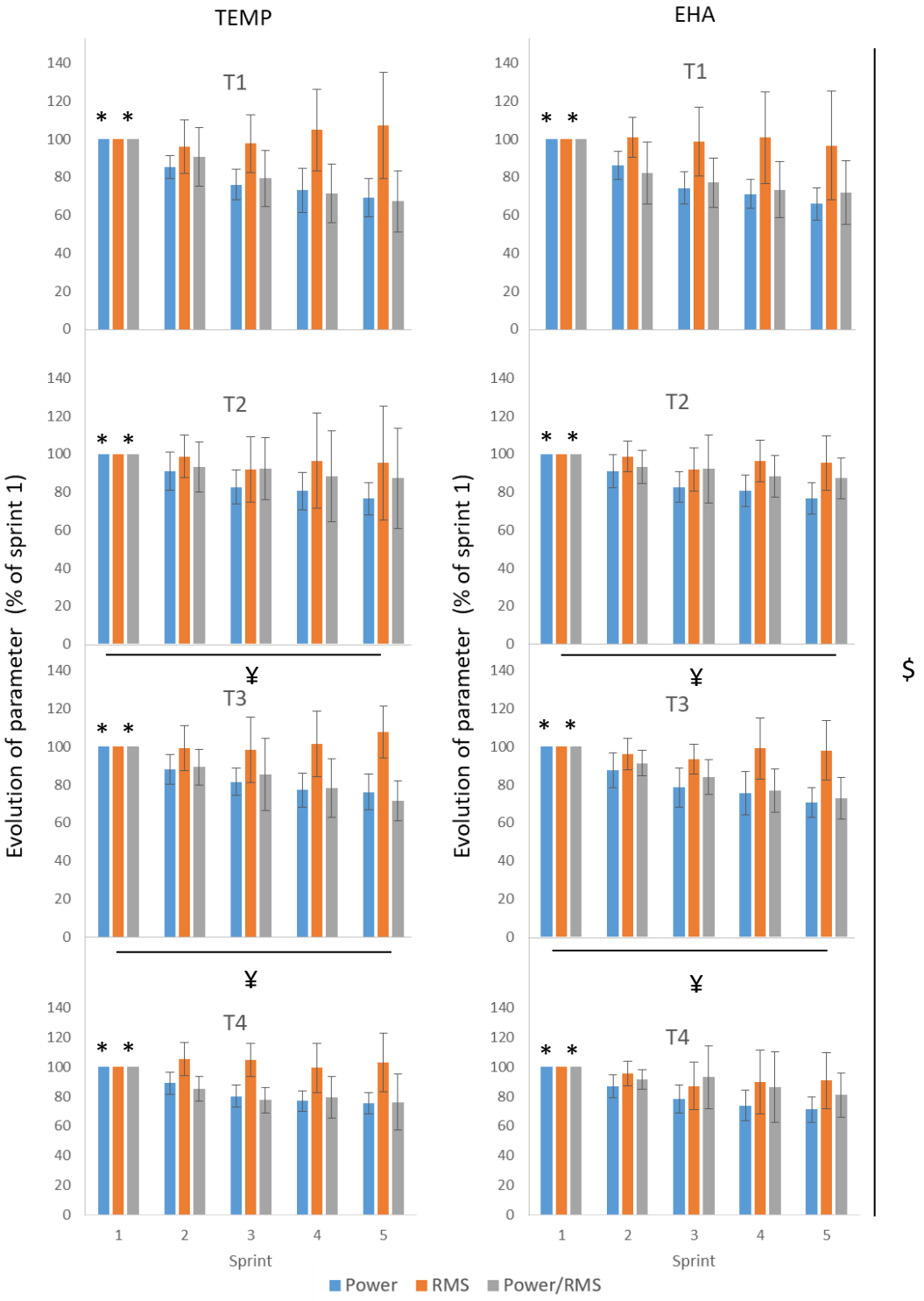


**Figure 6.4**: Evolution of average power output, RMS activity of the VL and power/RMS ratio. Data are normalized to sprint 1 of each trial. Data are presented as mean (columns) and SD (error bars), n = 8. $ Significant main effect of training condition for evolution of power. $ Significant main effect of trial for evolution of power. * Significant main effect of sprint, $p < 0.05$.

Evolution of fatigue as characterized by average power, EMG activity of the VL, and tissue saturation of the VL are presented in figure 6.4. Due to technical reasons, data for tissue saturation index is presented for eight participants only. There was a significant main effect of training condition for the evolution of fatigue as characterized by percentage of sprint 1 average power output, whereby the decrement was larger in EHA ($p$ = 0.030, $\eta_p^2$ = 0.423 ). There was a significant main effect of trial ($p$ = 0.001, $\eta_p^2$ = 0.450). There was a significant main effect of sprint, ($p <$ 0.001, $\eta_p^2$ = 0.919) with the decrement increasing progressively from sprint 1 to 5. There was no interaction effect (training condition*trial*sprint) ($p$ = 0.758, $\eta_p^2$ = 0.071). There was no effect of training condition, trial, sprint, or an interaction effect for the evolution of VL RMS ($p$ = 0.167, $\eta_p^2$ = 0.201, $p$ = 0.306, $\eta_p^2$ = 0.123, $p$ = 0.502, $\eta_p^2$ = 0.087, $p$ = 0.252, $\eta_p^2$ = 0.123, respectively). There was no main effect of training condition for the evolution of power to RMS ratio ($p$ = 0.911, $\eta_p^2$ = 0.001). There was a significant main effect of trial ($p$ = 0.009, $\eta_p^2$ = 0.347). There was a significant main effect of sprint ($p$ < 0.001, $\eta_p^2$ = 0.784), with the decrement increasing progressively from sprint 1 to 5. However, there was no interaction effect (training condition*trial*sprint, $P$ = 0.062, $\eta_p^2$ = 0.164).

### 6.3.4 *Tissue Saturation Index*

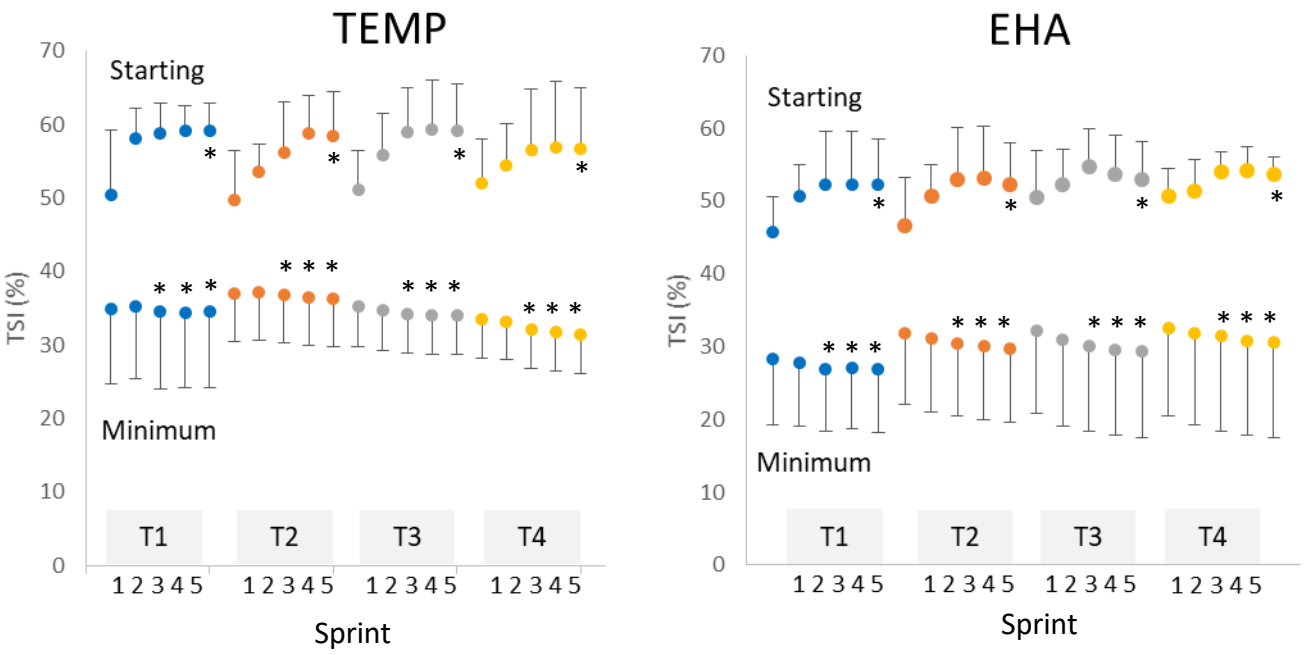


**Figure 6.5:** Starting and minimum tissue saturation index during each sprint, for each trial (T1 – T4). Data are presented as mean (solid circles) and SD (error bars), n = 8. * Significant main effect of sprint, compared to sprint 1, $p < 0.05$.

Tissue saturation index data are presented in figure 6.4. There was no main effect of training condition ($p = 0.054$, $\eta_p^2 = 0.432$), or trial ($p = 0.621$, $\eta_p^2 = 0.079$) for starting TSI of each sprint. There was a main effect of sprint ($p = 0.004$, $\eta_p^2 = 0.619$). There was no interaction effect (training condition*trial*sprint, $p = 0.738$, $\eta_p^2 = 0.092$). There was no main effect of training condition ($p = 0.161$, $\eta_p^2 = 0.259$), or trial ($p = 0.537$, $\eta_p^2 = 0.096$) for minimum TSI of each sprint. There was a main effect of sprint ($p < 0.001$, $\eta_p^2 = 0.790$). There was no interaction effect (training condition*trial*sprint, $p = 0.613$, $\eta_p^2 = 0.107$).

### 6.3.5 *Perceptual*

**Table 6.2;** Perceptual session data for each intermittent sprint trial during the TEMP and EHA interventions. Data are presented as median(interquartile range), n = 10. * Significantly higher than TEMP, $p < 0.05$)

| Trial | T1 | T2 | T3 | T4 | T1 | T2 | T3 | T4 |
|---|---|---|---|---|---|---|---|---|
| | | TEMP | | | | EHA | | |
| RPE | 19.8 (19.8-20.0) | 19.9 (19.6-20.0) | 19.9 (19.8-20.0) | 20.0 (19.7-20.0) | 19.9 (19.3-20.0) | 19.8 (19.6-20.0) | 20.0 (20.0-20.0) | 20.0 (19.9-20.0) |
| TS | 4.0 (4.0-4.8) | 4.0 (4.0-4.8) | 4.0 (4.0-4.9) | 4.0 (4.0-4.9) | 6.5 (5.6-6.6)* | 6.0 (5.2-6.3)* | 6.0 (5.4-6.4)* | 6.0 (5.3-6.4)* |
| TC | 4.3 (4.0-4.8) | 4.0 (4-4.8) | 4.0 (4.0-4.9) | 4.1 (4.0-4.9) | 5.9 (5.5-6.6)* | 6.0 (5.2-6.3)* | 6.1 (5.8-6.4)* | 6.0 (5.3-6.4)* |

Perceptual responses are presented in table 6.2. Differences between trials for each environment (TEMP vs EHA) were analysed using the Wilcoxon Signed Rank Test. There was no difference for RPE between T1, T2, T3, or T4 between TEMP and EHA ($p = 0.104$, $p = 0.713$, $p = 0.916$, $p = 0.144$, respectively. TS and TC were significantly higher in EHA than TEMP for all sessions ($P < 0.05$ for all).

## 6.4 **Discussion**

The purpose of this study was to investigate the neural, metabolic and performance adaptations to sprint interval training in warm and cool environments. Three weeks of training resulted in significantly greater peak and average power during a 30 s intermittent sprint protocol, with this improvement being independent of environmental condition (TEMP *vs.* EHA).

### 6.4.1 *Acute effect of intermittent sprint*

No effect of competing an intermittent sprint protocol in a warm compared to a cool environment was observed, as demonstrated by similar attainment of peak and average power outputs in trial one of both conditions (see Figure 6.4). Observations have been made between the rate of rise in core temperature and heat-induced reductions in intermittent sprint performance, with a strong correlation reported (r =

0.90) (Morris et al., 2005). In the present study, we intentionally designed an extended warm-up (~25mins), in order to elevate core temperature. However, participants generally commenced sprint 1 at only a mild level of hyperthermia with an average core temperature of ~38°C, and this rose only to ~38.7°C by the final sprint. It may be that core temperature elevation reached insufficient levels to observe reductions in intermittent sprint performance in the present study. Indeed, similarly to our findings, final values for core temperature of ~38.6 °C were reported after 2 min blocks of 4 s sprints followed by 100 s of active recovery and 10 s of passive rest when repeated for 80 minutes in 36°C. This level of hyperthermia is insufficient to adversely affect sprint performance (Almudehki et al. 2012).

### 6.4.2 *Effect of heat acclimation on intermittent sprint performance*

We observed a ~3 % improvement in intermittent sprint performance, as assessed by total work done, in both TEMP and EHA intervention. Our data suggests that similar performance gains can be made, regardless of the environmental condition (TEMP vs EHA) intermittent sprint training is performed in. However, whilst heat acclimation has been shown to induce advantageous neuromuscular modulation in an isolated muscle model after 11 days of passive heating (Racinais *et al.* 2017b), this may not translate into meaningful differences during whole body all-out sprint activity. This is significant, since athletes often undertake training camps in warm climates and are recommended to maintain high exercise intensity during a taper period prior to competition which may coincide with a heat acclimation strategy. It should be noted that we used trained cyclists, all of whom were highly familiar with all-out sprint training protocols on laboratory ergometers, having either taken part in previous research projects, or performed these sessions as part of standardized training prior to the experimental intervention. There is limited evidence available in the literature with which to compare

our performance data, with reports of improved (Castle *et al.* 2011) , and unaltered (Sunderland *et al.* 2008) performance. Heat acclimation has been shown to enhance high-intensity exercise performance, but not maximal, intermittent running performance in team-sport athletes (Sunderland *et al.* 2008; Petersen *et al.* 2010). Our data are in line with that of Castle and colleagues (Castle *et al.* 2011), who observed a 2% increase in peak power output during a 40 min intermittent sprint protocol in the heat after 10 days of heat acclimation. In the present study the average power output of each sprint progressively declined, regardless of environmental conditions, or acclimation status. While power output progressively decreased, the mean EMG activity (i.e. RMS) of the prime mover (vastus lateralis) remained unchanged during the intermittent sprints. This is in agreement with Hautier and colleagues (Hautier *et al.* 2000), who observed that mean EMG activity of both the vastus lateralis and glueteaus maximus remained unchanged during a repeated sprint task of 15 maximal 5 s sprints, interspersed with 25 s of recovery, despite large reductions in power output. In contrast with this, Racinais and colleagues (Racinais *et al.* 2007), observed a reduction in power output which coincided with a reduction of EMG amplitude (*i.e.* RMS) of the vastus lateralis during repeated maximal sprints, it was suggested that there was a possible decrement in neural drive to the muscle. It should be noted, that participants completed 10 x 6 s sprints with only 30 s of recovery, so differences in exercise task (i.e. repeated *vs.* intermittent sprint), may alter the physiological limitations to performance (Girard *et al.* 2015). There are conflicting reports in the literature, with some studies reporting improved performance in the heat (Falk et al. 1998; Ball et al. 1999), and others a reduction (Drust et al. 2005), although differences in protocols (repeated vs intermittent sprint) may account for these discrepancies (Girard et al. 2015). Indeed, intermittent sprint performance may be enhanced in

individuals exercising in warm environmental temperatures provided there is only a mild increase in body core temperature (Falk et al. 1998). However sprint tasks performed in the heat have also been shown to result in reduced performance towards the end of prolonged exercise tasks (Morris et al. 1998; 2000). Drust and colleagues (Drust et al. 2005) reported that repeated sprint performance after an intermittent protocol (5 x 15s sprints interspersed by 15s rest period) designed to elevate both core and muscle temperature was impaired in hyperthermic compared to normothermic participants, where exercise was undertaken in hot 40°C, and cool 20°C conditions, respectively. However, our data suggests that the etiology of fatigue during intermittent 30 s sprints with 4.5 min recovery is fundamentally peripheral in origin, rather than central, since RMS of the VL was unchanged with fatigue. This assertion is further supported by the observation that tissue oxygenation was largely unchanged in each sprint, confirming neither motor drive, nor muscle oxygen saturation were responsible for the power decrement in each subsequent sprint. It should be noted that while we observed a significantly larger delta change in muscle oxygenation in sprints 2, 3, 4, and 5 in comparison to sprint 1, this may be explained by a methodological artefact in the study protocol. Since we used a rolling start and isokinetic modality to clamp cadence at 105 rpm, participants could voluntarily (consciously, or sub-consciously) self-select power output prior to the start of the sprint, by exerting additional force against the 'unloaded' resistance supplied by the ergometer at 105 rpm. It is evident that participants commenced sprint 1 with a lower starting TSI than other sprints, due to a higher self-selected power output in the seconds prior to initiation of the first sprint. This anticipatory increase power output prior to the first sprint was not evidenced in subsequent sprints, possibly due to physiological fatigue, motivation, or other psychological factors. Therefore, the delta shift is smaller, due to a reduction in starting

TSI, rather than a shift in the sprint induced oxygenation kinetic. While heat acclimation did not influence the kinetics of electromyographic activity or muscle tissue oxygenation with fatigue within each session, our data does not allow for a comparison of absolute changes between sessions, and so the effect of heat acclimation on these parameters remains unknown. However, given that intermittent sprint performance was not acutely impaired by the heat in an un-acclimated state, when compared to an identical session performed in cool environments (*i.e.* trial 1), it is unlikely heat acclimation would yield any adaptive benefit.

## 6.5 **Conclusion**

We observed a similar improvement in overall session power in both TEMP and EHA. Whilst we were unable to identify a mechanistic explanation for this improvement, our data confirms that similar acute performances and performance gains can be made during intermittent sprint exercise in both warm and cool environments. Our data suggests that performance gains can be made, even when intermittent sprint training is undertaken in warm environmental conditions, which athletes may face during warm-weather training camps. Heat acclimation did not modulate any changes in muscle oxygenation, or the evolution of muscle recruitment with fatigue. The present study supports the existing literature that power output is not affected in the heat, in the absence of hyperthermia, and demonstrates that heat acclimation has no specific benefit over training in a temperate environment.

# CHAPTER 7

# EXERCISE PERFORMANCE AND CROSS-ACCLIMATION

## 7.1 Introduction

Heat acclimation (HA) has been well documented to improve $\dot{V}O_{2max}$ , and time trial performance in the heat in both laboratory (Sawka et al. 1985) and field (Racinais et al. 2015b) settings. The effect of heat acclimation on performance in cooler environments is highly contentious (Minson and Cotter 2016; Nybo and Lundby 2016). Heat acclimation is sometimes reported to improve performance in the cool (Scoon et al. 2007; Lorenzo et al. 2010), but not always (Karlsen et al. 2015b; Keiser et al. 2015; Neal et al. 2016). The proposed mechanisms by which HA could improve performance in cooler environments include: reduced oxygen uptake at a given power output (Sawka et al. 1983b; Young et al. 1985); increased lactate threshold (Young et al. 1985; Lorenzo et al. 2010, 2011); muscle glycogen sparing (Young et al. 1985; Febbraio et al. 1994); increased skeletal muscle force generation (Kodesh and Horowitz 2010); plasma volume expansion (Bass et al. 1958; Senay et al. 1976); improved myocardial efficiency (Horowitz et al. 1993); and increased ventricular compliance (Horowitz et al. 1986b).

There is limited literature available and methodological constraints associated with the majority of these studies. For example, several studies have reported performance improvements after a training camp in the heat but lack a control group (Hue et al. 2007; Buchheit et al. 2011; Racinais et al. 2014a). Given the reasonable expectation

of performance enhancement following a period of intensified training *per se*, regardless of environmental conditions (Laursen et al. 2002; Iaia et al. 2008), a control group is required to ascertain the true impact of heat acclimation on performance. Furthermore, several studies prescribe un-even training loads between heat and cool environments (Lorenzo et al. 2010). Where a similar absolute work-load is utilized, heat stress will increase the relative intensity as $\dot{V}O_{2max}$ progressively decreases throughout the session (Périard and Racinais 2015), thus increasing the training load. When heart rate is utilized to match the relative intensity (Keiser et al. 2015), a higher absolute workload is likely to be performed in the cool condition, so matching work done maybe a better alternative to matching training time. Furthermore, a correction factor should be implemented to match relative intensity, since heart rate is higher at the same relative intensity when exercise is performed in hot compared to cool environments (Périard and Racinais 2015). Accordingly, studies in the present thesis implemented a progressive, work-matched, HR based training approach, matching both the relative and absolute workloads between heat and cool training (see chapter 4). Whether heat acclimation improves performance in cool environments when training loads are adequately matched remains equivocal. Furthermore, the effect of long-term (*i.e.* 3 weeks) HA is also unknown.

In addition, cross-acclimation, whereby adaptation to the stressors of one environment might convey benefits in another, is not only limited to less stressful conditions (normothermic), but could also improve performance upon exposure to other environmental stressors such as altitude (Ely et al. 2014; Lee et al. 2016), provided they share a common adaptive response (Fregly et al. 2011). At a cellular level, both acute and chronic exposure to heat and/or hypoxia induce a heat shock protein (HSP) response (Morimoto 1998). HSPs are highly conserved molecular chaperones which

facilitate the synthesis and folding of proteins, conveying a cytoprotective effect against subsequent thermal (Hutter et al. 1994) or ischaemic stressful insult (Levi et al. 1993). Furthermore, heat shock factor (Hsf) transcription, the regulator of HSP's, is upregulated during hypoxia due to binding by hypoxia-indicible factor 1-$\alpha$ (HIF1-$\alpha$) (Baird et al. 2006). The interactions between the heat shock response (HSR) and HIF1-$\alpha$, the global regulator of cellular and systemic oxygen homeostasis, have also been suggested as a result of heat acclimation (Salgado et al. 2014; Lee et al. 2016). This could elicit down-stream effects on erythropoietin (EPO) expression, and consequently on HBmass (as detailed in chapter 5), a key determinant of endurance exercise performance (Garvican et al. 2011). Therefore, the aim of the present study was to examine the impact of heat acclimation on maximal oxygen uptake and cycle time trial performance in a hot environment, as well as in temperate and hypoxic environments, above that of matched-training in a cool environment.

## 7.2 **Materials and Methods**

### 7.2.1 *General procedure*

10 participants completed 3 weeks of exercise heat acclimation (EHA, 35°C, 60% RH) and 3 weeks of work-matched training in temperate conditions (TEMP, 18°C, 60% RH). Detailed descriptions of the participant characteristics and project overview are presented in general methods 3.1.2, and 3.2.1, respectively.

Prior to and after each training intervention, participants completed 3 performance testing sessions in control (CON - 18°C, 60% RH, FiO2 20.93%), hot (HOT: 35°C, 60% RH, FiO2 20.93%) and hypoxic (HYP: 18°C, 60% RH, FiO2 15.40%) environments, before and after 3-weeks of training. Each testing session was separated by 48 hours of rest, and including an incremental cycling test to exhaustion

and a 20-min time-trial (TT). Participants repeated the complete procedure (1 week testing + 3 weeks training + 1 week testing) with two different training interventions in a counterbalanced order separated by a minimum 4 week wash out period. Training interventions lasted for 3 weeks and included 5 training sessions per week in hot ambient conditions (35°C, 60% RH, (exercise heat acclimation - EHA) or work-matched training in temperate conditions (18°C, 60% RH, TEMP). Each 5-day training block included 4 HR-based moderate intensity endurance sessions (days 1, 2, 4 and 5), and 1 intermittent sprint session (day 3). Hereafter throughout the manuscript, CON and HOT relate to the performance trials, while TEMP and EHA relate to the training interventions.

### 7.2.2 *Testing procedure*

Participants attended the laboratory at the same time of day and were instructed to ingest 7 mL/kg body mass of plain water 1 h prior to the start of the trial. After instrumentation and baseline measures, participants rested in the environmental chamber for 15 min in the CON and HYP trials to allow the body to equilibrate to the environment before commencing the exercise tests. In the HOT trial, participants laid supine and were immersed in a custom-made temperature controlled bath (41.9 ± 0.9 °C) until reaching a $T_{core}$ of 38.5°C. The participants towel dried and changed into cycling kit and commenced the incremental cycling test after 5 min.

### 7.2.3 *Ramp test*

The incremental cycling test was based on a ramp protocol commencing from unloaded pedalling and increasing by 1 W every 2 s (equating to 30 $W.min^{-1}$) until volitional fatigue on the Lode ergometer (see Chapter 3). Participants were allowed to self-select pedal cadence but were instructed to maintain their preferred cadence throughout the test. Oxygen uptake was measured continuously using an online

breath-by-breath cardiopulmonary system (Oxycon Pro, CareFusion, Rolle, Switzerland). The breath-by-breath $\dot{V}O_2$ data was edited by removing artefacts (e.g., coughing, swallowing, sighing, etc.) that lay outside 4 SD of local mean, interpolated to give second-by-second values, aligned to the commencement of exercise and transformed into 5 sec bin averages. $\dot{V}O_{2max}$ and $W_{max}$ were determined as the highest 30 s average of the second by second $\dot{V}O_2$ and power output data, respectively.

### 7.2.4 *Time-trial*

Following the incremental test, participants rested in the environmental chamber for 30 minutes, including 20 min of passive rest and 10 min self-selected warm-up, before performing a 20 min TT. During the HOT trial, the environment was adjusted during this recovery time (37.4 ± 2.7 °C, 59.3 ± 6.5 % RH) to maintain a $T_{core}$ of 38.5°C prior to commencing the TT. The TT was performed on an SRM ergometer (Schoberer Rad Meßtechnik, Jülich, Germany) in open-ended mode and zero-offset immediately prior to the start of the TT. Participants were asked to produce a maximal effort over 20 min, designed to simulate the demands of a 16.1 km (10 mile) TT. Feedback during the TT was limited to the time remaining, with no other information provided. Participants were permitted to drink plain water *ad libitum*. Similar protocols employing trained cyclists in our laboratory have suggested a coefficient of variation of 1.1% (Cocking et al. 2018). Power output was measured at 2 Hz during the 20 min. Average power output was calculated as the main performance measure. A secondary analysis of pacing was also performed. The 20 min data was divided into 4, 5 min bin averages for quarterly pacing analysis.

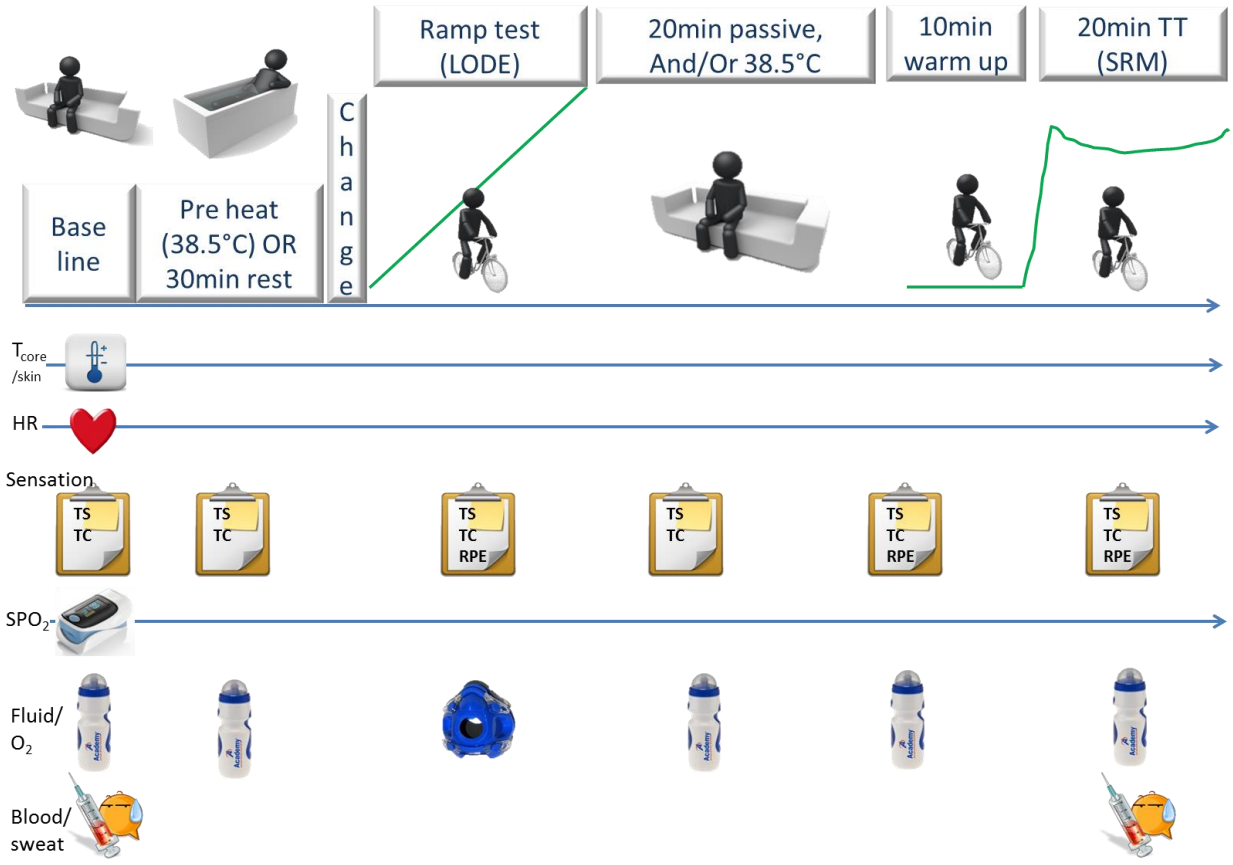


**Figure 7.1:** Schematic of the performance trials. Participants completed a $\dot{V}O_{2max}$ test and 20 min TT in hot, cool and hypoxic conditions in a randomized, counterbalanced cross-over design prior to and following each training intervention.

### 7.2.5 *Temperatures*

Rectal, skin, and environmental temperatures (Chapter 3) were recorded at baseline and at specified intervals throughout the trials. Specific timings are provided below for each part of the trial. Measurements were taken at 5 min intervals during the resting phase prior to the $\dot{V}O_{2max}$ test. In the HOT trial, the bath temperature was continuously measured and adjusted to ensure a stable water temperature (~42°C). Temperature measures were recorded at 2 min intervals during the $\dot{V}O_{2max}$ test and at volitional exhaustion. Thereafter during the 30 min recovery period and during the TT, measures were taken at 5 min intervals, including immediately prior to and upon completion of

the TT. Temperatures were recorded as previously described in general methods 3.3.2.

### 7.2.6 *Sweat rate*

Sweat rate was calculated from changes in nude body weight for each of the trials, as previously described in general methods 3.3.5.

### 7.2.7 *Perceptual responses*

Perceptual responses were recorded at baseline and periodically throughout the trials. Measurements of TS and TC were taken at 5 min intervals during the resting phase prior to the $\dot{V}O_{2max}$ test. Participants were asked for the RPE, TS and TC immediately prior to and upon completion of the $\dot{V}O_{2max}$ test. Thereafter during the 30 min recovery period, measures were taken at 5 min intervals. Participants were asked for their TS and TC immediately prior to and upon completion of the TT, and additionally were asked to give an RPE upon completion of the TT. Perceptual responses were recorded as previously described in general methods 3.3.6.

### 7.2.8 *Extracellular heat shock proteins*

Plasma samples collected prior to and upon completion of the performance trials were analysed using the ELISA technique for determination of extracellular protein content of extracellular heat shock protein 70 (HSP70*e*) and extracellular heat shock protein 90 (HSP90*e*), as previously described in general methods 3.3.3.

### 7.2.9 *Intracellular heat shock proteins*

Cell lysate samples collected prior to and upon completion of the performance trials were analysed using the ELISA technique for determination of intracellular protein content of heat shock protein 70 (HSP70i*)* , and heat shock protein 90 (HSP90i*)*, as previously described in general methods 3.3.3.

### 7.2.10 *Statistical analyses*

Data are presented as mean ± standard deviation (SD), unless otherwise stated. A three-way analysis of variance (ANOVA) with repeated measures design was used to assess differences in average power output and physiological responses [2 status (Pre, post intervention) x 3 testing environments (CON, HOT, HYP) x 2 training conditions (TEMP, EHA)]. A fourth way was added to the ANOVA for parameters recorded at different time points, before (rest), during and/or after exercise). 95% confidence intervals (CI), and Cohen's *d* effect sizes (ES) were also determined. Where a significant interaction effect was apparent, pair-wise differences were evaluated using the Sidak method. For non-parametric data, a Wilcoxon Signed Rank Test was used to determine differences between pre and post intervention. Data analysis was performed using Statistical Package for the Social Sciences for Windows version 22.0 software (SPSS Inc., Chicago, IL). Significance was accepted at $P < 0.05$.

## 7.3 **Results**

### 7.3.1 *Pre-Intervention Status*

All participants completed every trial in a randomized cross-over design. Initial aerobic performance results prior to commencing TEMP and EHA training are presented in table 7.1. Baseline measures were analysed by a 2-way repeated measures ANOVA (testing condition * training intervention). There were no significant differences in baseline fitness between TEMP and EHA as characterized by $\dot{V}O_{2max}$, $W_{max}$, or TT power output between training interventions ($p = 0.791$, $\eta_p^2 = 0.008$, $p = 0.449$, $\eta_p^2 = 0.065$, $p = 0.418$, $\eta_p^2 = 0.074$, respectively). $\dot{V}O_{2max}$, $W_{max}$, and TT power output were higher in CON, than HOT and HYP ($p < 0.001$, $\eta_p^2 = 0.761$, $p < 0.001$, $\eta_p^2 = 0.832$, $p < 0.001$, $\eta_p^2 = 0.783$, respectively).

**Table 7.1:** Initial aerobic performance prior to TEMP and EHA training interventions. Data are presented as mean ± SD, n = 10.

| | | CON | HOT | HYP |
|---|---|---|---|---|
| $\dot{V}O_{2max}$ , $L.min^{-1}$ | | | | |
| | TEMP | 4.5 ± 0.5* | 4.3 ± 0.5 | 4.0 ± 0.5 |
| | EHA | 4.6 ± 0.5* | 4.2 ± 0.5 | 3.9 ± 0.5 |
| $W_{max}$, W | | | | |
| | TEMP | 416 ± 39* | 370 ± 35 | 371 ± 31 |
| | EHA | 411 ± 38* | 364 ± 47 | 374 ± 38 |
| TT power, W | | | | |
| | TEMP | 288 ± 36* | 244 ± 41 | 251 ± 28 |
| | EHA | 285 ± 39* | 236 ± 42 | 252 ± 34 |

* Significantly different from HOT and HYP ($p < 0.05$).

### 7.3.2 *Ramp test*

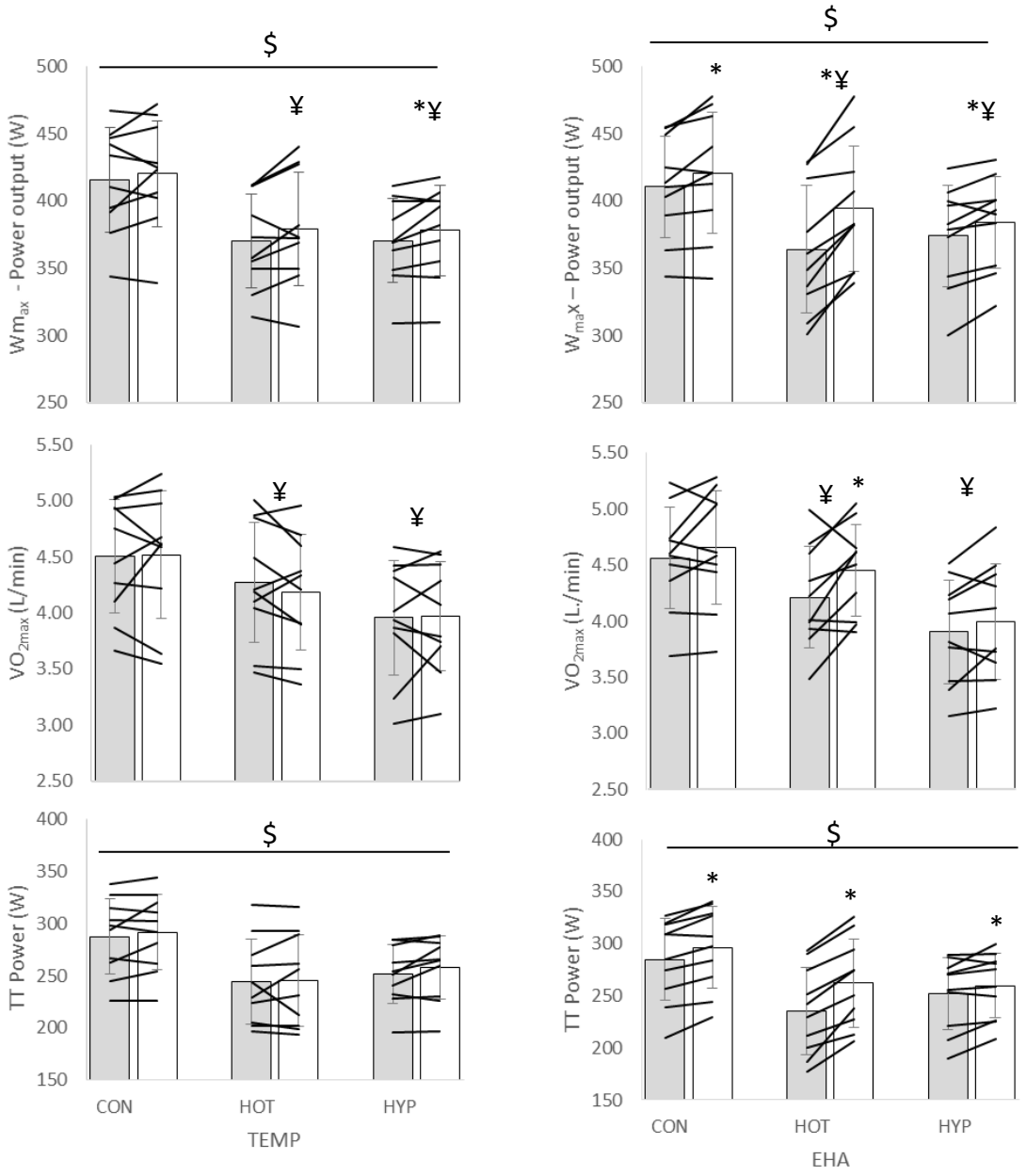


**Figure 7.2:** Responses in $\dot{V}O_{2max}$ , $W_{max}$ and TT performance before and after the temperate (TEMP) and exercise heat acclimation (EHA) training interventions. Data are presented as mean (columns), SD (error bars) and indivdual responses (black lines), n = 10. $ significant main effect of intervention status. ¥ Significant main effect of testing condition compared to CON. * Signifcant interaction effect versus pre-intervention. $p < 0.05$

Mean and individual responses in $W_{max}$ before and after each training intervention

are presented in figure 7.2. There was no main effect of training condition ($p = 0.513$,

$\eta_p^2$ = 0.049), but there was a significant main effect of intervention status ($p$ < 0.001, $\eta_p^2$ = 0.765) and testing environment ($p$ < 0.001, $\eta_p^2$ = 0.755), which increased from pre- to post-intervention, and was higher in CON than HOT and HYP. Importantly, there was a significant interaction effect (training condition*status* testing environment, $p$ = 0.036, $\eta_p^2$ = 0.309) for $W_{max}$ during the ramp test. Post-hoc analysis revealed no effect of the TEMP training on $W_{max}$ in CON (+5[-5;14]W, ES 0.12, $p$ = 0.358), HOT (+9[0;18]W, ES 0.24, $p$ = 0.076) and a significantly improved performance in HYP (+8[1;14]W, ES 0.23 $p$ = 0.038). After EHA, $W_{max}$ was improved in all conditions: CON (+10[3;18]W, ES 0.25, $p$ = 0.021), HOT (+30[21;39]W, ES 0.65, $p$ <0.001), and HYP (+10[4;16]W, ES 0.28, $p$ = 0.009). Mean and individual responses in $\dot{V}O_{2max}$ before and after each training intervention are presented in figure 7.2. There was no main effect of training condition ($p$ = 0.423, $\eta_p^2$ = 0.073) or intervention status ($p$ = 0.146, $\eta_p^2$ = 0.220) on $\dot{V}O_{2max}$. There was a significant main effect of testing environment ($p$ < 0.001, $\eta_p^2$ = 0.739) which was highest in CON. Albeit the interaction effect (training condition*status* testing environment) did not reach significance ($p$ = 0.103, $\eta_p^2$ = 0.223), post-hoc analysis revealed a significant increase in $\dot{V}O_{2max}$ in HOT after EHA (+0.23[0.06;0.42] L, ES 0.56, $p$ = 0.030).

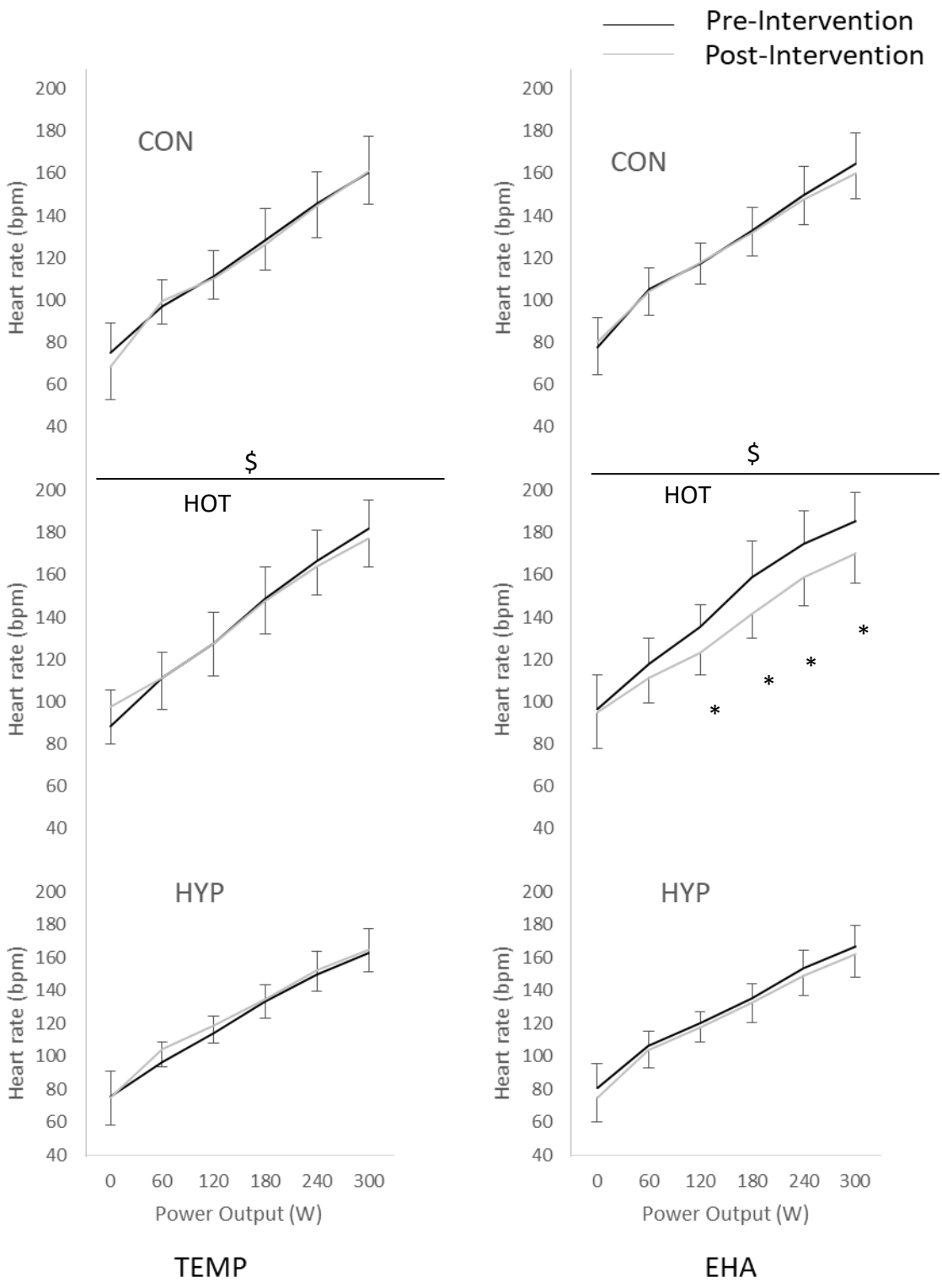


**Figure 7.3:** Sub-maximal heart rate response during the ramp test before (black line) and after (grey line) each intervention in CON, HOT and HYP trials. Data are presented to the highest common completed power output (300w). Data are presented as mean (solid line) and SD (error bars), n = 10. $ main effect of testing condition, * interaction effect (training condition*status), $p < 0.05$.

Heart rate during the ramp test is shown in figure 7.2. There was no main effect of training condition ($p = 0.209$, $\eta_p^2 = 0.169$) or intervention status ($p = 0.152$, $\eta_p^2 = 0.214$). There was a significant effect of testing environment ($p < 0.001$, $\eta_p^2 = 0.769$), whereby heart rate was highest in hot. Whilst there was no overall interaction effect (training condition * intervention status * testing environment * power, $p = 0.201$, $\eta_p^2 = 0.132$), there was an interaction effect between training condition and intervention status as heart rate was lower during the ramp test after EHA, than before ($p = 0.039$, $\eta_p^2 = 0.394$).

### 7.3.3 *Time trial performance*

**Table 7.2:** Quarterly pacing data during the TT before and after each training intervention in CON, HOT and HYP. Data are presented as mean ± SD, n = 10. $ main effect of testing environment, ¥ main effect of intervention status, * main effect of time, $p < 0.05$.

| | TEMP | | | | EHA | | | |
|---|---|---|---|---|---|---|---|---|
| Quarter | 1 | 2* | 3* | 4 | 1 | 2* | 3* | 4 |
| CON $ | | | | | | | | |
| Pre | 301 ± 41 | 284 ± 40 | 279 ± 36 | 286 ± 32 | 295 ± 51 | 281 ± 41 | 277 ± 36 | 288 ± 34 |
| Post ¥ | 305 ± 42 | 288 ± 37 | 282 ± 40 | 293 ± 32 | 302 ± 44 | 294 ± 43 | 291 ± 38 | 298 ± 36 |
| HOT | | | | | | | | |
| Pre | 258 ± 38 | 239 ± 39 | 234 ± 45 | 244 ± 48 | 246 ± 46 | 230 ± 44 | 227 ± 40 | 240 ± 43 |
| Post ¥ | 258 ± 45 | 240 ± 42 | 236 ± 47 | 250 ± 49 | 266 ± 43 | 259 ± 44 | 253 ± 44 | 270 ± 42 |
| HYP | | | | | | | | |
| Pre | 270 ± 37 | 244 ± 31 | 241 ± 26 | 250 ± 25 | 265 ± 38 | 246 ± 41 | 243 ± 34 | 254 ± 32 |
| Post ¥ | 276 ± 41 | 250 ± 33 | 248 ± 29 | 258 ± 27 | 273 ± 36 | 258 ± 35 | 250 ± 31 | 259 ± 26 |

Mean and individual responses in TT performance before and after each training intervention are presented in figure 7.2. There was no main effect of training condition on TT performance ($p = 0.563$, $\eta_p^2 = 0.039$). There was a main effect of intervention status ($p = 0.001$, $\eta_p^2 = 0.734$), and testing environment ($p < 0.001$, $\eta_p^2 = 0.785$),

whereby TT power was higher after the interventions, and was highest in CON. Importantly, there was a significant interaction effect for TT performance (training condition*status* testing environment, $p$ = 0.001 $\eta_p^2$ = 0.545). Post-hoc analysis revealed no effect of the TEMP training on TT performance in CON (+4[-2;11]W, ES 0.12, $P$ = 0.231) or HOT (+1[-8;1]1W, ES 0.03, $P$ = 0.785) and only a tendency for improved performance in HYP (+7[0;13]W, ES 0.22 $P$ = 0.071). Conversely, EHA increased TT performance in all conditions: CON (+12[7;16]W, ES 0.29, P < 0.001), HOT (+27[20;33]W, ES 0.63, P <0.001), and HYP (+8[1;14]W, ES 0.23, $P$ = 0.043).

Pacing characteristics during the TT are presented by quarter in Table 7.2. There was no main effect of training condition ($p$ = 0.554 $\eta_p^2$ = 0.040). There was a significant main effect of intervention status ($p$ = 0.001 $\eta_p^2$ = 0.740 and testing environment ($p$ < 0.001 $\eta_p^2$ = 0.784) whereby power was higher post interventions than pre, and was highest in CON. There was a significant main effect for time ($p$ = 0.003 $\eta_p^2$ = 0.530), There was no interaction effect (training condition*status*testing environment*time, $p$ = 0.848 $\eta_p^2$ = 0.047).

**Table 7.3** Physiological responses during the 20 min TT before and after each intervention. Data are presented as mean ± SD, n = 10.

| | | CON | | HOT | | HYP | | Main effect training condition | Main effect status | Main effect testing environment | Interaction (Condition*status* environment |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Pre | Post | Pre | Post | Pre | Post | | | | |
| $HR_{avg,}$ bpm | TEMP | 174 ± 11 | 174 ± 11 | 176 ± 13 | 178 ± 10 | 171 ± 12 | 174 ± 10 | $p = 0.654$ | $p = 0.758$ | $p < 0.001$ | $p = 0.872$ |
| | EHA | 176 ± 9 | 173 ± 10 | 178 ± 9 | 176 ± 9 | 172 ± 11 | 171 ± 10 | $\eta_p^2 = 0.023$ | $\eta_p^2 = 0.011$ | $\eta_p^2 = 0.779$ | $\eta_p^2 = 0.015$ |
| Starting $T_{core}$, °C | TEMP | 37.7 ± 0.3 | 37.8 ± 0.4 | 38.3 ± 0.2 | 38.4 ± 0.2 | 37.7 ± 0.3 | 37.7 ± 0.3 | $p = 0.787$ | $p = 0.556$ | $p < 0.001$ | $p = 0.237$ |
| | EHA | 37.7 ± 0.3 | 37.7 ± 0.3 | 38.3 ± 0.3 | 38.3 ± 0.2 | 37.7 ± 0.3 | 37.7 ± 0.2 | $\eta_p^2 = 0.009$ | $\eta_p^2 = 0.040$ | $\eta_p^2 = 0.874$ | $\eta_p^2 = 0.148$ |
| Ending $T_{core}$, °C | TEMP | 38.8 ± 0.4 | 38.8 ± 0.4 | 39.2 ± 0.2 | 39.3 ± 0.4 | 38.6 ± 0.3 | 38.7 ± 0.3 | $p = 0.136$ | $p = 0.804$ | $p < 0.001$ | $p = 0.178$ |
| | EHA | 38.7 ± 0.4 | 38.8 ± 0.3 | 39.2 ± 0.3 | 39.1 ± 0.2 | 38.7 ± 0.2 | 38.6 ± 0.4 | $\eta_p^2 = 0.230$ | $\eta_p^2 = 0.007$ | $\eta_p^2 = 0.816$ | $\eta_p^2 = 0.174$ |
| Starting $T_{skin}$, °C | TEMP | 30.1 ± 1.0 | 30.0 ± 0.8 | 35.0 ± 0.6 | 34.9 ± 0.8 | 30.2 ± 0.8 | 30.5 ± 0.2 | $p = 0.644$ | $p = 0.140$ | $p < 0.001$ | $p = 0.442$ |
| | EHA | 30.1 ± 0.6 | 30.0 ± 0.9 | 35.3 ± 0.5 | 34.5 ± 1.0 | 30.3 ± 0.9 | 30.2 ± 0.5 | $\eta_p^2 = 0.025$ | $\eta_p^2 = 0.225$ | $\eta_p^2 = 0.986$ | $\eta_p^2 = 0.087$ |
| Ending $T_{skin}$, °C | TEMP | 31.5 ± 1.0 | 30.8 ± 1.8 | 35.6 ± 1.1 | 35.2 ± 1.1 | 30.6 ± 1.1 | 31.1 ± 1.4 | $p = 0.630$ | $p = 0.026$ | $p < 0.001$ | $p = 0.332$ |
| | EHA | 31.6 ± 0.7 | 30.8 ± 1.7 | 36.2 ± 0.7 | 35.4 ± 0.8 | 31.2 ± 1.1 | 30.5 ± 1.0 | $\eta_p^2 = 0.027$ | $\eta_p^2 = 0.442$ | $\eta_p^2 = 0.952$ | $\eta_p^2 = 0.115$ |
| Starting $T_{whole}$, °C | TEMP | 35.1 ± 0.5 | 35.1 ± 0.3 | 37.2 ± 0.3 | 37.2 ± 0.4 | 35.1 ± 0.3 | 35.2 ± 0.2 | $p = 0.839$ | $p = 0.420$ | $p < 0.001$ | $p = 0.267$ |
| | EHA | 35.1 ± 0.3 | 35.1 ± 0.4 | 37.3 ± 0.3 | 37.0 ± 0.4 | 35.2 ± 0.4 | 35.2 ± 0.2 | $\eta_p^2 = 0.005$ | $\eta_p^2 = 0.074$ | $\eta_p^2 = 0.988$ | $\eta_p^2 = 0.137$ |
| Ending $T_{whole}$, °C | TEMP | 36.3 ± 0.5 | 36.1 ± 0.7 | 38.0 ± 0.4 | 37.9 ± 0.5 | 35.8 ± 0.5 | 36.1 ± 0.7 | $p = 0.973$ | $p = 0.086$ | $p < 0.001$ | $p = 0.144$ |
| | EHA | 36.3 ± 0.2 | 36.1 ± 0.7 | 38.2 ± 0.3 | 37.8 ± 0.3 | 36.1 ± 0.4 | 35.8 ± 0.5 | $\eta_p^2 < 0.001$ | $\eta_p^2 = 0.292$ | $\eta_p^2 = 0.954$ | $\eta_p^2 = 0.194$ |
| Start C-S gradient, °C | TEMP | 7.6 ± 1.0 | 7.8 ± 0.9 | 3.2 ± 0.6 | 3.5 ± 0.8 | 7.4 ± 0.9 | 7.2 ± 0.4 | $p = 0.620$ | $p = 0.082$ | $p < 0.001$ | $p = 0.592$ |
| | EHA | 7.6 ± 0.6 | 7.7 ± 0.9 | 3.0 ± 0.6 | 3.8 ± 1.0 | 7.5 ± 0.9 | 7.5 ± 0.6 | $\eta_p^2 = 0.028$ | $\eta_p^2 = 0.299$ | $\eta_p^2 = 0.976$ | $\eta_p^2 = 0.057$ |
| End C-S gradient,°C | TEMP | 7.3 ± 1.0 | 7.9 ± 1.7 | 3.6 ± 1.1 | 4.0 ± 1.1 | 8.0 ± 0.9 | 7.6 ± 1.2 | $p = 0.460$ | $p = 0.013$ | $p < 0.001$ | $p = 0.539$ |
| | EHA | 7.1 ± 1.0 | 8.0 ± 1.6 | 3.0 ± 0.7 | 3.7 ± 0.8 | 7.4 ± 1.1 | 8.1 ± 0.9 | $\eta_p^2 = 0.062$ | $\eta_p^2 = 0.511$ | $\eta_p^2 = 0.945$ | $\eta_p^2 = 0.066$ |

### 7.3.4 *Temperature*

Thermoregulatory responses during the TT are presented in Table 7.3. There was no main effect of training condition for any of the measured variables ($p > 0.05$ for all). There was a main effect of intervention status only for ending $T_{skin}$ ($p = 0.026$), and ending core-to-skin gradient ($p = 0.026$) which were lower during the post-tests compared with pre-tests. There was a main effect of testing environment on all thermoregulatory variables, which were always higher in HOT than CON and HYP ($p < 0.001$). No interaction effects (training condition*status* testing environment) were observed for any variable ($p > 0.05$).

### 7.3.5 *Sweat rate*

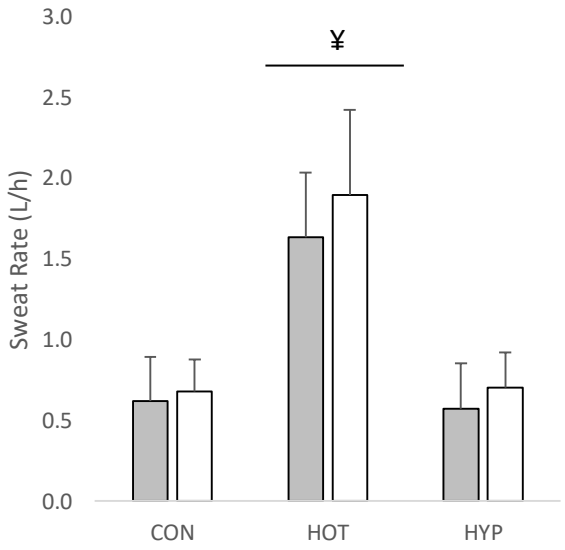


**Figure 7.4**: Sweat rate before (grey box) and after (white box) the exercise heat acclimation (EHA) training intervention in control (CON), hot (HOT) and hypoxic (HYP) testing environments. Data are presented as mean (columns) and SD (error bars), n = 10. ¥ main effect of testing environment, $p < 0.05$.

Sweat rates in each of the testing environments before and after EHA are presented in figure 7.4. Sweat rate tended to be higher in all post-tests after EHA than TEMP and

increase over time, but these failed to reach statistical significance (main effect of training condition: $p = 0.108$, $\eta_p^2 = 0.262$ and main effect of status: $p = 0.134$, $\eta_p^2 = 0231$). There was a main effect of testing environment ($p < 0.001$, $\eta_p^2 = 0.878$), whereby sweat rate was higher in HOT than CON and HYP, but no interaction effect (training condition*status* testing environment, $p = 0.724$, $\eta_p^2 = 0.035$).

### 7.3.6 *Plasma volume*

Resting plasma volume is presented in table 7.4. For percentage change in resting plasma volume using the relative formula, there was no main effect for training condition ($p = 0.845$, $\eta_p^2 = 0.004$) or testing environment ($p = 0.755$, $\eta_p^2 = 0.031$). There was no interaction effect (training condition*testing environment, $p = 0.960$, $\eta_p^2 = 0.005$). For percentage change in resting plasma volume using the absolute formula, there was no main effect for training condition ($p = 0.728$, $\eta_p^2 = 0.014$) or testing environment ($p = 0.244$, $\eta_p^2 = 0.145$). There was no interaction effect (training condition*testing environment, $p = 0.391$, $\eta_p^2 = 0.090$).

**Table 7.4** Percentage change in resting plasma volume prior to the experimental trials during the temperate (TEMP), and exercise heat acclimation (EHA) training interventions. Data are presented as mean ± SD, n = 10.

| | TEMP | | | EHA | | |
|---|---|---|---|---|---|---|
| | CON | HOT | HYP | CON | HOT | HYP |
| % Δ Resting PV (Rel) | 0.6 ± 4.8 | 1.4 ± 8.1 | 2.0 ± 6.9 | 1.2 ± 6.5 | 0.9 ± 4.6 | 2.6 ± 6.1 |
| % Δ Resting PV (Abs) | 2.5 ± 8.5 | -0.6 ± 5.2 | 1.5 ± 5.5 | 4.0 ± 9.0 | 2.7 ± 6.7 | -1.0 ± 5.7 |

*Data are presented as relative percentage change (Rel) using the formula of Dill and Costill (Dill and Costill 1974), and absolute percentage changes (Abs) derived from the CO-rebreathing (Schmidt and Prommer 2005).*

### 7.3.7 *Heat Shock Proteins*

Resting and post exercise intra (i) and extra-cellular (e) HSP concentrations are presented in figures 7.5 and 7.6 for HSP70 and HSP90, respectively.

### 7.3.8 *Intra and extra-cellular HSP70*

There was a main effect of training condition for HSP70i, which was higher in EHA than TEMP ($p$ = 0.017, $\eta_p^2$ = 0.484). There was also a main effect of pre- to post-intervention status, whereby resting HSP70i was higher post- interventions compared with pre-intervention ($P$ = 0.038, $\eta_p^2$ = 0.396). There was no effect of testing environment ($p$ = 0.149, $\eta_p^2$ = 0.191). HSP70i concentrations were higher post-exercise than at rest ($p$ = 0.001, $\eta_p^2$ = 0.717). There was no interaction effect ($p$ = 0.674, $\eta_p^2$ = 0.043). There was no main effect of training condition ($p$ = 0.580, $\eta_p^2$ = 0.035), pre- to post-intervention status ($p$ = 0.151, $\eta_p^2$ = 0.215), testing environment ($p$ = 0.678, $\eta_p^2$ = 0.042) for HSP70e. Post exercise samples were significantly higher than resting ($p$ = 0.002, $\eta_p^2$ = 0.665). There was no interaction effect ($p$ = 0.780, $\eta_p^2$ = 0.027).

### 7.3.9 *Intra and extra-cellular HSP90*

There was a main effect of training condition on HSP90i, whereby it was higher in EHA than TEMP ($p$ = 0.021, $\eta_p^2$ = 0.462). There was a main effect of pre- to post-intervention status, whereby HSP90i was higher post interventions than pre ($p$ = 0.040, $\eta_p^2$ = 0.611). There was no effect of testing environment ($p$ = 0.359, $\eta_p^2$ = 0.108). HSP90i was significantly higher post exercise than at rest ($p$ = 0.001, $\eta_p^2$ = 0.743). There was no interaction effect ($p$ = 0.666, $\eta_p^2$ = 0.044). There was no main effect of training condition on HSP90e ($p$ = 0.753, $\eta_p^2$ = 0.012), pre- to post-intervention status ($p$ = 0.902, $\eta_p^2$ = 0.002) or testing environment ($p$ = 0.174, $\eta_p^2$ = 0.176). Post exercise samples were

significantly higher than resting ($p$ = 0.001, $\eta_p^2$ = 0.692). There was no interaction effect ($p$ = 0.722, $\eta_p^2$ = 0.035).

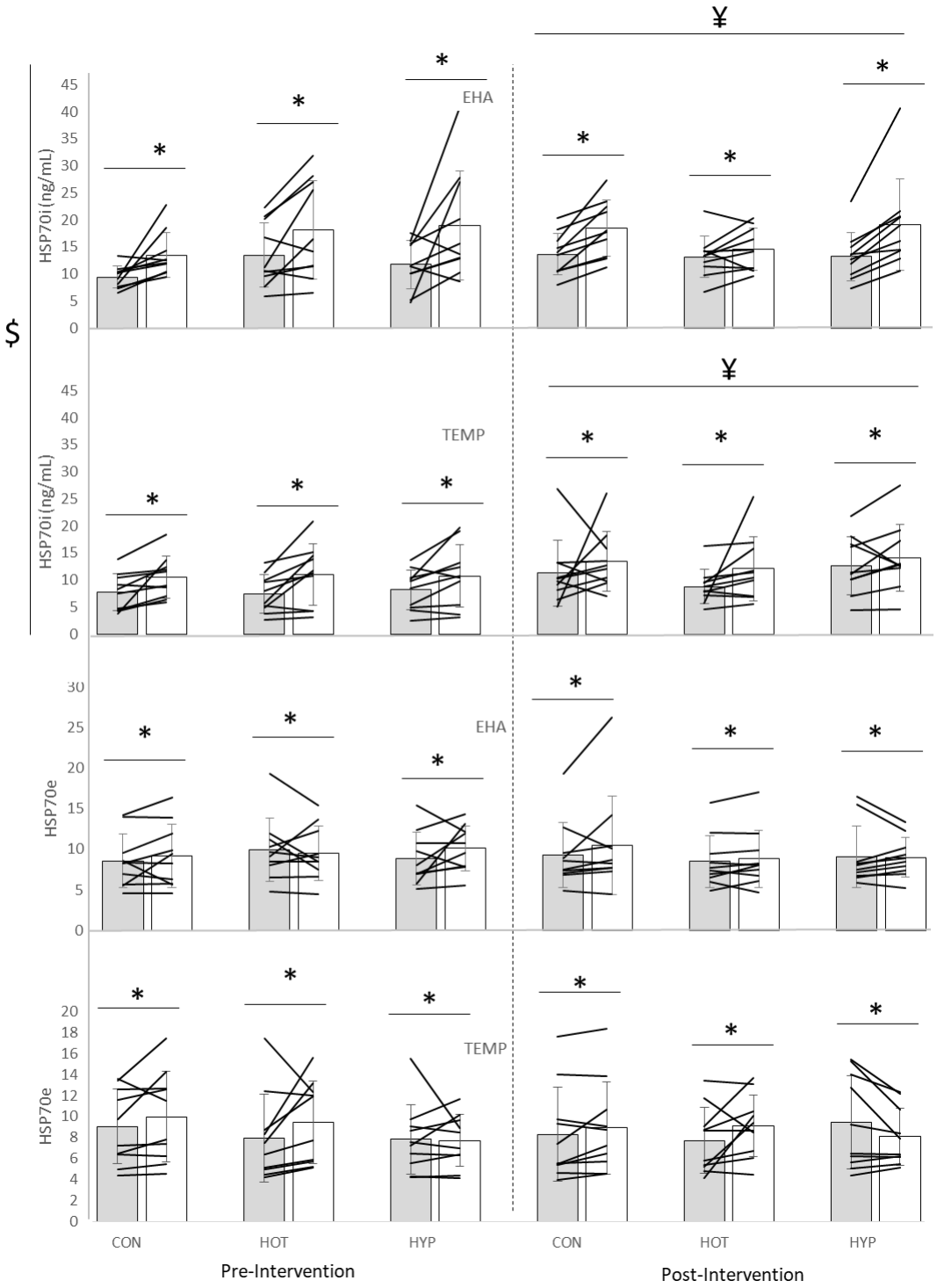


**Figure 7.5:** Resting (grey boxes) and post exercise (white boxes) concentrations of extracellular and intracellular HSP70, pre- and post-intervention for each testing environment. Data are presented as mean (columns), SD (error bars) and indivdual responses (black lines), n = 10. $ main effect of training condition, ¥ main effect of pre-post intervention status, * main effect of time (resting to post-exercise), P < 0.05.

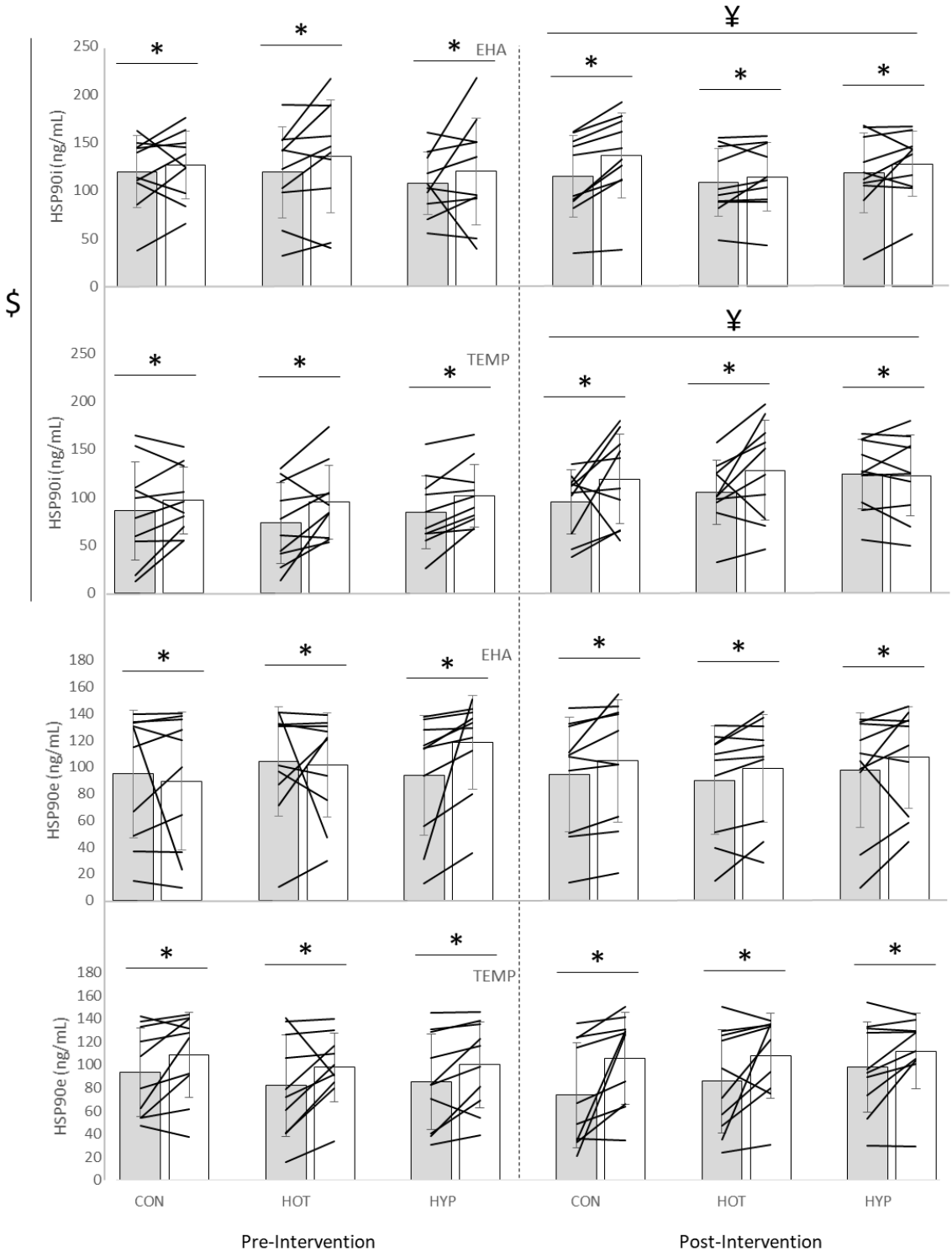


**Figure 7.6:** Resting (grey boxes) and post exercise (white boxes) concentrations of extracellular and intracellular HSP90, pre- and post-intervention for each testing environment. Data are presented as mean (columns), SD (error bars) and indivdual responses (black lines), n = 10. $ main effect of training condition, ¥ main effect of pre-post intervention status, * main effect of time (resting to post-exercise), $P < 0.05$.

### 7.3.10 *Perceptual*

Perceptual data from the TT are presented in Table 7.5. Wilcoxon signed rank tests were used to assess differences between pre and post intervention measures. There was no effect on RPE after the TEMP intervention in CON, HOT, or HYP ($p$ = 0.083, $p$ = 0.098, $p$ = 0.063, respectively). Similarly, there was no effect on RPE after the EHA intervention in CON, HOT, or HYP ($p$ = 0.317 for all). There was no effect on TS after the TEMP intervention in CON, HOT, or HYP ($p$ = 0.083, $p$ = 0.739, $p$ = 0.564, respectively). Similarly, there was no effect on TC after the EHA intervention in CON, HOT, or HYP ($p$ > 0.999, $p$ > 0.999, $p$ = 0.564, respectively). There was no effect on TC after the TEMP intervention in CON, HOT, or HYP ($p$ = 0.083, $p$ = 0.655, $p$ > 0.999, respectively). Similarly, there was no effect on TC after the EHA intervention in CON, HOT, or HYP ($p$ = 0.655, $p$ = 0.157, $p$ = 0.157, respectively).

**Table 7.5** Perceptual responses to the 20 min TT before and after each intervention. Data are presented as median(interquartile range), n = 10.

| | | CON | | HOT | | HYP | |
|---|---|---|---|---|---|---|---|
| | | Pre | Post | Pre | Post | Pre | Post |
| RPE | TEMP | 19.0 (19.0-20.0) | 20.0 (19.3-20.0) | 19.5 (18.3-20.0) | 20.0 (20.0-20.0) | 19.0 (18.0-19.8) | 19.3 (19.0-20.0) |
| | EHA | 20.0 (19.0-20.0) | 20.0 (19.0-20.0) | 20.0 (19.3-20.0) | 20.0 (19.3-20) | 19.8 (18.3-20.0) | 20.0 (19.3-20.0) |
| TS | TEMP | 5.0 (5.0-5.0) | 5.0 (4.3-5.0) | 7.0 (6.0-7.0) | 6.8 (6.0-7.0) | 4.0 (4.0-5.0) | 5.0 (4.0-5.0) |
| | EHA | 5.0 (5.0-5.0) | 5.0 (4.3-6.0) | 7.0 (6.3-7.0) | 7.0 (6.0-7.0) | 4.8 (4.0-5.0) | 4.8 (4.0-5.0) |
| TC | TEMP | 5.0 (5.0-5.0) | 5.0 (4.3-5.0) | 6.4 (6.0-7.0) | 7.0 (6.0-7.0) | 4.5 (4.0-5.0) | 5.0 (4.0-5.0) |
| | EHA | 5.0 (5.0-5.0) | 5.0 (4.3-6.0) | 7.0 (6.3-7.0) | 6.8 (6.0-7.0) | 5.0 (4.6-5.0) | 4.8 (4.0-5.0) |

## 7.4 **Discussion**

To the author's knowledge, this is the first study to compare the impact of heat acclimation on aerobic capacity and time-trial performance in hot, cool and hypoxic conditions in a single group of trained cyclists. Our data demonstrate a large improvement in time-trial performance in the heat after 3 weeks of heat acclimation, and also improvements in both cool and hypoxic environments when compared to matched training in a temperate environment.

### 7.4.1 *Exercise performance in hot environments*

During the HOT trial, participants were passively pre-heated to a rectal core temperature of 38.5°C prior to the $\dot{V}O_{2max}$ test, and maintained this core temperature prior to commencing the 20min TT, by manipulation of the ambient environment. Whilst this experimental design allowed us to determine the impact of heat acclimation at a given level of heat strain, it is likely to have minimized some of the benefits of heat acclimation (e.g. reductions in sub-maximal core temperature and HR response), since post acclimation it is expected that participants would have commenced the 20min TT at a lower level of heat strain (Périard et al. 2015). It was also necessary to ensure that participants commenced the $\dot{V}O_{2max}$ test and 20min TT in a hyperthermic state, since $\dot{V}O_{2max}$ and TT performance may be better defended in a hot environment if initiated at a normothermic temperature and especially over a relatively short duration (González-Alonso et al. 1999). The increase in $\dot{V}O_{2max}$ (~6%), $W_{max}$(~9%) and 20min TT power output ( ~12% ) in HOT after EHA concurs with previous literature reporting a ~7%-13% increase in $\dot{V}O_{2max}$ and TT performance in the heat as a result of heat acclimation (Sawka et al. 1985; Nielsen et al. 1997). Interestingly, the increase in $\dot{V}O_{2max}$ and $W_{max}$ almost

fully attenuated the decrement when compared to the CON trial prior to EHA, as evidenced by $\dot{V}O_{2max}$ increasing from 92% to 98% of CON, and $W_{max}$ increasing from 89% to 96% of CON (Figure7.2). Concurrently, TT performance increased from 83% to 92% of that achieved in CON. This near complete restoration of performance in warm vs cool conditions has previously been observed in the laboratory (Keiser et al. 2015) and field (Racinais et al. 2015b). In the study of Keiser and colleagues, the increase in maximal oxygen uptake fully compensated the initial heat-induced decrement, while maximum aerobic power and 30-min TT power after heat acclimation were almost completely restored to levels initially obtained at 18°C. Racinais and colleagues (Racinais et al. 2015b) found, following two weeks of acclimatization, highly trained cyclists were capable of producing a similar time over a 40km TT performance in the heat, compared to cool conditions. Since air density decreases ~3.4% per 10°C increase in ambient air temperature, this performance decrement is likely to be further attenuated in field TT observations as speed at a given power output is higher at a lower air density (Nybo 2010a; Racinais et al. 2015b). The data from the present study supports the consensus that heat acclimation is a key strategy to enhance performance in the heat (Racinais et al. 2015a).

### 7.4.2 *Exercise performance in temperate environments*

We also observed that heat acclimation improved TT performance in temperate environments by ~4%, and in the absence of any significant alteration in $\dot{V}O_{2max}$. Of note, this change in performance far exceeds the coefficient of variation of 1.1% observed in our laboratory following a similar protocol (Cocking et al. 2018). The transfer from heat acclimation to performance in a cooler environment remains a highly debated area in the

literature (Minson and Cotter 2016; Nybo and Lundby 2016). Corbett and colleagues (Corbett et al. 2014) highlighted the limited literature available and suggested limitations in many of the existing studies, including absence of an adequate control group (Sawka et al. 1985; Hue et al. 2007; Buchheit et al. 2011, 2013; Racinais et al. 2014a). Our performance data are broadly in line with that of Lorenzo and colleagues (Lorenzo et al. 2010). Whilst Lorenzo and colleagues reported a 5% and 6% improvement in $\dot{V}O_{2max}$ and TT performance respectively, at ambient environment of 13°C after 10days of heat acclimation, we observed no difference in $\dot{V}O_{2max}$ (+2.2%, $P$ = 0.24), but an increase in $W_{max}$ (+2.4%) and a 4.2% improvement in TT performance. Similarly, McCleave and colleagues (McCleave et al. 2017), observed an improvement in 3-km running time trial performance in temperate conditions 3 weeks after heat acclimation. This in contrast to Karlsen and colleagues (Karlsen et al. 2015b) and Keiser and colleagues (Keiser et al. 2015), who observed no benefit to performance in cooler conditions.

Improved sub-maximal responses to exercise has been proposed as one of the explanations that might support exercise performance in cool conditions, as previously demonstrated by an increased lactate threshold (Lorenzo et al. 2010, 2011). However, while gross efficiency was not assessed in the present study, we observed no change in heart rate for a given power output during the ramp test (Figure 7.2), although kinetics of HR response to non-steady state exercise may have masked potential subtle differences.

Plasma volume expansion has been proposed as a mechanisms associated with improvements in maximal oxygen uptake in cool environments (Lorenzo et al. 2010), by facilitating increased vascular filling, and therefore stroke volume and cardiac output. In the present study, we observed no change in resting plasma volume prior to any of the

post-intervention trials, using relative (Dill and Costill 1974) and absolute changes (Schmidt and Prommer 2005). stroke volume and cardiac output were not measured. We previously suggested that using relative measures may not be valid (see Chapter 4). The absolute percentage change in resting plasma volume, derived from the CO-rebreathing technique, response was highly individualized, with 7 participants exhibiting an average 8% increase, one exhibiting no change (~-1%), and 2 exhibiting a decrease (~-9%), with a range of -9 to + 17%. Since plasma volume was not measured prior to the TT, we cannot confirm the role of plasma volume in supporting TT performance, as it may have been conflicted by the previous test. Lorenzo and colleagues observed a 2.2 L/min increase in maximal cardiac output, coupled to a 6.5% increase in plasma volume and 3.3% haemodilution. However, given that neither acute plasma volume expansion (Kanstrup and Ekblom 1982; Keiser et al. 2015) nor the increase in plasma volume as a result of heat acclimation (Keiser et al. 2015) or acclimatization (Karlsen et al. 2015b) resulted in increased $\dot{V}O_{2max}$ or TT performance in trained athletes, the role of plasma volume expansion in supporting exercise performance in the cool remains questionable.

The improvement in time trial performance, despite no change in $\dot{V}O_{2max}$, raises further question as to the exact mechanisms by which heat acclimation augments exercise performance. This could be due, for example, to improved efficiency, an increase in anaerobic energy contribution during the 20 min TT, or psychological factors. Since we did not measure oxygen uptake during the TT, the fractional utilization of oxygen during the performance task is unknown. The addition of the intermittent sprint training (see chapter 6) during the intervention raises an interesting possibility that sprint training may have contributed to an increased anaerobic work capacity, which theoretically could

contribute to performance over a relatively short TT. However, since TT performance was unchanged after TEMP, this would imply that intermittent sprint training in the heat is more beneficial in supporting adaptations to increase anaerobic work capacity than in the cool. A supporting mechanism for this is unclear, and more research is required to investigate this hypothesis.

### 7.4.3 *Effect of heat acclimation on performance in hypoxia*

Cross-acclimation has received increased interest recently whereby adaptation to one environmental stressor might induce protective responses upon exposure to others, provided they share common adaptive pathways (Fregly et al. 2011; Ely et al. 2014; Lee et al. 2016). The findings from this study support this hypothesis since we observed a ~3.3% increase in TT performance in hypoxia after EHA. The improvement in performance was not due to a change in $\dot{V}O_{2max}$, since this was unaltered by EHA. This is in agreement with Heled and colleagues (Heled et al. 2012), who examined the effect of 12 days of HA on the onset of blood lactate accumulation (OBLA) during an acute exposure to moderate hypoxia (2400 m). They concluded that OBLA following HA was equally delayed during a graded exercise test in both normoxia and hypoxia conditions. Following HA maximal aerobic capacity did not change, and the authors suggested that exposure to heat and not a training effect (no change in $\dot{V}O_{2max}$) led to increased physiological efficiency during acute altitude exposure, although this was not measured. Two mechanisms were proposed to explain how HA may promote adaptation that could benefit an individual during altitude exposure: 1) a reduced metabolic rate (Sawka et al. 1983b), which could contribute to greater efficiency when exposed to altitude; and 2) HA increases the expression of HIF1-$\alpha$ (Maloyan et al. 2005) , which may promote expression

of EPO and contribute to the adaptive response during hypoxic exposure by increasing tissue oxygen delivery and ATP production (Benita et al. 2009). Given that we previously observed an increase in EPO with EHA with a transient reduction in HBmass which returned to baseline when EHA was ceased (see chapter 5), the role of the EPO pathway to facilitate performance by increased oxygen supply seems limited. There is some evidence that EPO is associated with a neuroprotective effect in brain tissue (Shein et al. 2005; Ponce et al. 2013) and heightened cognitive function in hypoxia after heat acclimation (Heled et al. 2012). Whether this may relate to exercise performance remains to be elucidated.

### 7.4.4 *Heat Shock Proteins*

The cellular pathways responsible for cross-acclimation between heat and altitude remains unclear (Gibson et al. 2017). No change in resting extracellular or intracellular HSP70 and HSP90 was observed after 3-weeks of heat acclimation. The effect of heat acclimation on the HSP70 response remains equivocal within the literature with studies showing contrasting results. Extracellular levels of HSP70 have been shown to decrease after the initial phase (2 days) (Marshall et al. 2006) and after 5 days of HA (Kresfelder et al. 2006), but no difference was found after a 10-day HA protocol (Yamada et al. 2007b).It also should be noted that the aforementioned studies utilised a constant work-rate model of heat acclimation, which has been criticized for diminishing the thermal load as adaptation develops. Moreover, Magalhães and colleagues (Magalhães et al. 2010) utilized a controlled hyperthermia model of heat acclimation, and similarly found no change in resting eHSP72 levels after 11 days of heat acclimation. However, Gibson and colleagues (Gibson et al. 2015a) observed an increase in HSP72 mRNA following

isothermic (controlled hyperthermia), and fixed-intensity heat acclimation bouts. It should however be noted, that in this study only transcriptional mRNA was measured, which may or may not contribute to an increase in the formed protein. The present study does not support any increase in resting eHSP70 or an attenuation of the acute response to exercise in cool, hot or hypoxic environments after 3 weeks of heat acclimation. Relatively few studies have examined the intracellular response of HSP's to heat acclimation. In the present study, an acute exercise-induced response of HSP70i expression was observed in all environmental conditions prior to both the TEMP and EHA training interventions. Exercising in warm environments is sometimes shown to induce a HSP70i response (Magalhães et al. 2010), but not always (Marshall et al. 2006; Yamada et al. 2007b). However, given that our exercise tests consisted of 2 maximal tasks, and induced marked increases in core body temperature, to a similar extent of those reported by Magalhães and colleagues (Magalhães et al. 2010) (Table 7.3), it is likely these trials were sufficiently stressful to induce a HSP70i response. It should be noted, however, that in the study of Magalhães and colleagues, only leukocytes were measured, in contrast we isolated peripheral blood mononuclear cells. However, it is unlikely that the cell population analyzed would impact these interpretations since it has been reported that the response of monocytes and granulocytes expression of HSP72 after a half-marathon run were similar (Fehrenbach et al. 2000). We observed no effect of either intervention on resting HSP70i levels during the post-tests. Heat acclimation has been demonstrated to both increase (Maloyan et al. 1999; Yamada et al. 2007a; Mcclung et al. 2008; Magalhães et al. 2010), and have no effect (Marshall et al. 2006; Watkins et al. 2007), on resting HSP72i levels. The reasons for these discrepancies are unclear but may be due to the timing of

measurements in the present study. Each trial was separated by only 48 h of rest, and the HSP response may still have been elevated during the resting value of the subsequent trials (Moloney et al. 2012). Interestingly in the present study, TEMP training alone attenuated the exercise-induced rise in HSP70i after CON, HOT, and HYP, while this increase was only blunted in HOT after the EHA intervention. The acute response of HSP70i to exercise after heat acclimation is not well researched, although it is generally observed that heat acclimation attenuates the acute rise in HSP70i after exercise (Mcclung et al. 2008; Magalhães et al. 2010; Maloyan et al. 1999), although this coincided with an increase in resting levels. Since we utilized a maximal effort, self-paced exercise trial, these results are not entirely surprising. $W_{max}$ and TT power output were markedly increased in all trials after EHA and the magnitude of expression may differ during exercise at different intensities, given that this elicits distinctive metabolic demands and induces different rates of heat storage and physiological load. Power-output was not different in CON, HOT and HYP after TEMP training, and so it appears the effect of training alone in a cool environment was sufficient to blunt this acute response to subsequent exercise. However, performance was dramatically improved in HOT after EHA, and the acute response of HSP70i to exercise was still blunted after EHA. This finding validates the observation that, despite marked increases in work rate, heat acclimation attenuated the rise in HSP70i.

The role of HSP90 during heat exposure has received, only recently, limited scientific interest, and most current data exists in ex vivo models (Mcclung et al. 2008). No change in HSP90e, either at rest throughout the interventions, or any change in response to exercise were observed in the present study. McClung and colleagues (Mcclung et al.

2008) observed similar responses of HSP90i and HSP72i, as exhibited by increases in resting levels, and a blunting of the response after heat acclimation ex vivo. However, findings from the present study failed to replicate these observations, since there was no clear pattern of the HSP90i response to heat acclimation. In fact, HSP90i was only increased from rest to post-exercise in the control condition after 3 weeks of heat acclimatization. An attenuation of the HSP90i response after the TEMP intervention in the HOT and HYP trials was observed in the present study. The cause of this response is unclear, but may reflect the self-paced nature of these tasks. Future research should explore the HSP90 response to constant load exercise, to better standardize the physiological strain in these environments.

## 7.5 **Conclusions**

Heat acclimation resulted in improved cycle TT performance after EHA in CON, HOT and HYP environments. However, $\dot{V}O_{2max}$ was only enhanced in HOT. This raises further questions regarding the mechanisms which support self-paced exercise performance after heat acclimation. While the present study demonstrated that heat acclimation improved performance in the absence of a training effect, it cannot be ruled out that participation in an un-blinded scientific study may have contributed to performance improvements. Whilst blinding to altitude exposure is relatively simple, participants were fully aware that they were training and testing in either a cool or warm environment, and so a placebo effect cannot be dismissed. However, given that this is the first study to have matched both absolute and relative training workloads between cool and hot environments (see chapter 4), and used trained cyclists with stable performances, our

data supports an ergogenic effect of heat acclimation on performance in hot, cool and hypoxic conditions.

# CHAPTER 8

# GENERAL DISCUSSION AND CONCLUSIONS

The primary aims of this thesis were to: 1) investigate a novel heart rate based, long-term HA protocol; 2) quantify the haematological adaptations to heat acclimation, specifically that of the HBmass; 3) to ascertain the neurological adaptations to intermittent sprint training during heat acclimation and the effect on intermittent sprint performance; and 4) to examine the phenomena of cross- acclimation where the stressors of one environment (heat), might convey a performance benefit in alternative environments (cool and hypoxia). Results from chapter 4 and 5 characterised the haematological adaptations of heat acclimation, and provided evidence of alterations in the red blood cell compartment of the total blood volume. While temperate (TEMP) training led to a stable HBmass (HBmass), exercise heat acclimation (EHA) led to a transient drop in HBmass within 4 days of heat exposure, and remained at this level through the 3-week intervention. HBmass returned to baseline 1 week after EHA in 7 of the 10 participants. In chapter 6, all out intermittent sprint performance was similar between TEMP and EHA, and heat acclimation had no additional performance benefits over the same training intervention completed in a cool environment. Despite the hypothesis that heat acclimation might convey enhanced neuromuscular adaptations (Racinais *et al.* 2017a, 2017b), the present thesis failed to observe any benefit during whole body sprint exercise. Chapter 7 revealed

that heat acclimation led to enhanced cycle time-trial performance in hot, cool and hypoxic environments, whereas completing work matched training in a cool environment had no effect on exercise performance. Given that an increase in maximal aerobic capacity was only observed in the heat after heat acclimation, but improvements in maximal aerobic power and time trial performance in all three environments, it is possible that heat acclimation led to an enhancement of anaerobic energy production, or increased sub-maximal cycling efficiency, beyond that which cool training alone achieved.

## 8.1 **Main findings**

### 8.1.1 *Long-term heat acclimation with a heart rate clamp*

The aim of chapter 4 was to characterise the typical adaptations to a novel long-term, work matched, heart rate clamped heat acclimation protocol in a group of trained cyclists. We were able to accept the hypothesis that utilizing a heart rate based protocol would induce and maintain the typical adaptations to heat acclimation over a long term (3 week) heat acclimation exposure. The novel work-matched heart rate based training design utilized in the present study successfully induced heat acclimation (see Figure 4.8). Similar to controlled hyperthermia, or isothermal protocols previously described the aim of the heart rate based approach was to provide a continual forcing function throughout the 3-week intervention. While constant work-rate protocols do induce heat acclimation, the thermal strain gradually diminishes over time, as relative exercise intensity decreases as adaptation progresses. An increase in power output for a given heart rate from weeks 1 to 3 was observed in the present thesis, and while haematological and sudomotor adaptations seemingly plateaued during the 2$^{nd}$ week, the average power sustained in the 3$^{rd}$ week was marginally, but significantly higher. The benefits of utilizing a protocol

longer term (*i.e.* > 3 weeks) is unknown, but is likely to yield only minimal, if any, further benefits. Furthermore, matching the heart rate allows for a better replication of the relative intensity of exercise between warm and cool environments, and extending this approach to also match total-work done also facilitates a matched absolute training load between conditions. This work-matched, HR clamp model allows a matching of both absolute and relative exercise load between environments. An extended benefit of utilizing a heart rate clamp over core temperature clamp is that it provides a practical application in the real world, and is more closely aligned to athletic training requirements. Indeed to maintain core temperature at a set level, participants have been noted to intermittently stop exercising entirely (Gibson et al. 2015a). This chapter provided a novel framework of a work-matched heart rate based model of heat acclimation, which successfully induced classic signs of heat acclimation and maintained these over an extended 3-week period.

**Key findings:**

Academic

Chapter 4 provides a novel framework to match both absolute and relative intensity where participants are exposed to hot and cool environments. The heart rate clamp protocol may not reflect true adaptive pressures on cardiac output where exercise is undertaken in the heat, and studies to compare heart rate vs cardiac output clamp are warranted to mechanistically confirm utilizing such an approach.

Practical

Heat acclimating with a heart rate clamp is a practically simple way to induce heat acclimation, with most benefits observed within 5 days of exposures. Heat acclimation

protocols should be individualized, especially within a team sport setting, since we observed large individual variation in the duration required to induce phenotypical adaptations to support exercise in the heat.

### 8.1.2 *Haematological adaptation to heat acclimation*

The aim of chapter 5 was to characterise the HBmass response to heat acclimation and repercussions this might have on relative estimates on changes in plasma volume. Data from the present thesis rejects the hypothesis that heat acclimation would induce and increase in HBmass. Rather, a transient decrease in HBmass was observed, which recovered to pre-intervention levels after the heat stimulus was withdrawn in 7 of the 10 participants. This led to significant discrepancies in plasma volume estimates using absolute methods (i.e. CO-rebreathing), and relative methods (i.e. Dill and Costill formula). The constant thermal strain from a heart rate clamp protocol suggested in chapter 4 may have contributed to the transient decline in HBmass reported in chapter 5. Within 4 days of heat acclimation, HBmass was reduced by 2.5% (see Figure 5.1). This reduction was maintained at a similar level at week 3. However, the average of HBmass on days 5 and 7 (post-test duplicate), following the daily heat acclimation intervention, HBmass had returned to baseline in 7 of the 10 participants, while 3 participants remained below baseline values. It was not possible to identify an analytical artefact that could explain this reduction; none of the physiological consequences of heat acclimation seem to affect the kinetics of carbon monoxide uptake, distribution or binding capacity to the haemoglobin, or myoglobin. The concomitant increase in plasma erythropoietin concentrations, reduction in plasma ferritin and unaltered reticulocyte response suggested that an active destruction of red blood cells (neocytolysis), or a

cessation in red blood cell production was unlikely. Instead, we suggest that either heat acclimation *per se*, or the inclusion of the all-out intermittent sprint session may have led to exercise-induced intravascular rupture of the red blood cells (haemolysis). Since haemolysis would simultaneously promote an erythropoetic response (Thorling and Erslev 1968), this would also explain the concurrent increase in erythropoietin after 1 week of heat acclimation. The novel observation of a reduction in HBmass with heat acclimation should facilitate future research and may have consequences in the planning of athlete training. The use of relative changes in plasma volumes is conventional in the literature (Dill and Costill 1974), yet the relationship between pre- and post-exercise measures of haematocrit and haemoglobin concentration for the determination of changes in plasma volume relies on the assumption that HBmass is a constant. Our data suggests that within the context of a heat training intervention, this could lead to biased results. Furthermore, the reduction in HBmass with heat acclimation has additional practical significance since there has recently been increased interest in the combination of environmental stressors of heat and altitude during specified training blocks. This is an interesting concept, since hypoxic exposure can result in an increase in red blood cells volume or HBmass, while heat acclimation generally increases plasma volume. Thus, theoretically, we may expect an augmented response and that by combining both stimuli would provide additional training stressors and afford substantial improvements in total blood volume and convective oxygen delivery. Whilst we did not implement an altitude training intervention in the present study, the fact that heat acclimation decreased HBmass might suggest that rather than

augment the adaptations to altitude (Buchheit et al. 2013), the adaptive pathways may in fact counter-act each other, and lead to maladaptation.

**Key findings:**

Academic

The reduction in HBmass should be confirmed through specific studies relating to HBmass breakdown (i.e. haptoglobin, bilirubin). Since it was hypothesizes that HBmass would increase as a result of heat acclimation, we are able only to speculate the causes of reduced HBmass. Absolute measures of plasma volume utilizing the CO-rebreathing technique should be used in favour of relative estimates (i.e. Dill and Costill formula), since any change in HBmass would lead to biased results.

Practical

Whilst there is some interest in combining environmental stressors (i.e. sleep high, train hot), heat and altitude acclimation appear to yield opposing rather than complementary effects on HBmass, and as such it may be prudent to add only one of these environmental extremes to a specified training block.

### 8.1.3 *Neuromuscular, and metabolic adaptations to intermittent sprint training in warm and cool environments.*

The aim of chapter 6 was to explore the acute effect on performance of intermittent sprint training in hot and cool environments, and to evaluate the neuromuscular and metabolic adaptations to heat acclimation, and its effect on intermittent sprint performance. Data from the present thesis rejects the hypotheses that heat exposure

would acutely impair intermittent sprint performance and that heat acclimation would improve intermittent sprint performance beyond that of training in a cool environment alone. Similar performances were observed in intermittent sprinting, regardless of whether these sessions were performed in the context of a temperate or warm climate (see Figure 6.4). Whilst heat acclimation did not improve intermittent sprint performance beyond training in a temperate environment, our data suggests that similar performance gains were made despite intermittent sprint training being undertaken in warm environmental conditions, which athletes may face during warm-weather training camps. Heat acclimation did not modulate any changes in muscle oxygenation, or the evolution of muscle recruitment with fatigue. Our data supports the existing literature that power output is not affected in the heat, in the absence of hyperthermia, and demonstrates that heat acclimation has no specific benefit over training in a temperate environment. However, this may offer an additional approach in training where heat acclimation is a primary goal. Heat acclimation is likely mostly used during periods of a taper towards a competition set to take place in warm environmental temperatures. Heat acclimation is generally undertaken at relatively low intensities for extended periods of time (>60min), to induce the required physiological adaptations. Yet optimal tapering strategies are generally recognized to maintain training quality, while progressively decreasing the training volume (Bosquet *et al.*, 2007). While high-intensity interval training is likely to be vastly impaired during exercise in the heat, data from the present thesis showed that intermittent, all-out sprint training is not impaired, and similar improvements are made whether exercise is undertaken in a warm or cool environment. The inclusion of a high-intensity session during heat acclimation is uncommon and may

have influenced the findings in chapter 5. While further research is required to identify whether all-out intermittent sprint training had a role in the observed reduction in HBmass during the study, the inclusion of high-intensity exercise in a heat acclimation regime appears to have its own merits. Athletes rarely train solely at low-moderate intensities and research suggests a polarized training approach may be the optimal training distribution for endurance athletes (Seiler and Kjerland 2006). Furthermore, deliberate heat acclimation is a strategy likely to occur in two scenarios: 1) during warm weather training camps where training loads are likely to be increased; and 2) during the taper period prior to competition in the heat, where volume is likely to be reduced but training intensity maintained. Thus, incorporating high-intensity loads into a heat acclimation plan is practically meaningful. While high-intensity interval training is likely to be impaired in the heat, and athletes may have to seek indoor training, or early morning or late night time opportunities to train, data from the present thesis suggests not only that all-out intermittent sprint training can be performed similarly in warm and cool climates, but also that the performance gains over time are similar. Accordingly, this is a strategy which could be utilized over a range of sporting environments to maintain training quality in the heat, avoiding the need to schedule high intensity training either indoors on stationary trainers, or early in the morning and late in the evening to avoid heat exposure.

**Key findings:**

Academic

Utilizing the intermittent training protocol presented in the current thesis (5 x 30s sprint, 4.5min recovery) was insufficient to induce large increases in core temperature, and as such no differences were observed between warm and cool environments. Since no differences were observed, heat acclimation had no added benefit over that of cool training alone.

Practical

Intermittent sprint training can be similarly performed in hot and cool environments, with similar performance improvements regardless of environmental conditions. This type of training could replace high-intensity intervals training during warm-weather camps or specific heat acclimation sessions, as training quality can be maintained without the need to perform high intensity sessions early in the morning or late at night to avoid heat exposure.

### 8.1.4 *Exercise Performance and Cross Acclimation*

The aim of chapter 7 was to examine the impact of heat acclimation on maximal oxygen uptake and cycle time trial performance in a hot, cool, and hypoxic environment. We were able to accept the hypothesis that heat acclimation would improve cycle time trial performance in hot, cool, and hypoxic environments, but only led to increased maximum oxygen uptake in hot environments. Data from chapter 7 revealed that three weeks of heat acclimation led to marked improvements in 20 min time trial performance in hot (13% improvement), cool (4% improvement) and hypoxic (3% improvement) environments (see figure 7.2). A performance enhancement in the cool (Lorenzo *et al.* 2010) and hypoxia (Lee *et al.* 2016) have previously been observed after heat acclimation, although this

remains a highly debated topic in the literature (Minson and Cotter 2016; Nybo and Lundby 2016). This is the first study to have tested performance in all 3 environments as part of the same intervention. Furthermore, given that this is the first study to have matched both absolute and relative training workloads between cool and hot environments, and that trained cyclists with stable performances were employed, the data supports an ergogenic effect of heat acclimation on performance in cool and hypoxic conditions. This was achieved through the use of a work-matched, heart rate based approach to the training stimulus. To match the relative intensity we utilized the heart rate derived from an incremental step test to identify the heart rate at 65% of $\dot{V}O_{2max}$. A 7 bpm correction was factored into the warm training since HR increases approximately 7 bpm for every 1°C rise in body temperature (Jose *et al.* 1970) and was implemented to account for the increased cardiac strain during exercise in the heat to maintain the same relative training intensity between conditions (Périard and Racinais 2015). Rather than set a target training volume each day, exercise completion was dependent on total work done, which was set so that training at the heart rate associated with 65% $\dot{V}O_{2max}$ in the cool condition would take 1 h. Of note, this daily work-load target was increased 5% each week to ensure a progressive training stimulus. This novel approach provides a frame-work for future heat acclimation research to better match the relative and absolute training loads.

**Key findings:**

Academic

Whist we were unable to identify the exact mechanisms, heat acclimation appears to enhance endurance exercise performance in cool and hypoxic environments,

independently of changes in maximum oxygen uptake suggesting that heat acclimation enhances sub-maximal efficiency, although this cannot be confirmed by the current data.

Practical

Heat acclimation should be performed prior to performing in the heat. We utilized a long term (3 week) heat acclimation period, and while adaptations to support exercise performance were observed in the first week, further adaptions were identified in the third week. Therefore long term heat acclimation (3 weeks) should be considered more efficacious than short term heat acclimation (1 – 2 weeks). Adding heat exposures to training might provide a stronger adaptive stimulus to cool training alone, and as such its application should not only be limited to pre-competition phases of training, but could also be added periodically through the training cycle to enhance training adaption in cool environments. Finally, whilst altitude acclimatization is the preferred protocol for preparing for competition at altitude, it is practically difficult and costly to implement. Heat acclimation may provide an alternative option and convey a small but meaningful performance benefit at altitude.

## 8.2 **Limitations**

### 8.2.1 *Sample size*

The sample size of the studies was relatively small (n = 10), and therefore potentially risked the possibility of Type II errors affecting statistical analysis. However, we used trained cyclists with highly consistent performances, in a complex experimental design

which limited the ability to examine greater number of athletes. Future studies should aim to increase the number of participants to confirm the findings from the present thesis

### 8.2.2 *Measurement error*

Sound scientific findings rely on the ability of measurement techniques used to accurately, precisely, and reliably document true changes in the variables being measured. All equipment used in this thesis were calibrated prior to use in line with the manufactures guidelines. Where environmental temperatures could acutely alter the calibration (*i.e.* expired gas measurements, or power meter strain gauges, the equipment was placed into the environment to equilibrate to environmental conditions prior to calibration. The coefficient of variation on the HBmass measure was small (~1.4%) (see Table 5.1) and is under the 2% reported in a meta-analysis of the CO-rebreathing technique (Gore *et al.* 2013), suggesting good reproducibility in our data. The consistency of the CO-rebreathing technique is further confirmed by the stability of the HBmass data during the TEMP training intervention (see Figure 5.1). Given the performance outcomes in the present thesis, it was important that all participants were well-trained and with stable performance. Participant inclusion criteria included training a minimum of 250 km per week for at least 6 weeks prior to the study, and a maximal oxygen uptake of at least 55 ml/kg/min. All participants followed a standardised training regime and were fully familiarised to the performance trials prior to both training interventions. Most notably all participants were highly familiar with the laboratory equipment used in the study and had previously undertaken research projects utilizing cycle time-trial methodology. As such there were no significant differences in baseline performance during the pre-test time-trials of TEMP

and EHA (see Table 7.1), and the changes in performance as a result of EHA are likely to represent true changes rather than variation in performance.

### 8.2.3 *Extrapolation of results*

The results of the present thesis are relevant to trained individuals (*i.e.* $\dot{V}O_{2max}$ ~60 ml.kg.min), but may not be applicable to elite athletes. It is well established that elite athletes, or those with high endurance training loads, already benefit from some of the phenotypical adaptations observed with heat acclimation (hypervolaemia, increased stroke volume and cardiac output). It is therefore likely that the magnitude of benefit from a heat acclimation intervention would have been smaller in elite populations, since some of the underlying physiological adjustments to support exercise performance may have already manifested from endurance training in a cool environment alone.

### 8.2.4 *Blinding of intervention.*

A common limitation in heat acclimation research is the inability to blind participants to whether they are exposed to a warm or cool environment, and participants are easily able to identify which training environment is the intervention which may enhance performance. Interestingly, deception trials have suggested that performance is improved in the heat, when participants are deliberately misinformed about the testing environment. Castle and colleagues (2012) asked participants to perform three, 30 min cycling time-trials in temperate (~22°C, ~43%rh) , and hot/humid conditions (~32°C, ~65%rh). In one of the hot conditions, participants were deceived into thinking the ambient environment was 26.0°C, 60%rh, and their core temperature was 0.3°C lower than it was. While performance was impaired by ~4.5% in the heat, when participants were deceived to the environmental temperature, the decrement was completely ameliorated. Therefore, a

placebo effect cannot be ruled out. Furthermore, during a heat acclimation training intervention, participants may hold pre-existing beliefs such that they may perceive that heat acclimation will lead to a performance improvement, regardless of testing environment (confirmation bias). Whilst the addition of a control intervention may help to identify the effect of training alone, we must consider the inability to blind participants to a warm or cool training environment as a limitation to the research question.

## 8.3 Directions for Future Research

Results from this thesis provide evidence that HBmass is transiently reduced during repeated exposures to warm environments, which has consequences for the accurate determination of blood volumes. One of the major considerations of the present thesis is that HBmass was used to calculate blood volumes, but also that changes in blood volume might influence the measure of HBmass from the CO-rebreathing technique. Further research is required to corroborate these findings utilizing independent measures of blood volumes and HBmass during heat acclimation. This finding has implications for the combined use of heat and altitude stressors. The combined use of hypoxic and heat training may provide an effective acclimation strategies to enhance performance (Buchheit *et al.* 2013). Hypoxic exposure (*e.g.* sleeping) can result in an increased HBmass (Millet *et al.* 2010), while heat acclimation is likely to increase plasma volume (see chapter 4). Thus, combining both stimuli (*i.e.* hypoxic exposure and training in the heat) might provide an additional training stress and afford an improvement in performance at sea level and in temperate environments (Buchheit *et al.*, 2013). However, data from the present thesis questions the suitability of such approaches where their respective adaptive pathways seem to counter-act, rather than augment each other.

Future research should closely examine the regulation of HBmass during such interventions. The rapid return to baseline of HBmass after cessation of heat acclimation raises the possibility that heat acclimation could be used as a 'primer' for altitude training, since the milieu of physiological factors to increase HBmass must be present for such an increase to occur. The time-trial performance improvements after heat acclimation occurred independently of changes in $\dot{V}O_{2max}$, but also improvements in $W_{max}$. While much focus has been on efficiency gains at sub-maximal levels, data from the present thesis suggests that heat acclimation may have enhanced endurance exercise performance through alternative mechanisms. The inclusion of an all-out intermittent sprint training session provides a clear pathway for this, but given that time-trial performance was only improved after EHA, this would suggest the adaption was specific to the sprints undertaken in the heat. Data from the present thesis cannot confirm these observations, and future studies should aim to elucidate the exact mechanisms by which heat acclimation augments exercise performance in alternative environments.

# CHAPTER 9

# REFERENCES


Alfrey CP, Rice L, Udden MM, Driscoll TB (1997) Neocytolysis: physiological down-regulator of red-cell mass. Lancet 349:1389–1390.

Alfrey CP, Udden MM, Leach-Huntoon C, et al. (1996) Control of red blood cell mass in spaceflight. J Appl Physiol 81:98–104.

Almudehki F, Girard O, Grantham J, Racinais S (2012) Hot ambient conditions do not alter intermittent cycling sprint performance. J Sci Med Sport 15:148–152.

Armstrong LE, Maresh CM (1991) The induction and decay of heat acclimatisation in trained athletes. Sports Med 12:302–12.

ASHRAE (1966) Thermal comfort conditions, ASHRAE standard. 55.66, New York.

Assayag M, Saada A, Gerstenblith G, et al. (2012) Mitochondrial performance in heat acclimation—a lesson from ischemia/reperfusion and calcium overload insults in the heart. Am J Physiol Integr Comp Physiol 303:R870–R881.

Backx K, Mc Naughton L, Crickmore L, et al. (2000) Effects of Differing Heat and Humidity on the Performance and Recovery from Multiple High Intensity, Intermittent Exercise Bouts. Int J Sports Med 21:400–405.

Baird NA, Turnbull DW, Johnson EA (2006) Induction of the Heat Shock Pathway during Hypoxia Requires Regulation of Heat Shock Factor by Hypoxia-inducible Factor-1. J Biol Chem 281:38675–38681.

Ball D, Burrows C, Sargeant A. J (1999) Human power output during repeated sprint cycle exercise: The influence of thermal stress. Eur J Appl Physiol Occup Physiol 79:360–366.

Barcroft J, Binger C, Bock A, et al. (1922) The relation of external temperature to blood volume. Phi Trans. R. Soc. London, Ser. B 221:455-464.

Barker SJ (1998) Blood Volume Measurement The Next Intraoperative Monitor? J Am Soc Anesthesiol 89:1310–1312.

Bass D, Buskirk E, Iampietro P, Mager M (1958) Comparison of blood volume during physical conditioning, heat acclimatization and sedentary living. J Appl Physiol 12:186–8.

Bass DE, Kleeman CR, Quinn M, et al. (1955) Mechanisms of acclimatization to heat in man. Medicine 34:323–380.

Bazett HC, Sunderman FW, Doupe J, Scott JC (1940) Climatic Effects on the Volume and COmposition of Blood in Man. Am J Physiol 129:69–83.

Bean, WB. and Eichna, L W (1943) 'Performance in relation to Environmental Temperature. Reactions of Normal Young Men to Simulated Desert Environment.', *Federation Proceedings. Federation of American Societies for Experimental Biology*. Baltimore., 2(3), pp. 144–58.

Bedford T (1936) The Warmth Factor in Comfort at Work. A Physiological Study of Heating and Ventilation. Medical Research Council Industrial Health Research Board, Report 36. London: HMSO.

Belcher JD, Beckman JD, Balla G, et al. (2010) Heme degradation and vascular injury. Antioxid Redox Signal 12:233–48.

Benita Y, Kikuchi H, Smith AD, et al. (2009) An integrative genomics approach identifies Hypoxia Inducible Factor-1 (HIF-1)-target genes that form the core response to hypoxia. Nucleic Acids Res 37:4587–4602.

Bergh U, Ekblom B (1979) Influence of muscle temperature on maximal muscle strength and power output in human skeletal muscles. Acta Physiol Scand 107:33–37.

Birchard G (1997) Optimal Hematocrit: Theory, Regulation and Implications. Integr Comp Biol 37:65–72.

Bishop D, Maxwell NS (2009) Effects of active warm up on thermoregulation and intermittent-sprint performance in hot conditions. J Sci Med Sport 12:196–204.

Bolton CF, Sawa GM, Carter K (1981) The effects of temperature on human compound action potentials. J Neurol Neurosurg Psychiatry 44:407–13.

Bonner RM, Edwards RJ (1976) Effect of heat acclimatization on intravascular responses to acute heat stress in man. J Appl Physiol 41:708-713.

Borg GA (1982) Psychophysical bases of perceived exertion. Med Sci Sports Exerc 14:377–81.

Bosquet L, Monpetit J, Arvisais D, Mujika I (2007) Effects of Tapering on Performance. Med Sci Sport Exerc 39:1358–1365.

Bradley EC, Barr JW (1968) Determination of blood volume using indocyanine green (Cardio-Green®) dye. Life Sci 7:1001–1007.

Broun GO (1922) Blood destruction during exercise: I. Blood changes occuring in the course of a single day of exercise. J Exp Med 36:481–500.

Broun GO (1923) Blood destruction during exercise : II. Demonstration of blood destruction in animals exercised after prolonged confinement. J Exp Med 37:113–

30.

Buchheit M, Racinais S, Bilsborough J, et al. (2013) Adding heat to the live-high train-low altitude model: a practical insight from professional football. Br J Sports Med 47 Suppl 1:i59-69.

Buchheit M, Voss SC, Nybo L, et al. (2011) Physiological and performance adaptations to an in-season soccer camp in the heat: associations with heart rate and heart rate variability. Scand J Med Sci Sports 21:e477-85.

Buono MJ, Ball KD, Kolkhorst FW (2007) Sodium ion concentration vs. sweat rate relationship in humans. J Appl Physiol 103:990–4.

Buono MJ, Heaney JH, Canine KM (1998) Acclimation to humid heat lowers resting core temperature. Am J Physiol 274:R1295-9.

Buono MJ, Numan TR, Claros RM, et al. (2009) Is active sweating during heat acclimation required for improvements in peripheral sweat gland function? Am J Physiol Regul Integr Comp Physiol 297:R1082-5.

Candas V, Libert JP and Vogt JJ. (1979) 'Influence of air velocity and heat acclimation on human skin wettedness and sweating efficiency.', Journal of applied physiology: respiratory, environmental and exercise physiology, 47(6), pp. 1194–200

Casa DJ, Stearns RL, Lopez RM, et al. (2010) Influence of Hydration on Physiological Function and Performance During Trail Running in the Heat. J Athl Train 45:147–156.

Castle P, Mackenzie RW, Maxwell N, et al. (2011) Heat acclimation improves intermittent sprinting in the heat but additional pre-cooling offers no further ergogenic effect. J Sports Sci 29:1125–1134.

Chada KE, Bruce EN (2012) Computational analyses of CO-rebreathing methods for estimating haemoglobin mass in humans. Exp Physiol 97:141–154.

Chalmers S, Esterman A, Eston R, et al. (2014) Short-Term Heat Acclimation Training Improves Physical Performance: A Systematic Review, and Exploration of Physiological Adaptations and Application for Team Sports. Sport Med 44:971–988.

Cheung SS, Sleivert GG (2004) Lowering of skin temperature decreases isokinetic maximal force production independent of core temperature. Eur J Appl Physiol 91:723–728.

Chinevere TD, Kenefick RW, Cheuvront SN, et al. (2008) Effect of heat acclimation on sweat minerals. Med Sci Sports Exerc 40:886–91.

Cipryan L, Tschakert G, Hofmann P (2017) Acute and Post-Exercise Physiological Responses to High-Intensity Interval Training in Endurance and Sprint Athletes. J Sports Sci Med 16:219–229.

Close R, Hoh JF (1968) Influence of temperature on isometric contractions of rat skeletal muscles. Nature 217:1179–80.

Cocking S, Wilson MG, Nichols D, et al (2018) Is There an Optimal Ischemic-Preconditioning Dose to Improve Cycling Performance? Int J Sports Physiol Perform 13:274–282

Colin J, Timbal J, Houdas Y, et al. (1971) Computation of mean body temperature from rectal and skin temperatures. J Appl Physiol 31:484–489.

Convertino VA (1991a) Blood volume: its adaptation to endurance training. Med Sci Sports Exerc 23:1338–48.

Convertino VA (1987) Fluid shifts and hydration state: effects of long-term exercise. Can J Sport Sci 12:136S–139S.

Convertino VA (1991b) Blood volume: its adaptation to endurance training. Med. Sci. Sports Exerc. 23:1338–48.

Corbett J, Neal RA, Lunt HC, Tipton MJ (2014) Adaptation to Heat and Exercise Performance Under Cooler Conditions: A New Hot Topic. Sport Med 44:1323–1331.

Coyle EF, González-Alonso J (2001) Cardiovascular drift during prolonged exercise: new perspectives. Exerc Sport Sci Rev 29:88–92.

Coyle EF, Hopper MK, Coggan a R (1990) Maximal oxygen uptake relative to plasma volume expansion. Int J Sports Med 11:116–9.

Crandall CG, Wilson TE, Marving J, et al. (2008) Effects of passive heating on central blood volume and ventricular dimensions in humans. J Physiol 586:293–301.

Daanen HAM, Racinais S, Périard JD (2018) Heat Acclimation Decay and Re-Induction: A Systematic Review and Meta-Analysis. Sport Med 48:409–430.

Damsgaard R, Munch T, Mørkeberg J, et al. (2006) Effects of blood withdrawal and reinfusion on biomarkers of erythropoiesis in humans: Implications for anti-doping strategies. Haematologica 91:1006–8.

De Ruiter CJ, De Haan A (2000) Temperature effect on the force/velocity relationship of the fresh and fatigued human adductor pollicis muscle. Pflugers Arch 440:163–70.

DeLorey DS, Kowalchuk JM, Paterson DH (2005) Adaptation of pulmonary O2 uptake kinetics and muscle deoxygenation at the onset of heavy-intensity exercise in young and older adults. J Appl Physiol 98:1697–704.

Deschamps A, Magder S (1990) Skin vascular bed is a potential blood reservoir during heat stress. Am J Physiol Hear Circ Physiol 259:H1796-1802.

Dill DB, Costill DL (1974) Calculation of percentage changes in volumes of blood , plasma , and red cells in dehydration. 37:247–248.

Dotan R, Bar-Or O (1980) Climatic heat stress and performance in the Wingate Anaerobic Test. Eur J Appl Physiol Occup Physiol 44:237–243.

Douglas CG, Haldane JS, Haldane JB (1912) The laws of combination of haemoglobin with carbon monoxide and oxygen. J Physiol 44:275–304.

Dresoti (1935) 'The results of some investigations into the medical aspects of deep mining on the Witwatersrand', J Chem Metall Min Soc S Afr, 6, pp. 102–129.

Drust B, Rasmussen P, Mohr M, et al. (2005) Elevations in Core and Muscle Temp Impairs Repeated Sprint Performance. Acta Physiol Scand 183:181–190.

Dunn A, Donnelly S, Donnelly S (2007) The Role of the Kidney in Blood Volume Regulation: The Kidney as a Regulator of the Hematocrit. Am J Med Sci 334:65–7.

Ebbeling CB, Clarkson PM (1989) Exercise-Induced Muscle Damage and Adaptation. Sport Med 7:207–234.

Eichna LW et al. (1945) 'Performance in relation to environmental temperature. Reactions of normal young men to hot, humid (simulated jungle) environment.', Bulletin of the Johns Hopkins Hospital, 76, pp. 25–58.

Ekblom B, Wilson G, Astrand PO (1976) Central circulation during exercise after venesection and reinfusion of red blood cells. J Appl Physiol 40:379–83.

El-Sayed H, Goodall SR, Hainsworth R (1995) Re-evaluation of Evans blue dye dilution method of plasma volume measurement. Clin Lab Haematol 17:189–94.

Ely BR, Ely MR, Cheuvront SN, et al. (2009) Evidence against a 40 degrees C core temperature threshold for fatigue in humans. J Appl Physiol 107:1519–25.

Ely BR, Lovering AT, Horowitz M, Minson CT (2014) Heat acclimation and cross tolerance to hypoxia. Temperature 1:107–114.

Ely MR, Cheuvront SN, Roberts WO, Montain SJ (2007) Impact of weather on marathon-running performance. Med Sci Sports Exerc 39:487–93.

Fahraeus R (1921) The suspension stability of blood. Acta Med Scand 1:55.

Falk B, Radom-Isaac S, Hoffmann J, et al. (1998) The Effect of Heat Exposure on Performance of and Recovery from High-Intensity, Intermittent Exercise. Int J Sports Med 19:1–6.

Fan FC, Chen RY, Schuessler GB, Chien S (1980) Effects of hematocrit variations on regional hemodynamics and oxygen transport in the dog. Am J Physiol 238:H545-22.

Farina D, Arendt-Nielsen L, Graven-Nielsen T (2005) Effect of temperature on spike-triggered average torque and electrophysiological properties of low-threshold motor units. J Appl Physiol 99:197–203.

Faulkner SH, Ferguson RA, Gerrett N, et al. (2013) Reducing muscle temperature drop after warm-up improves sprint cycling performance. Med Sci Sports Exerc 45:359–365.

Febbraio MA, Carey MF, Snow RJ, et al. (1996a) Influence of elevated muscle temperature on metabolism during intense, dynamic exercise. Am J Physiol Integr Comp Physiol 271:R1251–R1255.

Febbraio MA, Murton P, Selig SE, et al. (1996b) Effect of CHO ingestion on exercise metabolism and performance in different ambient temperatures. Med Sci Sports Exerc 28:1380–7.

Febbraio MA, Snow RJ, Stathis CG, et al. (1994) Effect of heat stress on muscle energy metabolism during exercise. J Appl Physiol 77:2827–31.

Fehrenbach E, Passek F, Niess AM, et al. (2000) HSP expression in human leukocytes is modulated by endurance exercise. Med Sci Sports Exerc 32:592–600.

Fellmann N (1992) Hormonal and plasma volume alterations following endurance exercise. A brief review. Sports Med 13:37–49.

Finch CA, Harker LA, Cook JD (1977) Kinetics of the formed elements of human blood. Blood 50:699–707.

Fortney SM, Nadel ER, Wenger CB, Bove JR (1981a) Effect of acute alterations of blood volume on circulatory performance in humans. J Appl Physiol 50:292–298.

Fortney SM, Nadel ER, Wenger CB, Bove JR (1981b) Effect of blood volume on sweating rate and body fluids in exercising humans. J Appl Physiol 51:1594–1600.

Fox B, Goldsmith R, Kidd D, Lewis H (1963) Acclimatization to heat in man by controlled elevation of body temperature. J Physiol 166:530–547.

Fox RH, Goldsmith R, Hampton IF, Hunt TJ (1967) Heat acclimatization by controlled hyperthermia in hot-dry and hot-wet climates. J Appl Physiol 22:39–46.

Franco RS (2009) The measurement and importance of red cell survival. Am J Hematol 84:109–114.

Frank A, Belokopytov M, Shapiro Y, Epstein Y (2001) The cumulative heat strain index - a novel approach to assess the physiological strain induced by exercise-heat stress. Eur J Appl Physiol 84:527–532.

Fregly MJ, Fregly, J. M (2011) Adaptations: Some General Characteristics. In: Comprehensive Physiology. John Wiley & Sons, Inc., Hoboken, NJ, USA.

Frye AJ, Kamon E (1983) Sweating efficiency in acclimated men and women exercising in humid and dry heat. J Appl Physiol 54:972–977.

G. Morris J, Nevill ME, Lakomy HKA, et al. (1998) Effect of a hot environment on performance of prolonged, intermittent, high-intensity shuttle running. J Sports Sci

16:677–686.

Galloway SD, Maughan RJ (1997) Effects of ambient temperature on the capacity to perform prolonged cycle exercise in man. Med Sci Sports Exerc 29:1240–9.

Garrett AT, Goosens NG, Rehrer NJ, et al. (2009) Induction and decay of short-term heat acclimation. Eur J Appl Physiol 107:659–70.

Garvican LA, Burge CM, Cox AJ, et al. (2010) Carbon monoxide uptake kinetics of arterial, venous and capillary blood during CO rebreathing. Exp Physiol 95:1156–1166.

Garvican LA, Pottgiesser T, Martin DT, et al. (2011) The contribution of haemoglobin mass to increases in cycling performance induced by simulated LHTL. Eur J Appl Physiol 111:1089–1101.

Garvican L, Martin D, Quod M, et al. (2012) Time course of the hemoglobin mass response to natural altitude training in elite endurance cyclists. Scand J Med Sci Sport 22:95–103.

Gibson JG, Evans WA (1937) Clinical studies of the blood volume. ii. the relation of plasma and total blood volume to venous pressure, blood velocity rate, physical measurements, age and sex in ninety normal humans. J Clin Invest 16:317–28.

Gibson OR, Mee J, Tuttle J, et al. (2015a) Isothermic and fixed intensity heat acclimation methods induce similar heat adaptation following short and long-term timescales. J Therm Biol 49–50:55–65.

Gibson OR, Taylor L, Watt PW, Maxwell NS (2017) Cross-Adaptation: Heat and Cold Adaptation to Improve Physiological and Cellular Responses to Hypoxia. Sport Med 47:1751–176.

Gibson OR, Turner G, Tuttle JA, et al. (2015b) Heat acclimation attenuates physiological strain and the HSP72, but not HSP90α, mRNA response to acute normobaric hypoxia. J Appl Physiol 119:889–899.

Girard O, Bishop DJ, Racinais S (2013) Hot conditions improve power output during repeated cycling sprints without modifying neuromuscular fatigue characteristics. Eur J Appl Physiol 113:359–369.

Girard O, Brocherie F, Bishop DJ (2015) Sprint performance under heat stress: A review. Scand J Med Sci Sport 25:79–89.

Givoni B, Goldman RF (1972) Predicting rectal temperature response to work, environment, and clothing. J Appl Physiol 32:812–822.

Glaser E (1949) Acclimatization to heat and cold. J Physiol 110:330–7.

Gómez Perales JL (2015) Blood volume analysis by radioisotopic dilution techniques: state of the art. Appl Radiat Isot 96:71–82.

González-Alonso J, Crandall CG, Johnson JM (2008) The cardiovascular challenge of exercising in the heat. J Physiol 586:45–53.

Gonzalez-Alonso J, Mora-Rodriguez R, Coyle EF (2000) Stroke volume during exercise: interaction of environment and hydration. Am J Physiol Hear Circ Physiol 278:H321-330.

González-Alonso J, Teller C, Andersen SL, et al. (1999) Influence of body temperature on the development of fatigue during prolonged exercise in the heat. J Appl Physiol 86:1032–9.

Gorelov V (2004) Theoretical value of Hüfner's constant. Anaesthesia 59:97–97.

Goto K, Oda H, Kondo H, et al. (2011) Responses of muscle mass, strength and gene transcripts to long-term heat stress in healthy human subjects. Eur J Appl Physiol 111:17–27.

Gough C, Eastwood A, Saunders P, et al. (2012) Spurious Hb Mass Increases Following Exercise. Int J Sports Med 33:691–695.

Gray SR, De Vito G, Nimmo MA, et al. (2006a) Skeletal muscle ATP turnover and muscle fiber conduction velocity are elevated at higher muscle temperatures during maximal power output development in humans. Am J Physiol Integr Comp Physiol 290:R376–R382.

Gray SR, De Vito G, Nimmo MA, et al. (2006b) Skeletal muscle ATP turnover and muscle fiber conduction velocity are elevated at higher muscle temperatures during maximal power output development in humans. Am J Physiol Integr Comp Physiol 290:R376–R382.

Greenleaf JE, Looft-Wilson R, Wisherd JL, et al. (1997) Pre-exercise hypervolemia and cycle ergometer endurance in men. Biol Sport 14:103–14.

Haldane J, Smith JL (1900) The mass and oxygen capacity of the blood in man. J Physiol 25:331–343.

Haneda K, Horiuchi T (1986) A method for measurement of total circulating blood volume using indocyanine green. Tohoku J Exp Med 148:49–56.

Harrison H (1985) Effects of Thermal Stress and Exercise on Blood Volume in Humans. Physiol Rev 65:149-209.

Harrison MH, Edwards RJ, Cochrane LA, Graveney MJ (1983) Blood volume and protein responses to skin heating and cooling in resting subjects. J Appl Physiol 54:515–23.

Harrison MH, Edwards RJ, Graveney MJ, et al. (1981) Blood volume and plasma protein responses to heat acclimatization in humans. J Appl Physiol 50:597–604.

Hautier CA, Arsac LM, Deghdegh K, et al. (2000) Influence of fatigue on EMG/force ratio and cocontraction in cycling. Med Sci Sports Exerc 32:839–843.

Heled Y, Peled A, Yanovich R, et al. (2012) Heat acclimation and performance in hypoxic conditions. Aviat Sp Environ Med 83:649–653.

Helyar R, Green H, Zappe D, Sutton J (1996) Comparative effects of acute volume expansion and short-term training on thermal and cardiovascular responses to prolonged exercise. Can J Physiol Pharmacol 74:1087–94.

Henschen S, Busse MW, Zisowsky S, Panning B (1993) Determination of plasma volume and total blood volume using indocyanine green: a short review. J Med 24:10–27.

Hiestand WA, Stemler FW, Jasper RL (1955) Increased Anoxic Resistance Resulting from Short Period Heat Adaptation. Exp Biol Med 88:94–95.

Hiro T (1982) Studies on the Osmotic Fragility of Erythrocytes Influenced by a Metabolic Acidosis. Japanese J Phys Fit Sport Med 31:279–290.

Hom LL, Lee EC-H, Apicella JM, et al. (2012) Eleven days of moderate exercise and heat exposure induces acclimation without significant HSP70 and apoptosis responses of lymphocytes in college-aged males. Cell Stress Chaperones 17:29–39.

Horowitz M (2002) From molecular and cellular to integrative heat defense during exposure to chronic heat. Comp Biochem Physiol Part A Mol Integr Physiol 131:475–483.

Horowitz M (2003) Matching the heart to heat-induced circulatory load: heat-acclimatory responses. News Physiol Sci 18:215–21.

Horowitz M, Parnes S, Hasin Y Mechanical and metabolic performance of the rat heart: effects of combined stress of heat acclimation and swimming training. J Basic Clin Physiol Pharmacol 4:139–56.

Horowitz M, Peyser YM, Muhlrad A (1986a) Alterations in cardiac myosin isoenzymes distribution as an adaptation to chronic environmental heat stress in the rat. J Mol Cell Cardiol 18:511–5.

Horowitz M, Shimoni Y, Parnes S, et al. (1986b) Heat acclimation: cardiac performance of isolated rat heart. J Appl Physiol 60:9–13.

Houmard JA, Costill DL, Davis JA, et al. (1990) The influence of exercise intensity on heat acclimation in trained subjects. Med Sci Sports Exerc 22:615–20.

Hsu C-Y, Bates DW, Kuperman GJ, Curhan GC (2001) Relationship between hematocrit and renal function in men and women. Kidney Int 59:725–731.

Hue O, Antoine-Jonville S, Sara F (2007) The effect of 8 days of training in tropical environment on performance in neutral climate in swimmers. Int J Sports Med 28:48–52.

Hutter MM, Sievers RE, Barbosa V, Wolfe CL (1994) Heat-shock protein induction in rat hearts. A direct correlation between the amount of heat-shock protein induced and

the degree of myocardial protection. Circulation 89:355–60.

Iaia FM, Thomassen M, Kolding H, et al. (2008) Reduced volume but increased training intensity elevates muscle $Na^+$-$K^+$ pump $\alpha_1$-subunit and NHE1 expression as well as short-term work capacity in humans. Am J Physiol Integr Comp Physiol 294:R966–R974.

Inoue Y, Nakao M, Ishizashi H, et al. (1998) Regional differences in the $Na^+$ reabsorption of sweat glands. Appl Human Sci 17:219–21.

Jelkmann W (1992) Erythropoietin: structure, control of production, and function. Physiol Rev 72:449–489.

Jentjens RLPG, Wagenmakers AJM, Jeukendrup AE (2002) Heat stress increases muscle glycogen use but reduces the oxidation of ingested carbohydrates during exercise. J Appl Physiol 92:1562–1572.

Jewell UR, Kvietikova I, Scheid A, et al. (2001) Induction of HIF-1alpha in response to hypoxia is instantaneous. FASEB J 15:1312–4.

Johnson JM, Kellogg DL (2010) Thermoregulatory and thermal control in the human cutaneous circulation. Front Biosci (Schol Ed) 2:825–53.

Jones S, D'Andrea A, Haines L, Wong G (1990) Human erythropoietin receptor: cloning, expression, and biologic characterization. Blood 76:31-35.

Jose AD, Stitt F, Collison D (1970) The effects of exercise and changes in body temperature on the intrinsic heart rate in man. Am Heart J 79:488–98.

Kampmann B, Bröde P, Schütte M, Griefahn B (2008) Lowering of resting core temperature during acclimation is influenced by exercise stimulus. Eur J Appl Physiol 104:321–7.

Kanstrup IL, Ekblom B (1982) Acute hypervolemia, cardiac performance, and aerobic power during exercise. J Appl Physiol 52:1186–1191.

Karatzaferi C, Chinn MK, Cooke R (2004) The Force Exerted by a Muscle Cross-Bridge Depends Directly on the Strength of the Actomyosin Bond. Biophys J 87:2532–2544.

Kargotich S, Goodman C, Keast D, Morton AR (1998) The influence of exercise-induced plasma volume changes on the interpretation of biochemical parameters used for monitoring exercise, training and sport. Sport Med 26:101–117.

Karlsen A, Nybo L, Nyrgaard SJ, et al. (2015a) Time course of natural heat acclimatization in well-trained cyclists during a 2-week training camp in the heat. Scand J Med Sci Sport 25:240–249

Karlsen A, Racinais S, Jensen M V., et al. (2015b) Heat acclimatization does not improve $VO_{2max}$ or cycling performance in a cool climate in trained cyclists. Scand J Med Sci Sports 25:269–276.

Keiser S, Flück D, Hüppin F, et al. (2015) Heat training increases exercise capacity in hot but not in temperate conditions: a mechanistic counter-balanced cross-over study. Am J Physiol Heart Circ Physiol. 309:H750-761.

Kirby CR, Convertino VA (1986) Plasma aldosterone and sweat sodium concentrations after exercise and heat acclimation. J Appl Physiol 61:967–970.

Kobayashi Y, Ando Y, Takeuchi S, et al. (1980) Effects of heat acclimation of distance runners in a moderately hot environment. Eur J Appl Physiol Occup Physiol. 45:189–198.

Kodesh E, Horowitz M (2010) Soleus adaptation to combined exercise and heat acclimation: physiogenomic aspects. Med Sci Sports Exerc 42:943–52.

Koury MJ, Bondurant MC (1988) Maintenance by erythropoietin of viability and maturation of murine erythroid precursor cells. J Cell Physiol 137:65–74.

Kresfelder TL, Claassen N, Cronjé MJ (2006) Hsp70 Induction and hsp70 Gene polymorphisms as Indicators of acclimatization under hyperthermic conditions. J Therm Biol 31:406–415.

Kuennen M, Gillum T, Dokladny K, et al. (2011) Thermotolerance and heat acclimation may share a common mechanism in humans. Am J Physiol Integr Comp Physiol 301:R524–R533.

Lacombe C, Da Silva JL, Bruneval P, et al. (1988) Peritubular cells are the site of erythropoietin synthesis in the murine hypoxic kidney. J Clin Invest 81:620–623.

Länne T, Lundvall J (1992) Mechanisms in man for rapid refill of the circulatory system in hypovolaemia. Acta Physiol Scand 146:299–306.

Laursen PB, Shing CM, Peake JM, et al. (2002) Interval training program optimization in highly trained endurance cyclists. Med Sci Sports Exerc 34:1801–7.

Lee BJ, Emery-Sinclair EL, Mackenzie RW, et al. (2014) The impact of submaximal exercise during heat and/or hypoxia on the cardiovascular and monocyte HSP72 responses to subsequent (post 24 h) exercise in hypoxia. Extrem Physiol Med 3:15.

Lee BJ, Mackenzie RWA, Cox V, et al. (2015) Human monocyte heat shock protein 72 responses to acute hypoxic exercise after 3 days of exercise heat acclimation. Biomed Res Int 2015:849809.

Lee BJ, Miller A, James RS, Thake CD (2016) Cross acclimation between heat and hypoxia: Heat acclimation improves cellular tolerance and exercise performance in acute normobaric hypoxia. Front Physiol 7:1–15.

Levi E, Vivi A, Hasin Y, et al. (1993) Heat acclimation improves cardiac mechanics and metabolic performance during ischemia and reperfusion. J Appl Physiol 75:833–9.

Levick JR (1991) Capillary filtration-absorption balance reconsidered in light of dynamic

extravascular factors. Exp Physiol 76:825–57.

Lind AR (1964). Physiologic responses to heat. In: Licht S, ed. Medical climatology. Baltimore, MD: Waverly Press. pp. 164–195.

Linnane DM, Bracken RM, Brooks S, et al. (2004) Effects of hyperthermia on the metabolic responses to repeated high-intensity exercise. Eur J Appl Physiol 93:159–166.

Lorenzo S, Halliwill JR, Sawka MN, Minson CT (2010) Heat acclimation improves exercise performance. J Appl Physiol 109:1140–7.

Lorenzo S, Minson CT, Babb TG, Halliwill JR (2011) Lactate threshold predicting time-trial performance: impact of heat and acclimation. J Appl Physiol 111:221–7.

Mabjeesh NJ, Post DE, Willard MT, et al. (2002) Geldanamycin induces degradation of hypoxia-inducible factor 1alpha protein via the proteosome pathway in prostate cancer cells. Cancer Res 62:2478–82.

Macintosh BR, Neptune RR, Horton JF (2000) Cadence, power, and muscle activation in cycle ergometry. Med Sci Sport Exerc 32:1281–1287.

Magalhães FDC, Amorim FT, Passos RLF, et al. (2010) Heat and exercise acclimation increases intracellular levels of Hsp72 and inhibits exercise-induced increase in intracellular and plasma Hsp72 in humans. Cell Stress Chaperones 15:885–95.

Mairbäurl H (2013) Red blood cells in sports: effects of exercise and training on oxygen supply by red blood cells. Front Physiol 4:332.

Maloyan A, Eli-Berchoer L, Semenza GL, et al. (2005) HIF-1α-targeted pathways are activated by heat acclimation and contribute to acclimation-ischemic cross-tolerance in the heart. Physiol Genomics 23:79–88.

Maloyan A, Palmon A, Horowitz M (1999) Heat acclimation increases the basal HSP72 level and alters its production dynamics during heat stress. Am J Physiol 276:R1506-15.

Marsh SA, Jenkins DG (2002) Physiological Responses to the Menstrual Cycle. Sport Med 32:601–614.

Marshall HC, Ferguson R a., Nimmo M a. (2006a) Human resting extracellular heat shock protein 72 concentration decreases during the initial adaptation to exercise in a hot, humid environment. Cell Stress Chaperones 11:129.

Martin D, Watts P, Newbury V (1992) The Effect of Alpine Mountaineering on Acute Erythrocyte Hemolysis. Int J Sports Med 13:31–35.

Matsushita K, Homma S, Okada E (1998) Influence of adipose tissue on muscle oxygenation measurement with an NIRS instrument. In: Benaron DA, Chance B, Ferrari M (eds). International Society for Optics and Photonics, pp 159–165.

Maughan RJ, Shirreffs SM, Watson P (2007) Exercise, heat, hydration and the brain. J Am Coll Nutr 26:604S–612S.

Mccleave E, Slattery K, Duffield R, et al. (2017) Temperate Performance Benefits after Heat, but Not Combined Heat and Hypoxic Training. Med Sci Sport Exerc 49:509–517.

McClung JP, Hasday JD, He J, et al. (2008) Exercise-heat acclimation in humans alters baseline levels and ex vivo heat inducibility of HSP72 and HSP90 in peripheral blood mononuclear cells. Am J Physiol Regul Integr Comp Physiol 294:R185-9.

Meek WJ, Gasser HS (1918) Blood volume: A Method for its Determination with Data for Dogs, Cats and Rabbits. Am J Physiol. 47:302–317.

Miller B, Pate R, Burgess W (1988) Foot Impact Force and Intravascular Hemolysis During Distance Running. Int J Sports Med 9:56–60.

Millet GP, Roels B, Schmitt L, et al. (2010) Combining Hypoxic Methods for Peak Performance. Sport Med 40:1–25.

Minson CT, Cotter JD (2016) CrossTalk proposal: Heat acclimatization does improve performance in a cool condition. J Physiol 594:241–243.

Mitchell D, Senay LC, Wyndham CH, et al. (1976) Acclimatization in a hot, humid environment: energy exchange, body temperature, and sweating. J Appl Physiol 40:768–778.

Moloney TC, Hoban DB, Barry FP, et al. (2012) Kinetics of thermally induced heat shock protein 27 and 70 expression by bone marrow-derived mesenchymal stem cells. Protein Sci. 21:904-909.

Morimoto RI (1998) Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. Genes Dev 12:3788–96.

Morris JG, Nevill ME, Boobis LH, et al. (2005) Muscle Metabolism, Temperature, and Function During Prolonged, Intermittent, High-Intensity Running in Air Temperatures of 33 ° and 17 °C. Int J Sports Med 26:805–814.

Morris JG, Nevill ME, Williams C (2000) Physiological and metabolic responses of female games and endurance athletes to prolonged, intermittent, high-intensity running at 30° and 16°C ambient temperatures. Eur J Appl Physiol Occup Physiol 81:84–92.

Morrison S, Sleivert GG, Cheung SS (2004) Passive hyperthermia reduces voluntary activation and isometric force production. Eur J Appl Physiol 91:729–736.

Neal RA, Corbett J, Massey HC, Tipton MJ (2016) Effect of short-term heat acclimation with permissive dehydration on thermoregulation and temperate exercise performance. Scand J Med Sci Sport 26:875–884.

Nelson MD, Stuart-Hill LA, Sleivert GG (2008) Hypervolemia and blood alkalinity: effect on physiological strain in a warm environment. Int J Sports Physiol Perform 3:501–15.

Nielsen B (1998) Heat Acclimation - Mechanisms of Adaptation to Exercise in the Heat. Int J Sport Med 19:S154–S156.

Nielsen B, Hyldig T, Bidstrup F, et al. (2001) Brain activity and fatigue during prolonged exercise in the heat. Pflügers Arch 442:41–48.

Nielsen B, Strange S, Christensen NJ, et al. (1997) Acute and adaptive responses in humans to exercise in a warm, humid environment. Pflugers Arch 434:49–56.

Nielsen BYB, Hales JRS, Strange S, Christensen NJ, Warberg J, Saltin ,B (1993) Human circulatory and thermoregulatory adaptations with heat acclimation and exercise in a hot, dry environment. J Physiol. 460:467–485.

Nielsen M (1938) Die Regulation der Korpertemperatur bei Muskelarbeit. Skand Arch Physiol 79:193–230.

Nikolac N, Krleza JL, Simundic AM (2017) Preanalytical external quality assessment of the Croatian Society of Medical Biochemistry and Laboratory Medicine and CROQALM: finding undetected weak spots. Biochem medica 27:131–143.

Nose H, Mack GW, Shi XR, et al. (1990) Effect of saline infusion during exercise on thermal and circulatory regulations. J Appl Physiol 69:609–616.

Nybo L (2010a) Cycling in the heat: performance perspectives and cerebral challenges. Scand J Med Sci Sports 20:71–79.

Nybo L (2010b) CNS fatigue provoked by prolonged exercise in the heat. Front Biosci (Elite Ed) 2:779–92.

Nybo L, Lundby C (2016) CrossTalk opposing view: Heat acclimatization does not improve exercise performance in a cool condition. J Physiol 594:245–247.

Nybo L, Nielsen B (2001a) Hyperthermia and central fatigue during prolonged exercise in humans. J Appl Physiol 91:1055–1060.

Nybo L, Nielsen B (2001b) Perceived exertion is associated with an altered brain activity during exercise with progressive hyperthermia. J Appl Physiol 91:2017–2023.

Nybo L, Rasmussen P, Sawka MN (2014) Performance in the heat-physiological factors of importance for hyperthermia-induced fatigue. Compr Physiol 4:657–689.

Pandolf K (1998) Time Course of Heat Acclimation and its Decay. Int J Sports Med 19:S157–S160.

Pandolf K, Young A (1992) Environmental extremes and endurance performance. In: Astrand PO, Shephard RJ (eds) Endurance in sport. Blackwell Scientific Publications, Oxford, pp 287–300.

Pandolf KB, Burse RL, Goldman RF (1977) Role of Physical Fitness in Heat Acclimatisation , Decay and Reinduction. Ergonomics. 20(4):399–408.

Parkin JM, Carey MF, Zhao S, Febbraio MA (1999) Effect of ambient temperature on human skeletal muscle metabolism during fatiguing submaximal exercise. J Appl Physiol 86:902–908.

Patterson MJ, Stocks JM, Taylor NAS (2014) Whole-body fluid distribution in humans during dehydration and recovery, before and after humid-heat acclimation induced using controlled hyperthermia. Acta Physiol (Oxf) 210:899–912.

Patterson MJ, Stocks JM, Taylor NAS (2004) Sustained and generalized extracellular fluid expansion following heat acclimation. J Physiol 559:327–34.

Périard JD, Racinais S (2015) Self-paced exercise in hot and cool conditions is associated with the maintenance of %V̇ o $_{2peak}$ within a narrow range. J Appl Physiol 118:1258–1265.

Périard JD, Racinais S, Sawka MN (2015) Adaptations and mechanisms of human heat acclimation: Applications for competitive athletes and sports. Scand J Med Sci Sports 25 Suppl 1:20–38.

Petersen CJ, Portus MR, Pyne DB, et al. (2010) Partial heat acclimation in cricketers using a 4-day high intensity cycling protocol. Int J Sports Physiol Perform 5:535–45.

Ponce LL, Navarro JC, Ahmed O, Robertson CS (2013) Erythropoietin neuroprotection with traumatic brain injury. Pathophysiology 20:31–38.

Pottgiesser T, Specker W, Umhau M, et al. (2008) Recovery of hemoglobin mass after blood donation. Transfusion 48:1390–1397.

Prommer N, Schmidt W (2007) Loss of CO from the intravascular bed and its impact on the optimised CO-rebreathing method. Eur J Appl Physiol 100:383–391.

Racinais S, Alonso J-M, Coutts AJ, et al. (2015a) Consensus Recommendations on Training and Competing in the Heat. Sport Med 45:925–938.

Racinais S, Bishop D, Denis R, et al. (2007) Muscle Deoxygenation and Neural Drive to the Muscle during Repeated Sprint Cycling. Med Sci Sport Exerc 39:268–274.

Racinais S, Buchheit M, Bilsborough J, et al. (2014a) Physiological and Performance Responses to a Training Camp in the Heat in Professional Australian Football Players. Int J Sports Physiol Perform 9:598–603.

Racinais S, Mohr M, Buchheit M, et al. (2012) Individual responses to short-term heat acclimatisation as predictors of football performance in a hot, dry environment. Br J Sports Med 46:810–815.

Racinais S, Périard JD, Karlsen A, Nybo L (2014b) Effect of Heat and Heat-Acclimatization on Cycling Time-Trial Performance and Pacing. Med Sci Sports

Exerc 47:606-606.

Racinais S, Wilson MG, Gaoua N, Périard JD (2017a) Heat acclimation has a protective effect on the central but not peripheral nervous system. J Appl Physiol jap.00430.2017.

Racinais S, Wilson MG, Périard JD (2017b) Passive heat acclimation improves skeletal muscle contractility in humans. Am J Physiol - Regul Integr Comp Physiol 312:R101–R107.

Ramanathan NL (1964) A new weighting system for mean surface temperature of the human body. J Appl Physiol 19:531–3.

Recommended methods for measurement of red-cell and plasma volume: International Committee for Standardization in Haematology (1980) J Nucl Med 21:793–800.

Reljic D, Feist J, Jost J, et al. (2016) Rapid body mass loss affects erythropoiesis and hemolysis but does not impair aerobic performance in combat athletes. Scand J Med Sci Sports 26:507–517.

Reljic D, Hässler E, Jost J, Friedmann-Bette B (2013) Rapid Weight Loss and the Body Fluid Balance and Hemoglobin Mass of Elite Amateur Boxers. J Athl Train 48:109–117.

Rendell MS, Kelly ST, Bamisedun O, et al. (1993) The effect of increasing temperature on skin blood flow and red cell deformability. Clin Physiol 13:235–45.

Rendell RA, Prout J, Costello J, et al. (2017) The effects of 10 days of separate heat and hypoxic exposure on heat acclimation and temperate exercise performance. Am J Physiol - Regul Integr Comp Physiol 313(3):R191-R201.

Rice L, Alfrey C (2005) The Negative Regulation of Red Cell Mass by Neocytolysis: Physiologic and Pathophysiologic Manifestations. Cell Physiol Biochem 15:245–250.

Rice L, Ruiz W, Driscoll T, et al. (2001) Neocytolysis on descent from altitude: a newly recognized mechanism for the control of red cell mass. Ann Intern Med 134:652–6.

Risso A, Ciana A, Achilli C, et al. (2014) Neocytolysis: none, one or many? A reappraisal and future perspectives. Front Physiol 5:54.

Robinson N, Giraud S, Saudan C, et al. (2006) Erythropoietin and blood doping. Br J Sports Med 40 Suppl 1:i30-4.

Roelands B, Hasegawa H, Watson P, et al. (2008) The effects of acute dopamine reuptake inhibition on performance. Med Sci Sports Exerc 40:879–85.

Rowell LB (1974) Human cardiovascular adjustments to exercise and thermal stress. Physiol Rev 54:75–159.

Ryan BJ, Goodrich JA, Schmidt WF, et al. (2016) Haemoglobin mass alterations in healthy humans following four-day head-down tilt bed rest. Exp Physiol 101:628–

640.

Ryan BJ, Wachsmuth NB, Schmidt WF, et al. (2014) Altitudeomics: Rapid hemoglobin mass alterations with early acclimatization to and de-acclimatization from 5260 m in healthy humans. PLoS One 9 (10): e108788.

Saat M, Sirisinghe RG, Singh R, Tochihara Y (2005) Decay of heat acclimation during exercise in cold and exposure to cold environment. Eur J Appl Physiol 95:313–320.

Salgado RM, White AC, Schneider SM, Mermier CM (2014) A Novel Mechanism for Cross-Adaptation between Heat and Altitude Acclimation: The Role of Heat Shock Protein 90. Physiol J 2014:1–12.

Sargeant A, Rademaker A (1996) Human muscle power in the locomotory range of contraction velocities increases with temperature due to an increase in power generated by type I fibres. J Physiol 128–129.

Sargeant AJ (1987) Effect of muscle temperature on leg extension force and short-term power output in humans. Eur J Appl Physiol Occup Physiol 56:693–698.

Sawka MN, Convertino VA, Eichner ER, et al. (2000) Blood volume: importance and adaptations to exercise training, environmental stresses, and trauma/sickness. Med Sci Sports Exerc 32:332–48.

Sawka MN, Coyle EF (1999) Influence of body water and blood volume on thermoregulation and exercise performance in thae heat. Exerc Sport Sci Rev 27:167–218.

Sawka MN, Hubbard RW, Francesconi RP, Horstman DH (1983a) Effects of acute plasma volume expansion on altering exercise-heat performance. Eur J Appl Physiol Occup Physiol 51:303–312.

Sawka MN, Leon LR, Montain SJ, Sonna L (2011) Integrated physiological mechanisms of exercise performance, adaptation, and maladaptation to heat stress. Compr Physiol 1:1883–928.

Sawka MN, Pandolf KB, Avellini BA, Shapiro Y (1983b) Does heat acclimation lower the rate of metabolism elicited by muscular exercise? Aviat Space Environ Med 54:27–31.

Sawka MN, Young AJ, Cadarette BS, et al. (1985) Influence of heat stress and acclimation on maximal aerobic power. Eur J Appl Physiol Occup Physiol 53:294–8.

Schmidt W, Prommer N (2005) The optimised CO-rebreathing method: a new tool to determine total haemoglobin mass routinely. Eur J Appl Physiol 95:486–95.

Schmidt W, Prommer N (2010) Impact of Alterations in Total Hemoglobin Mass on $V\dot{O}_{2max}$. Exerc Sport Sci Rev 38:68–75.

Schobersberger W, Tschann M, Hasibeder W, et al. (1990) Consequences of 6 weeks of

strength training on red cell O2 transport and iron status. Eur J Appl Physiol Occup Physiol 60:163–8.

Schultz A, Hammarsten J, Heller B, Ebert R (1953) A critical comparison of the T-1824 dye and iodinated albumin methods for plasma volume measurement. J Clin Invest 32:107–12.

Scoon GSM, Hopkins WG, Mayhew S, Cotter JD (2007) Effect of post-exercise sauna bathing on the endurance performance of competitive male runners. J Sci Med Sport 10:259–62.

Seiler KS, Kjerland GØ (2006) Quantifying training intensity distribution in elite endurance athletes: Is there evidence for an “optimal” distribution? Scand J Med Sci Sport 16:49–56

Selby GB, Eichner ER (1986) Endurance swimming, intravascular hemolysis, anemia, and iron depletion. New perspective on athlete’s anemia. Am J Med 81:791–4.

Senay LC (1972) Changes in plasma volume and protein content during exposures of working men to various temperatures before and after acclimatization to heat: separation of the roles of cutaneous and skeletal muscle circulation. J Physiol 224:61–81.

Senay LC (1975) Plasma volumes and constituents of heat-exposed men before and after acclimatization. J Appl Physiol 38:570–5.

Senay LC, Mitchell D, Wyndham CH (1976) Acclimatization in a hot, humid environment: body fluid adjustments. J Appl Physiol 40:786–96.

Şentürk ÜK, Gündüz F, Kuru O, et al. (2005) Exercise-induced oxidative stress leads hemolysis in sedentary but not trained humans. J Appl Physiol 99:1434–1441.

Shamsuddin AKM, Yanagimoto S, Kuwahara T, et al. (2005) Changes in the index of sweat ion concentration with increasing sweat during passive heat stress in humans. Eur J Appl Physiol 94:292–7.

Shapiro Y, Hubbard RW, Kimbrough CM, Pandolf KB (1981) Physiological and hematologic responses to summer and winter dry-heat acclimation. J Appl Physiol 50:792–8.

Shein NA, Horowitz M, Alexandrovich AG, et al. (2005) Heat Acclimation Increases Hypoxia-Inducible Factor 1α and Erythropoietin Receptor Expression: Implication for Neuroprotection after Closed Head Injury in Mice. J Cereb Blood Flow Metab 25:1456–1465.

Shido O, Sugimoto N, Tanabe M, Sakurada S (1999) Core temperature and sweating onset in humans acclimated to heat given at a fixed daily time. Am J Physiol 276:R1095-101.

Shvartz E, Saar E, Meyerstein N, Benor D (1973) A comparison of three methods of

acclimatization to dry heat. J Appl Physiol 34:214–219.

Smith JA (1995) Exercise, Training and Red Blood Cell Turnover. Sport Med 19:9–31.

Stephenson LA, Kolka MA (1988) Plasma volume during heat stress and exercise in women. Eur J Appl Physiol Occup Physiol 57:373–81

Stienen GJ, Kiers JL, Bottinelli R, Reggiani C (1996) Myofibrillar ATPase activity in skinned human skeletal muscle fibres: fibre type and temperature dependence. J Physiol 493 ( Pt 2):299–307.

Streeton JA (1967) Traumatic haemogloinuria caused by karate exercises. Lancet (London, England) 2:191–2.

Sunderland C, Morris JG, Nevill ME (2008) A heat acclimation protocol for team sports. Br J Sports Med 42:327–33.

Tamura Y, Matsunaga Y, Masuda H, et al. (2014) Postexercise whole body heat stress additively enhances endurance training-induced mitochondrial adaptations in mouse skeletal muscle. AJP Regul Integr Comp Physiol 307:R931–R943.

Taylor N AS (2014) Human heat adaptation. Compr Physiol 4:325–65.

Telford RD, Sly GJ, Hahn AG, et al. (2003) Footstrike is the major cause of hemolysis during running. J Appl Physiol 94:38–42.

Tetievsky A, Cohen O, Eli-Berchoer L, et al. (2008) Physiological and molecular evidence of heat acclimation memory: a lesson from thermal responses and ischemic cross-tolerance in the heart. Physiol Genomics 34:78–87.

Thomas MM, Cheung SS, Elder GC, Sleivert GG (2005) Voluntary muscle activation is impaired by core temperature rather than local muscle temperature. J Appl Physiol 100:1361–1369.

Thomsen JK, Fogh-Andersen N, Bülow K, Devantier A (1991) Blood and plasma volumes determined by carbon monoxide gas, 99mTc-labelled erythrocytes, 125I-albumin and the T 1824 technique. Scand J Clin Lab Invest 51:185–90.

Thorling EB, Erslev AJ (1968) The "tissue" tension of oxygen and its relation to hematocrit and erythropoiesis. Blood 31:332–43.

Tølløfsrud S, Svennevig JL, Breivik H, et al. (1995) Fluid balance and pulmonary functions during and after coronary artery bypass surgery: Ringer's acetate compared with dextran, polygeline, or albumin. Acta Anaesthesiol Scand 39:671–7.

Tølløfsrud S, Tønnessen T, Skraastad ø., Noddeland H (1998) Hypertonic saline and dextran in normovolaemic and hypovolaemic healthy volunteers increases interstitial and intravascular fluid volumes. Acta Anaesthesiol Scand 42:145–153.

Tschaikowsky K, Meisner M, Durst R, Rügheimer E (1997) Blood volume determination using hydroxyethyl starch: a rapid and simple intravenous injection method. Crit Care

Med 25:599–606.

Tyler CJ, Reeve T, Hodges GJ, Cheung SS (2016) Erratum to: The Effects of Heat Adaptation on Physiology, Perception and Exercise Performance in the Heat: A Meta-Analysis. Sport Med 46:1771.

Uehara K, Goto K, Kobayashi T, et al. (2004) Heat-Stress Enhances Proliferative Potential in Rat Soleus Muscle. Jpn J Physiol 54:263–271.

Umschweif G, Alexandrovich AG, Trembovler V, et al. (2013) Hypoxia-inducible factor 1 is essential for spontaneous recovery from traumatic brain injury and is a key mediator of heat acclimation induced neuroprotection. J Cereb Blood Flow Metab 33:524–31.

Van Beaumont W (1973) Red cell volume with changes in plasma osmolarity during maximal exercise. J Appl Physiol 35:47–50.

van Beaumont W, Underkofler S, van Beaumont S (1981) Erythrocyte volume, plasma volume, and acid-base changes in exercise and heat dehydration. J Appl Physiol 50:1255–62.

Voss SC, Alsayrafi M, Alsowaidi N, et al. (2011) The influence of exercise and circadian rhythm of haemoglobin concentration associated changes in plasma volume on the biological passport. Br J Sports Med 45:A7–A7.

Watkins AM, Cheek DJ, Harvey AE, et al. (2007) Heat shock protein (HSP-72) levels in skeletal muscle following work in heat. Aviat Space Environ Med 78:901–5.

Watson P, Hasegawa H, Roelands B, et al. (2005) Acute dopamine/noradrenaline reuptake inhibition enhances human exercise performance in warm, but not temperate conditions. J Physiol 565:873–83.

Watt MJ, Garnham AP, Febbraio MA, Hargreaves M (2000) Effect of acute plasma volume expansion on thermoregulation and exercise performance in the heat. Med Sci Sports Exerc 32:958–62.

Welker H (1854) Prager Vrtljshr. Prager Vrtljshr 4:145.

White AC, Salgado RM, Astorino TA, et al. (2016) The effect of 10 days of heat acclimation on exercise performance in acute hypobaric hypoxia (4350 m). Temp 3:176–85.

Williams CG, Wyndham CH, Morrison JF (1967). Rate of loss of acclimatization in summer and winter. J Appl Physiol 22: 21–26

Wilson TE, Brothers RM, Tollund C, et al. (2009) Effect of thermal stress on Frank-Starling relations in humans. J Physiol 587:3383–3392.

Wilson TE, Crandall CG (2011) Effect of thermal stress on cardiac function. Exerc Sport Sci Rev 39:12–7.

Wu TC, He HZ, Tanguay RM, et al. (1995) The combined effects of high temperature and carbon monoxide on heat stress response. J Tongji Med Univ 15:178–83.

Wyndham CH, Benade AJ, Williams CG, et al. (1968) Changes in central circulation and body fluid spaces during acclimatization to heat. J Appl Physiol 25:586–93.

Yaicharoen P, Wallman K, Bishop D, Morton A (2012a) The effect of warm up on single and intermittent-sprint performance. J Sports Sci 30:833–840.

Yaicharoen P, Wallman K, Morton A, et al. (2012b) The effects of warm-up on intermittent sprint performance in a hot and humid environment. J Sports Sci 30:967–974.

Yamada PM, Amorim FT, Moseley P, et al. (2007a) Effect of heat acclimation on heat shock protein 72 and interleukin-10 in humans. J Appl Physiol 103:1196–1204.

Yamada PM, Amorim FT, Moseley P, et al. (2007b) Effect of heat acclimation on heat shock protein 72 and interleukin-10 in humans. J Appl Physiol 103:1196–204.

Yoshimura H, Inoue T, Yamada T, Shiraki K (1980) Anemia during hard physical training (sports anemia) and its causal mechanism with special reference to protein nutrition. World Rev Nutr Diet 35:1–86.

Young AJ, Sawka MN, Levine L, et al. (1985) Skeletal muscle metabolism during exercise is influenced by heat acclimation. J Appl Physiol 59:1929–1935.

Yusof A, Leithauser RM, Roth HJ, et al. (2007) Exercise-induced hemolysis is caused by protein modification and most evident during the early phase of an ultraendurance race. J Appl Physiol 102:582–586.

Zappe DH, Tankersley CG, Meister TG, Kenney WL (1993) Fluid restriction prior to cycle exercise: effects on plasma volume and plasma proteins. Med Sci Sports Exerc 25:1225–30.