The Effect of Progressive Heat Acclimation on Games Players Performing Intermittent-Sprint Exercise in the Heat

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To the people that challenge me to be better

“Energy and persistence conquer all things”

-Benjamin Franklin-
ABSTRACT

Intermittent-sprint performance is reduced in hot environments and the impairment occurs without significant difference in metabolic responses to exercise. Reductions in cerebral blood flow and voluntary activation occur with hyperthermia and neuromuscular impairment has been posited to explain decreased performance in the heat. Heat acclimation is used to minimise heat mediated performance impairment, yet traditional protocols may limit adaptation. The purpose of this thesis was to examine the efficacy of a novel progressive heat acclimation model for games players performing intermittent-sprint exercise in the heat and to examine if this method can ameliorate neuromuscular fatigue following exercise of this type.

In study one the reliability of the cycling intermittent-sprint protocol (CISP), a 40 minute test of intermittent sprinting was determined. Eleven male team-sport athletes completed two trials of the CISP in temperate conditions and reliability of the protocol was determined from typical error of measurement (TEM), Intraclass correlation (ICC) and least product regression analysis. TEM and ICC for peak power output (PPO, 2.9%, 0.96), mean power output (MPO, 4.2%, 0.96) and least product regression analysis demonstrated the CISP was a reliable protocol for assessment of prolonged intermittent-sprint exercise.

Study two examined the effect of hot humid (HH) and hot dry environments (HD), matched for heat stress using wet bulb globe temperature (WBGT), on intermittent-sprint performance to ascertain whether the composition of thermal factors constituting the heat stress is important when developing a heat acclimation protocol. Eleven male team-sport athletes completed three CISPs (HH, HD and temperate (TEMP)). In HH and HD three participants failed to complete the full protocol but all completed the CISP in TEMP. Peak power output was reduced in all conditions (P < 0.05) but was not different between trials (sprints 1 – 14 (n = 11); HH, 1073 ± 150 W; HD, 1104 ± 127 W; TEMP, 1074 ± 134 W;
sprints 15–20 (n = 8); HH, 954 ± 114 W; HD, 997 ± 115 W; TEMP, 993 ± 94 W; P > 0.05). Further, physiological strain was not significantly different in HH compared to HD but HH was greater than TEMP (P < 0.05). Findings indicated the importance of heat acclimation for games players but suggest the composition of thermal factors contributing to the heat stress is not a primary concern when developing a heat acclimation protocol.

Study three compared the effect of a novel, progressive heat acclimation protocol (PA) to a traditional protocol (TA), on heat adaptation and intermittent-sprint exercise in the heat. Before and after twelve days of acclimation or training twenty four participants (PA, n = 9; TA, n = 6; Training (TG), n = 9) completed the CISP in 33°C, 50% rh. TA reduced resting and peak exercise heart rate (-11 b.min⁻¹, -17 b.min⁻¹) and exercise rectal temperature (T_{re}) (-0.5°C) and increased sweat rate (0.41 L.hr⁻¹, all P values < 0.05). In contrast, while PA reduced resting heart rate (-12 b.min⁻¹, P < 0.05), the elevated heat stress in PA increased peak T_{re} and sweat rate (+0.4°C, +0.31 L.hr⁻¹, P < 0.05) and maintained mean and peak heart rate during acclimation (P > 0.05). During intermittent-sprint exercise post acclimation mean heart rate and T_{re} were reduced (TA, -19 b.min⁻¹, -0.3°C; PA, -16 b.min⁻¹, -0.5°C, P < 0.05). In addition, T_{sk} was reduced by both acclimation regimes (TA, -0.95°C; PA, -0.7°C, P < 0.05) but not TG (P > 0.05). Both acclimation regimes also reduced RPE and T_{sen} during CISP 2 (P < 0.05) but TG had no effect (P > 0.05). Finally, PPO was reduced by 19, 6 and 8% in TA, TG and PA prior to acclimation and negatively correlated with T_{re} for TA and PA (r > 0.90, P < 0.001). Post acclimation, TA increased PPO and ameliorated the negative correlation between PPO and T_{re}. PA or TG had no such effect. Based on the ability to evoke classic acclimation responses compared to TA, PA was judged an effective method for conferring the heat acclimated phenotype but optimisation is required to improve acclimation response and intermittent-sprint performance.

Study four examined neuromuscular fatigue following intermittent sprinting in the heat and the ability of PA to ameliorate fatigue of this type. This study was the first to quantify neuromuscular fatigue following intermittent sprinting in the heat using transcranial
magnetic stimulation (TMS) and both central and peripheral fatigue were observed when seventeen team-sport athletes completed the CISP in 33°C, 50% rh before twelve days of PA or training (TG) (MVC, -19.8%; cortical voluntary activation, -6.3%; potentiated twitch, -21.5%; all P values < 0.05). PA decreased resting heart rate and $T_{re}$ (-10 b.min$^{-1}$, -0.3°C, P < 0.05) prior to intermittent sprinting in the heat and reduced exercise heart rate (-17 b.min$^{-1}$, P < 0.05) but performance was unchanged (1058 ± 148 vs 1060 ± 120 W, P > 0.05, CISP 1 vs 2 respectively). Prior to acclimation, forty minutes of intermittent sprinting induced neuromuscular fatigue (MVC, -20.3%, potentiated twitch, -24.7%, P < 0.05) and twelve days of PA did not reduce this (MVC, -16.6%, potentiated twitch, -17.6%, P < 0.05). Findings indicate that during forty minutes of intermittent-sprint exercise in the heat, neuromuscular fatigue may be primarily peripheral in origin and PA or TG does not reduce the extent of fatigue despite a reduced physiological strain.

**Key words**

Intermittent-sprint exercise, reliability, progressive heat acclimation, transcranial magnetic stimulation, neuromuscular fatigue.
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<td>Description</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
<td></td>
</tr>
<tr>
<td>AMP</td>
<td>Adenosine monophosphate</td>
<td></td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
<td></td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
<td></td>
</tr>
<tr>
<td>b.min⁻¹</td>
<td>Beats per minute</td>
<td></td>
</tr>
<tr>
<td>BGT</td>
<td>Black globe temperature</td>
<td></td>
</tr>
<tr>
<td>BHC</td>
<td>Body heat content</td>
<td></td>
</tr>
<tr>
<td>BM</td>
<td>Body Mass</td>
<td></td>
</tr>
<tr>
<td>[B₈₆]</td>
<td>Blood lactate concentration</td>
<td></td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>Calcium</td>
<td></td>
</tr>
<tr>
<td>CISP</td>
<td>Cycling intermittent-sprint protocol</td>
<td></td>
</tr>
<tr>
<td>CK</td>
<td>Creatine kinase</td>
<td></td>
</tr>
<tr>
<td>Cl⁻</td>
<td>Chloride</td>
<td></td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
<td></td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
<td></td>
</tr>
<tr>
<td>CSP</td>
<td>Cortical silent period</td>
<td></td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
<td></td>
</tr>
<tr>
<td>CVA</td>
<td>Cortical voluntary activation</td>
<td></td>
</tr>
<tr>
<td>DBT</td>
<td>Dry bulb temperature</td>
<td></td>
</tr>
<tr>
<td>EMG</td>
<td>Electromyography</td>
<td></td>
</tr>
<tr>
<td>ERT</td>
<td>Estimated resting twitch</td>
<td></td>
</tr>
<tr>
<td>FAD</td>
<td>Flavin adenine dinucleotide</td>
<td></td>
</tr>
<tr>
<td>GPS</td>
<td>Global positioning system</td>
<td></td>
</tr>
<tr>
<td>H reflex</td>
<td>Hoffmann’s reflex</td>
<td></td>
</tr>
<tr>
<td>HD</td>
<td>Hot dry</td>
<td></td>
</tr>
<tr>
<td>HH</td>
<td>Hot humid</td>
<td></td>
</tr>
<tr>
<td>Hmax/Mmax</td>
<td>Ratio of maximum H wave to maximum M wave</td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td>Heart rate</td>
<td></td>
</tr>
<tr>
<td>ICC</td>
<td>Intraclass correlation</td>
<td></td>
</tr>
<tr>
<td>IMP</td>
<td>Inosine monophosphate</td>
<td></td>
</tr>
<tr>
<td>K⁺</td>
<td>Potassium</td>
<td></td>
</tr>
<tr>
<td>Kg</td>
<td>Kilograms</td>
<td></td>
</tr>
<tr>
<td>km.hr⁻¹</td>
<td>Kilometers per hour</td>
<td></td>
</tr>
<tr>
<td>l.min⁻¹</td>
<td>Litres per minute</td>
<td></td>
</tr>
<tr>
<td>LIST</td>
<td>Loughborough intermittent shuttle test</td>
<td></td>
</tr>
<tr>
<td>LOA</td>
<td>Limits of agreement</td>
<td></td>
</tr>
<tr>
<td>M Wave</td>
<td>Compound muscle action potential</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>Metabolic heat production</td>
<td></td>
</tr>
<tr>
<td>MEP</td>
<td>Motor evoked potential</td>
<td></td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>Magnesium</td>
<td></td>
</tr>
<tr>
<td>mmHg</td>
<td>Millimeter of mercury</td>
<td></td>
</tr>
<tr>
<td>MPO</td>
<td>Mean power output</td>
<td></td>
</tr>
<tr>
<td>MRFD</td>
<td>Maximum rate of force development</td>
<td></td>
</tr>
<tr>
<td>MRR</td>
<td>Maximum rate of relaxation</td>
<td></td>
</tr>
<tr>
<td>ms</td>
<td>Milliseconds</td>
<td></td>
</tr>
</tbody>
</table>
mV  Millivolts  
MVC  Maximum voluntary contraction  
Na  Sodium  
NAD$^+$  Nicotinamide adenine dinucleotide  
NMF  Neuromuscular fatigue protocol  
O$_2$  Oxygen  
PA  Progressive acclimation  
PCr  phosphocreatine  
Pi  Inorganic phosphate  
$[\text{Pi}]$  Inorganic phosphate concentration  
PPO  Peak power output  
PSI  Physiological strain index  
$Q_{\text{tw,pot}}$  Potentiated twitch  
Qc  Heat content of the human  
r.min$^{-1}$  Revolutions per minute  
RER  Respiratory exchange ratio  
rh  Relative humidity  
RMS  Root mean squared  
RPE  Rating of perceived exertion  
RT$_{0.5}$  Half relaxation time  
SIT  Superimposed twitch  
TA  Traditional acclimation  
$T_{\text{body}}$  Mean body temperature  
$T_{\text{core}}$  Core temperature  
TEM  Typical error of measurement  
TEMP  Temperate  
TG  Training group  
TMS  Transcranial magnetic stimulation  
$T_{\text{re}}$  Rectal temperature  
$T_{\text{sen}}$  Thermal sensation  
$T_{\text{sk}}$  Mean skin temperature  
$U_{\text{osm}}$  Urine osmolality  
$U_{\text{sg}}$  Urine specific gravity  
$V\text{O}_2$  Oxygen uptake  
$V\text{O}_{2\text{max}}$  Maximum oxygen uptake  
$V\text{O}_{2\text{peak}}$  Peak oxygen uptake  
W  Watts  
WBGT  Wet bulb globe temperature  
WD  Work done
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Firstly, to my family: Sue, for the six years this work has been in development you have been a source of constant support. You have provided me with the encouragement to finally see it through and patiently endured many evenings alone as I sat at a computer in the lab or the front room. I look forward to our evenings together but first it’s my turn to help you. Mam and dad you worked very hard to give me the opportunity to study abroad and were selfless in letting me go. Thank you for everything that you have done and your constant support. Finally, to Pat, Sandra, Holly and Niall, thank you for the motivation and perspective that kept me going when it all seemed a bit grim.

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To Sussex Downs College and the University of Brighton for allowing me the opportunity to combine my PhD with full-time work and providing me with excellent facilities and the funding to complete my thesis.
DECLARATION

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

Signed

Dated 12th March 2014

The following published articles have been due to the work contained within this thesis:

Chapter IV


Chapter V

CHAPTER I. INTRODUCTION

Endothermy is suggested to have evolved from increased atmospheric oxygen content coupled with a need to survive repeated cooling from climate fluctuations (Portner, 2004). Consequently, in contrast to ectotherms that rely on the external environment for temperature control, endotherms have the capacity to maintain body temperature by generation of heat through metabolism. As endothermic homeotherms, humans maintain body temperature at rest over the course of their lifecycle at 37 ± 1.0°C (Cheung, 2010) and it is suggested this temperature reflects an evolutionary adaptation to maximise performance capacity (Portner, 2004). Such homeostatic control is achieved through autonomic and behavioural mechanisms that promote thermoregulation through afferent thermal sensing, central regulation and efferent responses (Nagashima, 2006; Kurz, 2008).

An inherent advantage of endothermy is the uncoupling of physical performance from exogenous temperature which permits, for example, predatory activity independent of normal environmental conditions (Lieberman et al., 2009). A consequence of such activity is the conversion of metabolic energy to mechanical energy in an inefficient process that results in 30 - 70% of the energy liberated appearing as thermal energy that will increase body temperature and therefore, must be dissipated (Gleeson, 1998; Gonzalez-Alonso, 2012). In contrast to fur covered mammals that rely on panting to liberate metabolic heat, humans have evolved to an essentially glabrous state with a highly developed sudomotor system that maximises heat loss during physical activity, even in a hot environment (Jessen, 2001; Lieberman et al., 2009). Such thermoregulatory evolution has conferred humans with distinct advantages from a survival perspective. Firstly, the ability to succeed when competing for food in hot conditions with other hair covered mammals that are forced to slow running speed to maintain body temperature due to inferior heat loss mechanisms (Jessen et al., 2001; Piantadosi et al., 2003; Lieberman et al., 2009). Secondly, the ability to outrun and exhaust prey who, due to the coupling of respiration with locomotion that is incompatible with panting during galloping, are forced to change
behaviour, decrease running speed and eventually stop (Jessen, 2001; Lieberman et al., 2009).

Compared to other mammals humans display superior body temperature control during exercise in the heat, but are not immune to the debilitating effects of heat stress particularly when environmental conditions are severe and motivation is high, for example, during competition. During exercise body temperature increases at a rate proportional to the exercise intensity and percent of maximal aerobic capacity ($\text{VO}_{2\text{max}}$) and this is consistent within a wide range of environmental conditions, termed the “prescriptive zone” (Nielsen, 1996). During exercise, evaporation of sweat produced by the 1.6 - 4.0 million eccrine glands (Shibasaki et al., 2006) is the primary mechanism for maintenance of body temperature, with each litre of sweat evaporated liberating 2.4 MJ or 680 W.hr$^{-1}$ of thermal energy (Gleeson, 1998; Kenney, 1998). During high intensity exercise, heat production can be 800 - 1500 W (Verdaguer-Codina et al, 1993; Gleeson, 1998; Brotherhood, 2008) and when combined with high heat stress, the evaporative requirement of the exercising athlete to maintain body temperature can exceed the evaporative potential of the environment. Under such conditions, thermal energy is stored, body temperature rises and hyperthermia ensues contributing to fatigue and compromised exercise performance (Morris et al., 1998, 2000; Drust et al., 2005).

Heat acclimation, described as the physiological adaptations to heat brought about by repeated exposure to a controlled laboratory environment (Armstrong and Maresh, 1991), has been used as a strategy to alleviate the deleterious effect of heat in occupational and sporting settings for over seventy years. From the initial pioneering research of Robinson et al., (1943) and Bean and Eichna (1943, 1945) that characterised the pattern of response to dry and humid heat, to the work of Lind and Bass (1963) that identified the ‘optimal’ exposure time for development of heat acclimation, our understanding of physiological adaptation to heat acclimation and how this may be manipulated to optimise responses has developed considerably. More research, however, is required to elucidate fully the interplay of key protocol variables and genotypic markers
and how manipulation of the former ultimately affects acquisition of the heat acclimated phenotype. Typically, based on current evidence, acquisition of the heat-acclimated phenotype is considered to require 10 - 14 days of exercise under heat stress conditions, when exercise is performed for 60 – 100 minutes at ≥ 50% VO\textsubscript{2max} (Armstrong and Maresh, 1991; Maughan and Shirreffs, 2004). However, 66 - 75% of adaptation has been shown to occur within 4 - 6 days and nearly complete acclimation has been demonstrated within 7 - 10 days (Pandolf, 1998). Many classic responses to heat acclimation including improved cardiovascular stability, decreased resting and exercise core temperature and heart rate, increased sweat rate and plasma volume display a biphasic response pattern. Current research, however, suggests this may be protocol dependent and an artefact of decreased strain inherent in traditional constant work-rate heat acclimation methods (Patterson et al., 2004). Isothermal strain heat acclimation circumvents this limitation by necessitating exercise at a target core temperature to maintain physiological strain (Taylor, 2000). Traditional and isothermal strain protocols constitute the primary means by which heat acclimation has been induced. However, they necessitate exposure to high heat stress from the outset, evoke a decaying physiological strain (traditional) and require a high work rate (isothermal) and may, therefore, be untenable for certain individuals, for example, those who are less heat tolerant. Progressive heat acclimation, where exercise at a constant work-rate is performed under a gradually increasing heat stress may circumvent many of these limitations and emerging evidence (Daanen et al., 2011; Burk et al., 2012; Costa et al., 2014), in addition to comparative evidence from intermittent-hypoxic training, provides support for progressive models of acclimation (Hamlin and Hellemans, 2007).

Heat acclimation is used frequently to confer adaptation to the heat in athletes involved in endurance and submaximal exercise. However, globalisation has increased the incidence of major team-sport competition in hot humid and hot dry environments. As such, many field-based team-sport athletes frequently compete in conditions of high heat stress and will do so again in the 2014 World Cup, 2016 Olympic Games, 2018 Commonwealth Games and 2022 World Cup. Further, due to broadcasting pressure on tournament organisers these athletes may be required to compete at periods when
higher heat and humidity persist, for example the schedule of England versus Italy at the 2014 Football World Cup (Vickery, 2013). In controlled laboratory and field-based research, such environments have been shown to exacerbate fatigue and compromise intermittent-sprint exercise performance, especially when the duration and work:rest patterns of the protocols simulate those of field-based team-sports (Morris et al., 1998, 2000, 2005; Sunderland and Nevill, 2005). Further, intermittent-sprint exercise is known to produce greater thermal strain than continuous exercise of the same average work intensity (Nevill et al., 1995). Therefore, with the exception of a few recent studies (Sunderland et al., 2008; Petersen et al., 2010; Brade et al., 2013) it is surprising that more research has not examined heat acclimation to alleviate fatigue and performance decrements using an intermittent-sprint model of exercise. Those studies that have examined heat acclimation for intermittent-sprint exercise (Sunderland et al., 2008; Petersen et al., 2010; Brade et al., 2013) have used a short-term intermittent-exercise acclimation protocol that is suggested to provide incomplete heat acclimation responses (Pandolf, 1998) and may question the efficacy of intermittent heat acclimation models. As such, further work is required to determine how acclimation protocols may be manipulated to provide optimal benefit for intermittent-sprint exercise in the heat.

With regard to neuromuscular fatigue during intermittent-sprint exercise, the research is equivocal. Peripheral fatigue is considered the primary fatigue mechanism during exercise of this type, and there has been mixed evidence as to whether central fatigue exists during intermittent-sprinting (Racinais et al., 2007; Perrey et al., 2010b). Current research in this area has not however, (i) used protocols to simulate field-based team-sports, (ii) used a hot environment and (iii) used a method that allows the existence of central fatigue to be localised. Further, no work has examined whether heat acclimation can alleviate neuromuscular fatigue in an intermittent-sprint exercise model in the heat.

Considering the aforementioned, the primary aim of this thesis was to assess the efficacy of a novel progressive heat acclimation protocol using an intermittent-sprint model of exercise that simulated field-based team-sports. A secondary aim was to examine fatigue
during intermittent-sprint exercise in the heat and the efficacy of heat acclimation as an intervention to alleviate this fatigue.

This thesis is presented in the following chapters;

- Chapter II provides a review of the literature starting by examining the movement and general energetic demands of field-based intermittent-sprint exercise, followed by consideration of the reliability of protocols used to assess intermittent-sprint exercise and indices of heat stress. The review also examines the effect of heat stress on intermittent-sprint exercise performance, the mechanisms of central and peripheral fatigue that may contribute to exercise of this type and how neuromuscular fatigue is measured. Finally, this chapter culminates with a review of heat acclimation methods and adaptations.

- Chapter III provides the common methods that were used throughout all experimental chapters.

- Chapter IV presents the first study that examined the reliability of the cycling intermittent-sprint protocol (CISP). This protocol is designed to simulate the work:rest patterns of field-based team-sports over a duration equivalent to one half of a match and can be used to assess the effect of an intervention, for example, heat acclimation, on physiological, perceptual and performance responses during exercise of this type under conditions of heat stress.

- Chapter V uses the CISP to identify the effect of differing combinations of environmental factors (hot, humid and hot, dry) matched for heat stress on intermittent-sprint exercise to determine whether the composition of the
environment is important when determining the design of a heat acclimation protocol.

- Chapter VI presents a study that investigated the efficacy of a progressive heat acclimation protocol that used stepwise increases in heat stress to elevate physiological strain. The ability of this method to evoke classic heat acclimation responses was compared to a traditional, constant work-rate protocol. In addition, this study examined the effect of the progressive heat acclimation protocol compared to the traditional protocol on intermittent-sprint exercise under conditions of heat stress.

- Chapter VII examined neuromuscular fatigue before and after intermittent-sprint exercise under heat stress using transcranial magnetic stimulation (TMS) and the interpolated twitch technique. Further, the study investigated whether the progressive heat acclimation protocol in chapter VI alleviated neuromuscular fatigue that was experienced during intermittent-sprint exercise.

- Chapter VIII comparatively discusses the findings from the experimental studies with particular examination of the efficacy of progressive heat acclimation as a method to evoke classic heat acclimation responses, but also its effect on intermittent-sprint exercise and neuromuscular fatigue during exercise of this type. This chapter examines models of heat acclimation from an efficacy perspective and considers heat acclimation responses that may contribute to an optimal heat acclimation protocol.
CHAPTER II. LITERATURE REVIEW

2.1. Introduction

This literature review will provide a synopsis of the research examining intermittent-sprint exercise and the performance and physiological responses to heat stress. In addition, it will examine possible causes of fatigue during intermittent-sprint exercise and the potential for heat acclimation to alleviate the deleterious effects of heat stress. Specifically, the review will begin in section 2.2. by examining the movement demands and energetic requirements of common field-based team-sports and consider the reliability of protocols designed to assess performance and physiological responses during exercise of this type. In section 2.3., the process of thermoregulation and how exercise alters thermoregulation will be considered. From there the review will provide a historical overview of the quantification of heat stress in sport and exercise research and move to consider the impact of heat stress on physiological and performance responses during intermittent-sprint exercise in the heat.

Considering the deleterious effect of heat stress on exercise performance, section 2.4., will begin by examining neuromuscular fatigue and specifically central and peripheral fatigue in intermittent-sprint exercise in the heat. Finally, in section 2.5., the review will consider the development of heat acclimation methods and protocols. Adaptations to heat acclimation at a cellular and systemic level are then considered before focussing on the effect of heat acclimation upon the physiological and performance responses to intermittent-sprint exercise in the heat.
2.2. Intermittent-Sprint Exercise

2.2.1. Time-motion analysis of field-based team-sports

Many team sports are characterized by periods of intermittent high-intensity activity that require athletes to exercise maximally for short periods, interspersed with longer spells of recovery and lower intensity activity (Williams, 1990). Time-motion analysis research permits quantification of such activity in field-based sports and has developed from initial qualitative analysis to notational and video analysis to use of Global Positioning Systems (GPS) to characterize the movement patterns in the game. The following section will provide insight into time-motion analysis research for the field-based team-sports of hockey, rugby union and football. Accepting that positional differences exist, mean data derived from existing studies (Table 2.1.) in the field will be used to provide an overall insight to the general demands of intermittent field-based team-sports.
Table 2.1. Time-motion analysis data for field-hockey, rugby union and football (mean values).

<table>
<thead>
<tr>
<th>Study</th>
<th>Sport</th>
<th>Participants (n=)</th>
<th>Position</th>
<th>% time in HIA</th>
<th>% time in LMIA</th>
<th>Sprint Duration (s)</th>
<th>Sprint distance (m)</th>
<th>Sprint frequency</th>
<th>Recovery between sprints (s)</th>
<th>Total distance run (m)</th>
<th>CIA (s) (total number)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lothian and Farrally, (1994)</td>
<td>field-hockey</td>
<td>TF (12)</td>
<td>All</td>
<td>22</td>
<td>78</td>
<td>3.1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Boddington et al., (2002)</td>
<td>field-hockey</td>
<td>EF</td>
<td>-</td>
<td>2.6</td>
<td>97.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3901 (63 min GT)</td>
<td>-</td>
</tr>
<tr>
<td>Spencer et al., (2004)</td>
<td>field-hockey</td>
<td>EM (14)</td>
<td>All</td>
<td>5.6</td>
<td>94.4</td>
<td>1.8</td>
<td>-</td>
<td>30</td>
<td>140</td>
<td>-</td>
<td>5.4 (780)</td>
</tr>
<tr>
<td>MacLeod et al., (2007)</td>
<td>field-hockey</td>
<td>EF (12)</td>
<td>All</td>
<td>7.9</td>
<td>92.1</td>
<td>2.9</td>
<td>-</td>
<td>24 (48.5 ± 12.7 min GT)</td>
<td>120</td>
<td>-</td>
<td>3.0 (960)</td>
</tr>
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<td>Gabbett, (2010)</td>
<td>field-hockey</td>
<td>EF (14)</td>
<td>All</td>
<td>2.7</td>
<td>97.3</td>
<td>-</td>
<td>11 - 20</td>
<td>91</td>
<td>-</td>
<td>-</td>
<td>6600</td>
</tr>
<tr>
<td>Macutkiewicz &amp; Sunderland, (2011)</td>
<td>field-hockey</td>
<td>EF (25)</td>
<td>All</td>
<td>6.4</td>
<td>93.6</td>
<td>2.5</td>
<td>14 ± 3</td>
<td>17</td>
<td>-</td>
<td>5541 (48 ± 4 min GT)</td>
<td>-</td>
</tr>
<tr>
<td>Docherty et al., (1988)</td>
<td>Rugby Union</td>
<td>TM (13)</td>
<td>All</td>
<td>-</td>
<td>-</td>
<td>2.1</td>
<td>-</td>
<td>21</td>
<td>156</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Duthie et al., (2005)</td>
<td>Rugby Union</td>
<td>EM (47)</td>
<td>All</td>
<td>8.5</td>
<td>91.5</td>
<td>2.6</td>
<td>-</td>
<td>19</td>
<td>307</td>
<td>-</td>
<td>7.5 (711)</td>
</tr>
<tr>
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<td>Rugby Union</td>
<td>EM (25)</td>
<td>All</td>
<td>8.3</td>
<td>91.7</td>
<td>2.6</td>
<td>12 - 28</td>
<td>15</td>
<td>80 - 110</td>
<td>-</td>
<td>-</td>
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<td>EM (29)</td>
<td>All</td>
<td>7.7</td>
<td>92.3</td>
<td>1.2</td>
<td>8</td>
<td>20</td>
<td>-</td>
<td>6006</td>
<td>-</td>
</tr>
<tr>
<td>Cunniffe et al., (2009)</td>
<td>Rugby Union</td>
<td>EM (2)</td>
<td>All</td>
<td>11</td>
<td>89</td>
<td>-</td>
<td>15 - 20</td>
<td>27</td>
<td>-</td>
<td>6953</td>
<td>-</td>
</tr>
<tr>
<td>Coughlan et al., (2011)</td>
<td>Rugby Union</td>
<td>EM (2)</td>
<td>All</td>
<td>7.6</td>
<td>92.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9</td>
<td>6714</td>
<td>-</td>
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<tr>
<td>Cahill et al., (2013)</td>
<td>Rugby Union</td>
<td>EM (120)</td>
<td>All</td>
<td>0.7</td>
<td>98.8</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>Study</td>
<td>Sport</td>
<td>Participants (n=)</td>
<td>Position</td>
<td>% time in HIA</td>
<td>% time in LMIA</td>
<td>Sprint Duration (s)</td>
<td>Sprint distance (m)</td>
<td>Sprint frequency</td>
<td>Recovery time between sprints (s)</td>
<td>Total distance run (m)</td>
<td>CIA (s) (total number)</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-----------</td>
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<td>----------------</td>
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</tr>
<tr>
<td>Bangsbo et al., 1991</td>
<td>Football</td>
<td>EM (14)</td>
<td>All</td>
<td>-</td>
<td>-</td>
<td>2.0</td>
<td>-</td>
<td>19</td>
<td>284</td>
<td>10800</td>
<td>7</td>
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<tr>
<td>Reilly and Thomas, (1976)</td>
<td>Football</td>
<td>EM (40)</td>
<td>All</td>
<td>-</td>
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<td>-</td>
<td>15.7</td>
<td>62</td>
<td>90</td>
<td>-</td>
<td>6.4</td>
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<tr>
<td>Withers et al., (1982)</td>
<td>Football</td>
<td>EM (20)</td>
<td>All</td>
<td>-</td>
<td>-</td>
<td>3.7</td>
<td>22.4</td>
<td>30</td>
<td>180</td>
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<tr>
<td>Barros et al., (1999)</td>
<td>Football</td>
<td>EM (25)</td>
<td>All</td>
<td>-</td>
<td>-</td>
<td>13</td>
<td>55</td>
<td>98</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>Rienzi et al., 2000</td>
<td>Football</td>
<td>EM (17)</td>
<td>All</td>
<td>5</td>
<td>95</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>8638</td>
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</tr>
<tr>
<td>Mohr et al., (2003)</td>
<td>Football</td>
<td>EM (42)</td>
<td>All</td>
<td>9</td>
<td>91</td>
<td>2.0</td>
<td>-</td>
<td>33</td>
<td>-</td>
<td>10595</td>
<td>-</td>
</tr>
<tr>
<td>Bloomfield et al., (2007)</td>
<td>Football</td>
<td>EM (55)</td>
<td>All</td>
<td>4.8</td>
<td>95.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bradley et al., 2009</td>
<td>Football</td>
<td>EM (370)</td>
<td>All</td>
<td>9.0</td>
<td>91</td>
<td>1.2</td>
<td>14</td>
<td>18</td>
<td>72</td>
<td>10714</td>
<td>-</td>
</tr>
<tr>
<td>Di Salvo et al., 2010</td>
<td>Football</td>
<td>EM (717)</td>
<td>All</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8</td>
<td>27</td>
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<td>-</td>
</tr>
<tr>
<td>Rey et al., (2010)</td>
<td>Football</td>
<td>EM (42)</td>
<td>All</td>
<td>8</td>
<td>92</td>
<td>-</td>
<td>21.4</td>
<td>13</td>
<td>131</td>
<td>11053</td>
<td>-</td>
</tr>
<tr>
<td>Andrzejewski et al., (2013)</td>
<td>Football</td>
<td>EM (147)</td>
<td>All</td>
<td>-</td>
<td>-</td>
<td>90% &lt; 5</td>
<td>10-20(48%)</td>
<td>12</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>

HIA = high intensity activity; LMIA = low to moderate intensity activity; CIA = changes in activity; EM = elite males; EF = elite females; TM = trained males; GT = game time; - = data not available. All = data for all playing positions examined aggregated.
2.2.1.1. Time in low and high intensity activity

Early research in elite female field-hockey posited that 78% of match time involved low to moderate intensity activities (LMIA) such as standing, walking and jogging and 22% high intensity activity (HIA) (Lothian and Farrally, 1994, Table 2.1.). Recent work refutes this, suggesting greater than 90% of match time in elite field-hockey involves LMIA with the disparity attributed to differences in classification system and rule change (Boddington et al., 2002; Spencer et al., 2004; MacLeod et al., 2007, Table 2.1.). At elite female club level, 97.4% of game time is attributed to low intensity activity (Boddington et al., 2002), however, at international level figures for LMIA of 94 and 92% have been reported for males and females respectively (Spencer et al., 2004; MacLeod et al., 2007, Table 2.1.), but are based on analysis of a single match. When multiple matches are examined GPS data for elite club and international females indicates 97.3% and 93.6% of a match respectively, involves LMIA with differences in classification methods accounting for reported differences (Gabbet, 2010; Macutkiewicz and Sunderland, 2011).

Similar to field-hockey, in elite club rugby percentage of time in LMIA and HIA is reported as 91.5 and 92.3% and 8.4 and 7.7% respectively, when analysed across matches (Duthie et al., 2005; Deutsch et al., 2007; Roberts et al., 2008, Table 2.1.). More recently, GPS tracking of ninety-eight English Premiership players across forty-four matches has indicated the percentage of time in LMIA and HIA as 98.8 and 0.7% respectively; the greater emphasis on LMIA possibly being explained by the differing classification system used for GPS time-motion analysis (Cahill et al., 2013, Table 2.1.). In comparison to field-hockey, fewer data exist at international level, but one recent study using GPS reported 92.4 and 7.6% of match time involved LMIA and HIA respectively (Coughlan et al., 2011, Table 2.1.). While this study reflects elite club data, generalisability is limited by a small sample size (Table 2.1.).

The distribution of playing time across LMIA and HIA in elite football is similar at international and club level and reflects patterns observed in both field-hockey and rugby
union. At club level approximately 91 - 95% of time is dedicated to LMIA and 9 - 5% to HIA during a match (Mohr et al., 2003; Bloomfield et al., 2007; Bradley et al., 2009). At international level, although based on only one study, 95 and 5% of time are dedicated to LMIA and HIA respectively (Rienzi et al., 2000). Although few studies exist on elite females players similar distribution of time is reported for LMIA and HIA at 94 and 6% respectively (Krustrup et al., 2005, Table 2.1).

### 2.2.1.2. Sprint duration, distance and recovery

The contribution of HIA to the total activity profile in field-based sports is small. Yet, it has been demonstrated such activity precedes significant moments in game play and therefore, may be considered critical to the outcome of a match (Reilly, 1997; Spencer et al., 2004). Consequently, a number of studies have investigated the contribution of HIA such as sprinting and this activity type has received increasing attention in recent research. In field-hockey, sprinting is reported to comprise ~ 1.9 ± 0.8% of HIA, equating to 24 ± 7 sprints per game of 2.57 ± 0.57 s over 14 ± 4 m (Lothian and Farrally, 1994; Spencer et al., 2004; MacLeod et al., 2007; Gabbett, 2010; Macutkiewicz and Sunderland, 2011), but distances up to 20 m are suggested commonplace (Gabbett, 2010). Further, mean maximal data for elite males indicates maximal sprints of 4.1 ± 2.1 s and distances of 30 - 40 m (Spencer et al., 2004). Fifty percent of recovery times between sprints are reported to be > 60 s with mean values of 2 minutes observed (Spencer et al., 2004; MacLeod et al., 2007).

Similar to field-hockey, mean data for all playing positions in elite rugby union indicates ~ 0.8% HIA is sprinting based (Duthie et al., 2005; Deutsch et al., 2007; Cunniffe et al., 2009). The number of sprints completed during a rugby union match is suggested to range from 19 - 26 with a mean sprint time of 1.9 s and distance of ~ 16 m (Docherty et al., 1988; Duthie et al., 2005; Deutsch et al., 2007; Roberts et al., 2008; Cunniffe et al., 2009, Table 2.1.). Mean recovery between sprints is reported as 232 s (Docherty et al., 1988; Duthie et al., 2005; Deutsch et al., 2007; Roberts et al., 2008; Cunniffe et al., 2009, Table 2.1.).
1988; Duthie et al., 2005). With respect to total distance run at sprinting speed, recent in-game GPS data suggests ~ 346 m is covered during activity of this type (Cahill et al., 2013).

In football, elite players complete between 11 - 55 sprints, with the range probably reflecting the activity classification used (Bangsbo et al., 1991; Barros et al., 1999; Mohr et al., 2003; Andrzejewski et al., 2013, Table 2.1). In contrast to field-hockey and rugby union, O’Donoghue and Parker (2002) suggest the mean sprint duration as ~ 3.5 s and recent work using GPS indicates 90% of sprints in elite football are < 5 s (Andrzejewski et al., 2013). Mean individual sprint distance has been reported as ~ 13 - 23 m and players regularly cover a total sprint distance of ~ 160 - 300 m (Bangsbo et al., 1991; Rienzi et al., 2002; Di Salvo et al., 2009, 2010; Andrzejewski et al., 2013). Recovery periods are noted to range from 32.3 ± 7.9 s to 27.7 ± 4.5 s, but other research suggests recovery between sprints in elite football is likely to range from 72 - 284 s (Bangsbo et al., 1991; Barros et al., 1999; O’Donoghue and Parker, 2002; Bradley et al., 2009, Table 2.1.).

2.2.1.3. Total match distance and motion changes

Total distance covered in field-hockey by elite females has been reported as ~ 6500 m, but no indication of match duration was provided (Gabbett, 2010). Macutkiewicz et al., (2011) however, suggest in 48 ± 4 minutes of match time elite females cover 5541 ± 1141 m. During a match players are reported to complete 780 motion changes in a 71 minute game and a change in activity every 5.5 s (Spencer et al., 2004), but more recent work incorporating lunging as HIA reported 960 ± 272 motion changes in an average game time of 48.5 ± 12.7 minutes in females equating to an activity change every 3 s (MacLeod et al., 2007).

Similar to field-hockey, elite male rugby union players are reported to cover total distances of ~ 6430 ± 558 m during a match (Roberts et al., 2008; Cunniffe et al., 2009; Coughlan et al., 2011; Cahill et al., 2013). Work-to-rest ratios are reported as ranging from
1:5.8 to 1:14.6 depending on how the activity is classified (Deutsch et al., 2007; Cunniffe et al., 2009). With respect to changing patterns of play, 678 - 742 changes in activity have been reported during an 80 minute match representing a change in activity every 3 - 4 s (Duthie et al., 2005; Cunniffe et al., 2009).

Total distance travelled during an elite football match is ~ 10350 ± 874 m, with a range of 8 - 14 km (Reilly, Rienzi et al., 2000; Mohr et al., 2003; Reilly, 2003; Krstrup et al., 2005; Bangsbo et al., 2006; Bloomfield et al., 2007; Bradley et al., 2009; Rey et al., 2010, Table 2.1.). With respect to activity changes, elite players are reported to complete 750 - 1500 discrete movements during a ninety minute match, with a change in activity every 4 - 6 s (Reilly, 2003; Bangsbo et al., 2006; Bloomfield et al., 2007).

### 2.2.1.4. Progression of time-motion analysis variables during a match

In field-hockey, as a half progresses players walk and stand more and jog less, especially in the second half of a match (Spencer et al., 2004). In addition, the number of sprints completed in the second half is reduced compared to the first half and there is more time spent walking and less cruising post-sprint in the second half (MacLeod et al., 2007).

In elite rugby union much work has focused on positional differences as opposed to change in performance variables over time. Early work suggested that movement patterns were similar between halves and fatigue did not elicit a marked decrease in overall activity level (Duthie et al., 2005). Roberts et al., (2008) in part confirm this finding with no significant difference in high intensity activity between halve of a match, but did observe fewer bouts of high intensity running after the final five scrums compared to the first five. In addition, total distance covered was lower at 50 - 60 minutes and 70 - 80 minutes compared to the first 10 minutes (Roberts et al., 2008).
In football, Bradley et al., (2009) reported that, in FA Premier League players although average recovery time between very high intensity bouts was 72 ± 28 s, recovery time was 28% longer in the last 15 minutes compared to the first 15 minutes of a match. In addition, ~20% less high intensity running was completed in the last compared to the first 15 minutes of a game (Bradley et al., 2009). Similarly, Di Salvo et al., (2009) observed that total high intensity running and total sprint distance significantly decline in the second half of a match with the greatest decrements observed in wide midfielders and attackers.

2.2.1.5. Summary

Time-motion analysis of field-hockey, rugby union and football has developed significantly in the last forty years. From qualitative and notational analysis, performance is now assessed through video player tracking systems, but also the use of GPS in-game. This has resulted in significant data generation for a myriad of variables, but, as seen, comparison of findings is sometimes complicated by use of different classification systems or addition of different variables to an existing system. However, from the research presented the intermittent nature of team sports such as hockey, football and rugby union is clear with players completing between 5 and 14 km during a match incorporating up to 1500 movement changes during the game with a change every 4 - 6 s. Similarly, players in these sports are suggested to complete between ten and sixty sprints per game over an average distance of 10 and 20 m with recoveries up to 400 s covering a total sprint distance of up to 600 m. These data, combined with the observed deterioration in many indicators of HIA during a match, clearly indicate that intermittent high-intensity activity as observed in field-based team sports poses a significant physiological challenge for the exercising athlete.
2.2.2. Energetics of intermittent-sprint exercise

2.2.2.1. Metabolic pathways for ATP production

Intra muscular concentrations of ATP in humans have been estimated at ~ 5 - 6 mmol.kg$^{-1}$ wet muscle or 20 - 25 mmol.kg$^{-1}$ dry muscle (Glaister, 2005; Mougios, 2006; Baker et al., 2010). During brief, intense exercise an ATP turnover rate of ~ 10 - 15 mmol.kg$^{-1}$.s$^{-1}$ has been observed (Gaitanos et al., 1993; Bogdanis et al., 1996). Therefore, in brief maximal exercise, intramuscular stores of ATP can sustain exercise for ~ 1 - 3 s (Glaister, 2005; Mougios, 2006; Baker et al., 2010). Resting concentrations of ATP in skeletal muscle are optimal for maintaining cellular homeostasis and these levels are rigorously defended to avoid cellular catastrophe. As such, during brief maximal exercise, despite a 1000 fold increase in ATP demand, muscle ATP decreases only 1 - 2 mmol.kg$^{-1}$ wet weight or 4 - 8 mmol.kg$^{-1}$ dry weight, but a mismatch between ATP hydrolysis and supply persists (Glaister, 2005; Baker et al., 2010). Consequently, satisfying the energetic demands of brief maximal exercise requires the activation and integration of three energy-producing pathways (Figure 2.1.).

![Diagram of metabolic pathways](image)

**Figure 2.1.** Pathways for resynthesis of ATP including the phosphagen system (1), glycolysis (2) and aerobic respiration (3) (Baker et al., 2010).
2.2.2.2. Phosphocreatine, ADP and AMP

The primary mechanism for ATP resynthesis during intense exercise is degradation of phosphocreatine (PCr) by creatine kinase (CK) and subsequent phosphorylation of ADP (reaction 1).

\[
\text{PCr} + \text{ADP} + \text{H}^+ \xrightleftharpoons{\text{creatine kinase}} \text{ATP} + \text{Cr}
\]

(Reaction 1; Glaister, 2005)

As this process involves a single reaction to phosphorylate ADP to ATP coupled with the high activity of CK and proximity of PCr to myosin heads, it represents the fastest pathway for ATP resynthesis and permits rates of ATP resynthesis of up to 2.6 mmol.kg\(^{-1}\).s\(^{-1}\) to be achieved within 1 - 2 s (Mougios, 2006). With resting intramuscular PCr concentrations of 19 - 21 mmol.kg\(^{-1}\) wet muscle or 75 - 85 mmol.kg\(^{-1}\) dry muscle and maximal turnover rates between 7 - 9 mmol.kg\(^{-1}\).s\(^{-1}\), PCr provides for approximately 5 - 7 s of energy provision in intense exercise (Gaitanos et al., 1993; Parolin et al., 1999; Glaister, 2005; Spencer et al., 2005; Mougios, 2006). Effective resynthesis maintains intracellular ATP concentration at \(~73\%\) of resting immediately post 30 s of sprint exercise and at \(~68\%\) of baseline after ten 6 s sprints (Gaitanos et al., 1993; Bogdanis et al., 1996). Conversely, PCr concentrations are reduced to \(~16\%\) of pre-exercise concentrations after such exercise (Gaitanos et al., 1993; Bogdanis et al., 1996).

During intense exercise when ATP hydrolysis exceeds resynthesis intracellular ADP concentration is increased. In such circumstances additional resynthesis of ATP can occur through the adenylate kinase reaction (reaction 2).

\[
\text{ADP} + \text{ADP} \xrightleftharpoons{\text{adenylate kinase}} \text{ATP} + \text{AMP}
\]

(Reaction 2; Glaister, 2005)
Adenylate kinase catalyses the addition of a terminal phosphate of one ADP molecule to a second, promoting ATP resynthesis at rates up to 0.9 mmol.kg\(^{-1}\).s\(^{-1}\) (Mougios et al., 2006). As a consequence of adenylate kinase mediated ATP resynthesis, intracellular concentration of AMP is increased (Glaister, 2005; Mougios et al., 2006; Baker et al., 2010). Presence of increased intracellular AMP activates two important glycolytic enzymes, phosphorylase and phosphofructokinase increasing the glycogenolytic and glycolytic activity respectively, promoting increased rates of ATP resynthesis (Baker et al., 2010). In addition, exercise-induced metabolic acidosis and reduced ATP concentration activates adenylate deaminase that catalyses the conversion of AMP to IMP and eventually NH\(_3\). Although conversion of AMP to IMP does not directly contribute to ATP resynthesis elimination of AMP promotes enhanced resynthesis of ATP (Mougios, 2006; Baker et al., 2010). If intramuscular PCr declines below 60% of resting concentration further degradation of IMP may occur (Karatzaferi et al., 2001). Under these conditions, a portion of the IMP produced in intense exercise may be degraded to inosine and hypoxanthine (Stathis et al., 1999). In such circumstances, these compounds may be resynthesised to IMP via the purine salvage pathway or move to the plasma (Stathis et al., 1999). Loss of such compounds reduces the purine nucleotide pool making the resynthesis of ATP in recovery a protracted process (Mougios, 2006).

### 2.2.2.3. Anaerobic glycolysis

Anaerobic glycolysis is activated within 2 - 3 seconds of exercise, being initiated due to increase in P\(_i\) and activation of the glycolytic enzyme phosphorylase, by altered AMP:ATP ratio, increased cytosolic Ca\(^{2+}\) concentration and from the increase of epinephrine (Mougios, 2006; Baker et al., 2010, Figure 2.2.).
Anaerobic glycolytic production of ATP is initiated by either the phosphorylation of glycogen to glucose-1-phosphate and subsequent conversion to glucose-6-phosphate by phosphoglucomutase, or, the phosphorylation of glucose to glucose-6-phosphate by hexokinase (Mougios, 2006; Baker et al., 2010, Figure 2.2., phase 1). Subsequently, phosphoglucone isomerase catalyses glucose-6-phosphate conversion to fructose-6-phosphate and increased phosphofructokinase activity, due to low ATP and PCr concentrations coupled with increased AMP concentration, results in Fructose-1,6-bisphosphate production (Mougios, 2006; Baker et al., 2010). From this compound a further seven reactions are required to achieve ATP production and the end product pyruvate (Figure 2.2., phase 2). As a consequence the maximal glycolytic ATP turnover rate is \( \sim 5 - 9 \text{ mmol.kg}^{-1}.\text{s}^{-1} \) which is achieved after 10 - 15 s of exercise (Glaister, 2005; Spencer et al., 2005; Baker et al., 2010). During anaerobic glycolysis the conversion of pyruvate to lactate reduces end product inhibition of glycolysis and ensures regeneration.
of cytosolic NAD$^+$ as the hydrogen accepted by NAD$^+$ during stage six (Figure 2.2., Phase 2) is donated to pyruvate via lactate dehydrogenase (Astrand et al., 2003; Baker et al., 2010).

### 2.2.2.4. Aerobic respiration

Evidence suggests that in repeated sprint exercise there is an increasing contribution of aerobic metabolism to ATP regeneration with up to 49% of energy being provided aerobically during 30 s repeated efforts (Gaitanos et al., 1993; Bogdanis et al., 1996).

![Figure 2.3](image)

**Figure 2.3.** The aerobic pathway demonstrating the conversion of carbohydrate to pyruvate (1), the Krebs cycle (2) and electron transport (3) (Baker et al., 2010).

The conversion of carbohydrate to pyruvate signifies the initial step in aerobic metabolism (Figure 2.3., part 1) Subsequently, Pyruvate and the electrons and protons of glycolysis mediated NAD$^+$-NADH reduction enter the mitochondria where pyruvate reacts with coenzyme A in the mitochondrial matrix to produce Acetyl coenzyme A (Mougios, 2006; Baker et al., 2010). Acetyl coenzyme A enters the Krebs cycle (Figure 2.3., part 2) in
the mitochondria where a series of nine reactions produce oxaloacetate. In addition, three \( \text{NAD}^+ \) are converted to \( \text{NADH} \), one \( \text{FAD} \) is converted to \( \text{FADH}_2 \) and one ATP is produced (Mougios, 2006). The compounds \( \text{NADH} \) and \( \text{FADH}_2 \) are then oxidized by transferring their electrons to oxygen in the electron transport chain (Figure 2.3., part 3) to produce large amounts of energy (Mougious, 2006). This energy is then used in oxidative phosphorylation to synthesise large quantities of ATP (Mougios, 2006).

### 2.2.2.5. Energy production in intermittent-sprint exercise

When ten 6 s sprints are completed with 30 s recovery glycolysis and \( \text{PCr} \) contribute 44 and 56% respectively, to ATP production in the initial sprint. By the final sprint, however, glycolytic contribution to ATP requirement is decreased to 16% with an 80% contribution from \( \text{PCr} \) (Gaitanos et al., 1993). Overall, in the latter stages of intermittent sprinting, ATP production rate from anaerobic sources is reduced to a greater extent than power output and lactate concentration remains unchanged suggesting that ATP resynthesis in the final stages of this activity is mainly derived from \( \text{PCr} \) degradation and aerobic metabolism (Gaitanos et al., 1993). It is clear therefore, that during intermittent-sprint exercise, the contribution of energy producing pathways to ATP provision is altered as the activity progresses. However, it is important to note that much of the research examining energetics in intermittent-sprint exercise has used sprinting frequencies, durations and recoveries that do not replicate those observed in team sports. Spencer et al., (2005) propose that in a 3 s sprint 55% of energy required will come from \( \text{PCr} \) degradation, 32% from anaerobic glycolysis, 10% from stored ATP and 3% from aerobic metabolism. However, with the varying sprint demands of team-sports it is likely that this profile will alter with the sprint duration, number of sprints and the recovery period allowed.

When sprint distances similar to those in field-based team-sports are interspersed with 30 s recoveries post-sprint blood lactate values are higher after 30 and 40 m sprints compared to 15 m (Balsom et al., 1992a). Similarly, for 30 and 40 m sprints final times are significantly slower and plasma hypoxanthine, uric acid and oxygen uptake are
significantly elevated compared to the 15 m sprints (Balsom et al., 1992). Further, when repeated 5 s sprints are completed with 25, 50 and 100 s of passive or active recovery, peak power and muscle oxygenation is lower and percentage decrease in peak power higher in the 25 and 50 s recoveries during active compared to passive recovery (Ohya et al., 2013). Similar findings were reported by Dupont et al., (2004) using a different intermittent protocol and together these studies indicate that active recovery may interfere with PCr resynthesis and reoxygenation of myoglobin during intermittent sprinting. Taken together, these data serve to illustrate the importance of recovery duration and while it is suggested that short active recoveries up to sixty seconds can impede intermittent sprinting it is also important to note that during intermittent-sprint work using sprints similar to those observed in team-sports, performance can be sustained with recovery durations of 100 s and above even when fifteen sprints are completed.

Some of the decrease in performance in intermittent sprint exercise can be attributed to the decrease in the absolute contribution of PCr to the total ATP production during successive sprints. In addition, the reduction in glycogenolytic and glycolytic activity may contribute to the decrement in performance during exercise of this type (Glaister, 2005; Billaut and Bishop, 2009).

2.2.3. Reliability assessment in intermittent-sprint exercise

As previously identified (section 2.2.) the contribution of sprinting to the overall activity profile in field-based team-sports is small (1 - 1.5%; Mohr et al., 2003; Duthie et al., 2005; Cunniffe et al., 2009), but this activity frequently precedes significant moments in play and may be critical to the outcome of a match (Reilly, 1997; Spencer et al., 2004). Consequently, a number of performance-based protocols have been devised to examine the physiological and metabolic demands of activity in field-based sports. Many of these assess repeated sprinting ability, e.g. the Loughborough Intermittent Shuttle Test (LIST), but recent tests also incorporate sport specific movements e.g. lunging, tackling,
scrummaging, rucking and mauling (Nicholas et al., 2000; Roberts et al., 2010; Singh et al., 2010; MacLeod and Sunderland, 2012). Knowledge and understanding of the reliability or repeatability\(^1\) of such tests is essential to accurate interpretation of interventional change and the ability to distinguish worthwhile change from variation inherent in the test. Therefore, the reliability of these tests will be considered in this section.

As detailed in table 2.2., a number of statistical techniques have been used either singularly or in combination to determine reliability in intermittent sprint exercise, including typical error of measurement (TEM), coefficient of variation expressed as a percentage (CV,\%) , limits of agreement (LOA) and intra-class correlation coefficient (ICC). While LOA provide evidence of absolute reliability, it is different to TEM and CV as it assumes a population of individual test-retest differences (Atkinson and Nevill, 1998). Hopkins (2000a), however, suggests that LOA are restricted as they are biased by sample size, cannot be used in single trials and 95% limits are too stringent within an athletic population. Considering the aforementioned, TEM and ICC are being used more frequently to report reliability of measures. TEM is the average deviation from the mean and is the standard deviation in each participant’s measurements between tests after any shifts in the mean have been taken into account (Hopkins, 2000a). Although a low TEM is associated with reliable measures, there is limited evidence in the literature as to how this statistic should be interpreted (Atkinson and Nevill, 2001). However, guidelines for expected TEM for a range of physical tests are reported in the literature and a TEM score of less than 5% is recommended for physical tests (Tanner and Gore, 2013). ICC is designed to analyse repeated measures data on the same variable and is sensitive to changes in both the order and magnitude of the repeated values (Hopkins, 2000a). An ICC of a minimum of 0.80 is considered acceptable for physical measures (Lemmink et al., 2004).

\(^{1}\) Repeatability relates to the variation in repeat measurements on the same subject under identical conditions. Reliability relates the magnitude of the measurement error in observed measurements to the inherent variability in the ‘error-free’ or underlying level of the quantity between subjects (Bartlett and Frost, 2008). For the purposes of this thesis these terms will be used synonymously.
Table 2.2. Experimental design and statistical methods for reliability assessment of intermittent-sprint tests.

<table>
<thead>
<tr>
<th>Study</th>
<th>Sport (n=)</th>
<th>Design</th>
<th>Statistical method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capriotti et al., (1999)</td>
<td>Cycling (5M)</td>
<td>RM (10 x 7 s cycling sprints with 30 s recovery on six occasions)</td>
<td>RMA + CV(%)</td>
</tr>
<tr>
<td>Wragg et al., (2000)</td>
<td>Football (7 M)</td>
<td>RM (Modified Bangsbo running sprint test on six occasions)</td>
<td>CV(%) + CI; RMA</td>
</tr>
<tr>
<td>Nicholas et al., (2000)</td>
<td>Football (7M)</td>
<td>RM (LIST on two occasions)</td>
<td>LOA + RMA</td>
</tr>
<tr>
<td>Tong et al., (2001)</td>
<td>Rugby (27 M)</td>
<td>RM (3 x 6 s treadmill running sprints on two occasions)</td>
<td>CV(%) + RMA</td>
</tr>
<tr>
<td>Watt et al., (2002)</td>
<td>Cycling(8 M)</td>
<td>RM (2 x 30 s cycling sprints, 4 min rec on 4 occasions)</td>
<td>TEM</td>
</tr>
<tr>
<td>Lemmink et al.,(2004)</td>
<td>Field Hockey (22 M, 12F)</td>
<td>RM (3 x 30 m running sprints, 20 s rec, x 2 sets on two occasions)</td>
<td>LOA, RMA, ICC</td>
</tr>
<tr>
<td>McGawley &amp; Bishop  (2006)</td>
<td>Football (9 F)</td>
<td>RM (5 x 6 s cycling sprints, 24 s rec on five occasions)</td>
<td>RMA, SEM, CV(%) + ICC</td>
</tr>
<tr>
<td>Oliver et al., (2006)</td>
<td>(12 AM)</td>
<td>RM (field test – 7 x 30 m sprints + lab test – 7 x 5 s sprints on five occasions)</td>
<td>RMA + CV(%)</td>
</tr>
<tr>
<td>Spencer et al., (2006)</td>
<td>Field-hockey (10 M)</td>
<td>RM (6 x 30 m running sprints on two occasions)</td>
<td>TEM</td>
</tr>
<tr>
<td>Glaister et al., (2007)</td>
<td>(11M)</td>
<td>RM (12 x 30 m running sprints, 35 s rec on two occasions)</td>
<td>CV(%) + CI; ICC</td>
</tr>
<tr>
<td>Oliver et al., (2007)</td>
<td>(12AM)</td>
<td>RM (SSIET on two occasions)</td>
<td>CV(%) + CI</td>
</tr>
<tr>
<td>Glaister et al., (2009)</td>
<td>(12M,4F)</td>
<td>RM (8 x 40 m running sprints, 20 s rec x 4 + 12 x 35 m running sprints, 35 s rec on two occasions)</td>
<td>CV (%), ICC</td>
</tr>
<tr>
<td>Gabbett et al., (2010)</td>
<td>Football (10 F)</td>
<td>RM (6 x 20 m running sprints, 15 s rec on two occasions)</td>
<td>TEM + ICC</td>
</tr>
<tr>
<td>Glaister et al., (2010)</td>
<td>(20M)</td>
<td>RM (12 x 30 m running sprints, self-selected recovery on four occasions)</td>
<td>CV(%) + CI; ICC</td>
</tr>
<tr>
<td>Singh et al., (2010)</td>
<td>(11M)</td>
<td>RM (4 sets of intermittent running and game specific activity 2 x 2 trials)</td>
<td>TEM + ICC</td>
</tr>
<tr>
<td>DaSilva et al., (2011)</td>
<td>Football (24M)</td>
<td>RM (Carminatti’s test on two occasions)</td>
<td>CV(%) + CI; LOA</td>
</tr>
<tr>
<td>Mooney et al., (2011)</td>
<td>Australian football (26 M)</td>
<td>RM (IPRST on two occasions)</td>
<td>CV(%) + ICC</td>
</tr>
<tr>
<td>MacLeod &amp; Sunderland (2012)</td>
<td>Field-hockey (8F)</td>
<td>RM (FHIIP – 50 min on two occasions)</td>
<td>CV(%) + CI; ICC</td>
</tr>
</tbody>
</table>

M = Males; F = Females; AM = Adolescent males; RM = Repeated measures; FHIITP = Field hockey intermittent treadmill protocol; IPRST = Intermittent peak running speed test; SSIET = Soccer specific intermittent exercise test; CISP = Cycling intermittent sprint protocol; TEM = Typical error of measurement; ICC = Intra-class correlation coefficient; LOA = Limits of Agreement; CV = Coefficient of variation; CI = Confidence interval; RMA = Repeated measures ANOVA.

The majority of protocols used to assess reliability of intermittent-sprint exercise, as displayed in table 2.2. are running based and use a variety of durations, intensities, and
work-rest ratios. From these data (Table 2.2.), it appears that in running-based protocols, athletes are generally required to complete between 5 and 45 sprints over distance of 20 - 40 m, interspersed with 15 - 100 s of active and / or passive recovery (Nicholas et al., 2000; Wragg et al., 2000; Oliver et al, 2007) . Based on the criteria identified in the above paragraph, such protocols have demonstrated repeatability, logical and construct validity and are specific to the demands of the game. However, ecological validity aside, field-based tests of intermittent-sprint exercise are not necessarily conducive to sophisticated measurement of complex physiological and metabolic variables necessary to develop understanding of the demands of high intensity activity (McGawley and Bishop, 2006). Brief and prolonged cycling-based protocols have also been used to assess reliability in intermittent-sprint exercise (Capriotti et al., 1999; McGawley and Bishop, 2006) and have been proposed to be valid measures of sprint activity in games play (Bishop et al., 2001). However, many cycling-based protocols, typically comprise only 5 - 10, 6 s sprints and due to the truncated nature of the design, do not replicate intermittent-sprint activity across durations consistent with aforementioned team sports.

As identified earlier, field-based team-sport athletes may complete up to ~ 1500 movement changes in a game with alterations in activity occurring every 4 – 6 s. Many of the movements are game-specific, including such activities as jumping, lunging, heading, scrummaging, rucking, tackling and mauling. As such, a number of protocols have emerged incorporating specific elements of game play. (Roberts et al., 2010; Singh et al., 2010; MacLeod et al., 2012). While these protocols enhance ecological validity, demonstrate reliability and generally improve our understanding of the true demand of match play in field-based team-sports, the effect of simulated actions on subsequent performance is questionable (Singh et al., 2010) and the reliability of treadmill running is generally lower than in other modes (Tong, et al., 2001; Oliver et al., 2007).

The cycling intermittent-sprint protocol (CISP) comprises twenty, two minutes blocks of exercise with each block incorporating 10 s of passive rest, a 5 s sprint against 7.5% of body mass and 105 s of active recovery at 35% VO\textsubscript{2peak}. With a duration of forty minutes
the protocol permits examination of the effect of differing environmental factors and interventions, for example, heat stress and pre-cooling on intermittent-sprint performance over a time period similar to one half of a field-based team-sport using sprint frequencies, sprint durations and recovery times that are reflective of those observed in such sports. Previously, the CISP has been used to examine the effect of pre-cooling and heat acclimation on intermittent-sprint performance under heat stress (Castle et al., 2006, 2011) and has demonstrated significant improvements in PPO of 4 and 2%, respectively, with these interventions. The reliability of the CISP has, however, not previously been determined and therefore, it is unknown whether such findings reflect a real effect of either intervention or simply the inherent error of the test.

2.3. Thermoregulation and Heat Stress

2.3.1. Overview

Humans are homeothermic and maintain body temperature within a narrow range independent of external environmental change (Cheung et al., 2000; Bicego et al., 2007). In 1965 the set point model was developed to explain such thermoregulatory homeostasis proposing that perturbations in internal temperature, the error signal, when compared to an organism specific set point would evoke a compensatory response if a threshold was exceeded (Hammel, 1965 in Cabanac, 2006). This model suggested that the set point was not fixed, but adjustable and could be altered in response to differing thermoregulatory effectors including circadian and menstrual cues (Boulant, 2006). As such, in humans, internal body temperature in the rested state is generally maintained at 37 ± 1°C over the lifecycle (Cheung, 2010). Such thermoregulatory homeostasis is achieved through negative feedback incorporating afferent, integrative and efferent processes (Bicego et al., 2007). Perturbations from a desired set point elicit autonomic defence mechanisms that are stressor-dependent and serve to maintain core temperature at or approximate to the set point (Figure 2.4.). Primary warm defences include active cutaneous vasodilation and sweating that are triggered at approximately
the same core temperature (Sessler, 2008). Cutaneous vasoconstriction represents the first autonomic response to cold stress followed by shivering, with a threshold for activation approximately 1°C lower that occurs when primary mechanisms are insufficient to maintain core temperature (Sessler, 2008). It has been suggested the thresholds for inducing warm and cold responses differ by approximately 0.2°C, referred to as the inter-threshold range (Kurz, 2008). Temperature changes that fall within the inter-threshold range do not evoke a thermoregulatory defence response, however, perturbations beyond 0.2°C evoke an autonomic response (Kurz, 2008). Consequently, in humans, core temperature can generally be maintained within 0.2°C of the desired set point (Kurz, 2008).

Figure 2.4. The thermoregulatory control system (Sawka et al., 2011)

### 2.3.1.1. Mechanical efficiency and heat production

The efficiency of the human body is approximately 25% and consequently, greater than 70% of metabolic energy liberated to perform work is converted to heat (Verdaguer-Codina et al., 1993; Cheung, 2010). Therefore, in endurance events such as the marathon where high relative exercise intensity combines with high aerobic capacities, heat production can reach approximately 1500 W (Verdaguer-Codina et al, 1993). In
intermittent sports such as tennis and football where a larger mass is combined with more moderate aerobic capacities, estimated heat production can be 800 – 1300 W (Brotherhood, 2008). The heat content of the human body (BHC) is derived from mean skin temperature $T_{sk}$ and rectal temperature ($T_{re}$) (equation 2.1 and 2.2) and calculated as depicted in equation 2.3. where $m$ is the mass of the body, 3.47 is the specific heat capacity of body tissues (kJ.kg$^{-1}$·°C$^{-1}$) and body temperature ($T_{body}$) is the weighted mean of $T_{re}$ and $T_{sk}$ and 0.4 is a constant to account for the underestimation of core temperature change, as depicted in equation 2.2. Hypothetically, in a male team-sport athlete resting body heat content (assuming mass = 72 kg, $T_{re}$ = 37.5°C and $T_{sk}$ = 33°C) can be calculated as 9244 J and at the end of exercise in hot humid conditions (33°C, 50% rh, assuming $T_{re}$ = 39.1°C and $T_{sk}$ = 36.4°C) may rise to 9734 J.

**Equation 2.1. $T_{sk}$**

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

(Ramanathan, 1964)

**Equation 2.2. $T_{body}$**

$$\bar{T}_{body} = 0.8(T_{re}) + 0.2 (T_{sk}) + 0.4$$

(Jay and Kenney, 2007)

**Equation 2.3. Body heat content (BHC)**

$$BHC = \bar{T}_{body} \times m \times 3.47$$

(Jay and Kenney, 2007)

The overall effect of metabolic heat production on body heat content and body temperature can be ascertained from the heat balance equation;
Equation 2.4. The heat balance equation

\[ S = M - W \pm C_{res} - E_{res} \pm R \pm C \pm K - E_{sk} \]

where \( S \) is heat storage in the body, \( M \) is metabolic heat production, \( W \) is external work performed, \( C_{res} \) is respiratory convective heat exchange, \( E_{res} \) is respiratory evaporative heat loss and \( R, C, K \) and \( E \) represent radiative, convective and conductive heat exchange and evaporative heat loss, respectively (Aoyagi et al, 1997; Cheung et al., 2000).

2.3.1.2. Mechanisms of heat exchange

When thermoregulatory homeostasis is challenged heat loss or gain occur primarily via dry (conduction, convection, radiation) and wet (evaporation) heat exchange. During rest and exercise the relative contributions of these mechanisms to heat exchange varies. Radiative heat exchange involves the transfer of heat in the form of electromagnetic waves between the environment and the body. Effective functioning of this process is dependent upon a thermal gradient between the body and surrounding environment. At rest, radiation accounts for approximately 60% of total heat loss to the surroundings, however this is reduced to 5% during exercise. Skin temperature normally achieves balance at 34 – 37°C (Armstrong, 2000). When environmental temperature exceeds skin temperature, the thermal gradient between the body and the environment is reversed and radiation may represent a source of net heat gain.

Conductive heat exchange requires direct physical contact between objects. Heat exchange in this process is achieved by molecular agitation as molecules of greater heat collide with those that are cooler ensuring a net transfer of heat to the cooler body. As such, in the human body conduction provides a mechanism for heat exchange by transfer of heat from deep body tissues of greater temperature to cooler peripheral tissues. Convection also requires direct surface contact, but in this process the medium is usually air or water. In convection, heat transfer is by mass motion of air or water as the
molecules in contact with the body are heated, expand, become less dense and move away causing a convective current that transports heat away. In addition convective heat loss in the body can be achieved by varying cutaneous blood flow that can at rest reach flow levels of 7.5 l.min\(^{-1}\) (Sessler, 2008). At rest, conduction and convection contribute approximately 20% to heat loss, but this is reduced to 15% during exercise (Wendt et al., 2007).

Evaporation represents the primary defence mechanism to maintain thermoregulatory homeostasis during exercise. Each gram of sweat liberates approximately 0.6 kcal of heat while each litre of sweat evaporated, removes 2.4 MJ or 680 W.hr\(^{-1}\) of heat energy (Gleeson, 1998; Kenney, 1998; Kurz, 2008; Cheung 2010). Consequently, under appropriate environmental conditions a sweat rate of 1.3 – 2.0 L.hr\(^{-1}\) is required to maintain heat balance and thermoregulatory homeostasis in the aforementioned intermittent sports (Brotherhood, 2008). The primary factor determining the effectiveness of evaporative heat loss is the moisture content in the air, commonly expressed as relative humidity; the ratio of ambient vapour pressure to the saturated water vapour pressure (Kenney, 1998). Although evaporation can account for up to 80% of heat loss during exercise, a high ambient relative humidity increases sweat drippage, decreases vapoourisation of sweat and reduces the effectiveness of the evaporative process.

2.3.1.3. Temperature regulation

As previously described, thermoregulatory homeostasis is maintained through a mechanism of negative feedback involving afferent, integrative and efferent processes. Although thermoreceptors are present in the hypothalamus, cortex, spinal cord, deep abdominal and thoracic tissue (Insler and Sessler, 2006), Boulant (2000), proposes a hierarchy of structures with the hypothalamus, brainstem and spinal cord exerting control over thermoregulation. Lesion studies with animals demonstrate, however, that the brainstem and spinal cord provide only crude control of thermoregulation and that the
hypothalamus, including specifically the preoptic and anterior nuclei, is required for precise control and coordination of afferent and efferent signals (Hammel et al., 1960; Boulant, 2000; Cooper, 2002; Nagashima, 2006).

Forming the floor of the third ventricle, the hypothalamus is considered the primary regulator of thermoregulation (Kumar and Clark, 2012). It is bathed in cerebrospinal fluid, is thermally sensitive and therefore, can also be judged a central thermoreceptor (Benzinger, 1969). The preoptic area, a region at the junction of the telencephalon and diencephalon is considered the thermointegrative and thermosensitive site of the central nervous system (Bicego et al., 2007). This region contains a predominance of warm sensitive neurons enabling sensitivity to hypothalamic temperature to an accuracy of 0.01°C. It is proposed the thermosensitivity of such neurons is due in part to ion channels located in the cell body that are activated by thermal stimuli (Insler and Sessler, 2006; Nakamura, 2011). Increased signaling of warm sensitive neurons due to temperature change causes these ionic channels to open permitting charged ions to create an electrical potential that subsequently evokes a thermoregulatory response that is temperature dependent, for example, vasomotion (Nagashima, 2006; Nakamura, 2011).

The preoptic area of the hypothalamus also responds to afferent information from receptors in other body parts facilitated by perpendicular orientation of dendrites in warm sensitive neurons to the third ventricle (Bicego et al., 2007). Peripheral afferent thermal inputs are derived from cutaneous thermal receptors located up to 2.5 mm below the skin surface that exist as free nerve endings (Insler and Sessler 2006). Signals from warm and cold sensitive receptors are transmitted by unmyelinated C fibres and transverse A-delta fibres respectively (Kurz, 2008). Compared to warm receptors that are sensitive to temperatures of greater than 30°C, cutaneous cold receptors are sensitive to temperatures in the range of -5 to 43°C and number about ten times as many as warm receptors (Insler and Sessler, 2006). Thus a dominance of information from cold receptors highlights that peripheral thermosensitivity is more concerned with cold temperatures
allowing for immediate autonomic reflexes, for example cutaneous vasoconstriction and shivering, when skin cooling occurs (Insler and Sessler, 2006; Bicego et al., 2007).

Afferent signaling of thermoregulatory status may be derived from both core and peripheral cutaneous receptors. Nadel et al., (1971) and Frank et al., (1999), however, demonstrated that, with respect to autonomic defence mechanisms such as vasomotion, changes in core temperature exert a greater regulatory influence. In contrast, peripheral input from skin temperature influences thermal comfort more, providing earlier initiation of behavioural responses (Frank et al., 1999). As such, perception of low skin temperature, possibly via transient receptor potential M8 (TRPM8), or low core temperature by neurons in the preoptic anterior hypothalamus provides afferent feedforward signaling that initiates effector output (Nakamura, 2011). Consequently, when low skin or core temperature is perceived, cutaneous vasoconstriction, the first autonomic response to cold, is invoked to minimise heat loss by reducing skin blood flow. Cutaneous vasoconstriction is sympathetically mediated and reduced skin temperatures augment this response by inhibition of the nitric oxide system, vasoconstrictor postsynaptic up-regulation of α 2c receptors and cold sensitive afferents (Charkoudian, 2010; Johnson and Kellogg, 2010). In addition to cutaneous vasoconstriction, involuntary skeletal muscle tremor, that can increase metabolic heat production by 200%, is invoked in response to decreased skin or core temperature but the threshold is approximately 1°C lower than for cutaneous vasoconstriction (Bicego et al., 2007; Kurz, 2008). Heightened activity of temperature sensitive neurons in the preoptic area in response to decreased core temperature also evoke further heat generating mechanisms that are regulated by the posterior hypothalamus (Piantadosi, 2003). These include upregulation of thyroxine production due to hypothalamic neuropeptides that promote thyroid stimulating hormone release from the pituitary gland, and, sympathetically mediated release of cortisol (Piantadosi, 2003).

Perturbations in thermoregulatory homeostasis that evoke an increase in heat storage e.g. physical activity or high ambient temperature elicit autonomic and behavioural
responses designed to maintain core temperature at a desired set point (Jessen, 2001; Piantadosi, 2003). Such responses include the major autonomic effector responses of vasodilation and sweating that are activated at approximately the same time (Sessler, 2008). Although based on a muroid model, recently, Nakamura and Morrison (2010) demonstrated that cutaneous vasodilation may be mediated by neurons in the dorsal lateral parabrachial nucleus that glutaminergically transmit signals to the pre-optic anterior hypothalamus with subsequent efferent output via sympathetic cholinergic nerves. Mechanistically, cutaneous vasodilation is considered a response to actions of a number of factors including, nitric oxide, intestinal peptide, prostaglandins, and substance P (Charkoudian, 2010).

In addition to vasodilation, when core temperature increases above a desired set point efferent output from the preoptic hypothalamus is relayed by sympathetic cholinergic fibres that innervate the ducts and coils of the 1.6 – 4.0 million eccrine sweat glands in the human body (Shibasaki et al., 2006). Acetylcholine released from these cholinergic fibres binds to muscarine receptors, increases Ca^{2+} concentration in the gland and the permeability to K^+ and Cl^- and an isotonic precursor fluid is released from the secretory cells (Shibasaki et al., 2006).

2.3.1.4. Thermoregulation during exercise in the heat

During exercise muscular work promotes heat generation. Consequently, body temperature increases and the extent of increase is in direct proportion to the exercise intensity and percentage of VO_{2\text{max}} required (Nielsen, 1996). Theoretical estimates of heat production suggest core temperature could increase 1°C every 5 – 6 minutes in exercise, producing critical core temperatures within 10 – 15 min (Kenney and Johnson, 1992; Verdaguer-Codina et al., 1993). As such, effective functioning of thermoregulation is essential to maintain homeostasis and prevent a deleterious effect on performance or the occurrence of a heat related illness.
During exercise in a hot environment regulation of elevated body temperature is through activation of the autonomic heat loss mechanisms of vasodilation and increased sudomotor activity that are regulated predominantly by changes in core temperature (Franks et al., 1999; Sessler, 2008). In the heat the cardiovascular system is challenged by both thermoregulatory and metabolic demands dependent upon the duration, type and intensity of exercise and the environment (Gonzalez-Alonso, 2012). Thermoregulatory requirements necessitate an elevated skin blood flow yet active muscle oxygenation must be maintained. In maximal exercise under high heat stress maximal cutaneous blood flow may reach 7 - 8 L.min\(^{-1}\) or 300 - 400 ml.min\(^{-1}\).100g of skin and muscle blood flow 2.5 - 4 L.kg\(^{-1}.min\(^{-1}\) (Kenney and Johnson, 1992; Gonzalez-Alonso et al., 2008; Charkoudian, 2010; Johnson and Kellogg, 2010;). Combined, these would exceed cardiac pumping capacity and consequently, effective regulation is required during exercise in the heat (Gonzalez-Alonso et al., 2008; Crandall and Gonzalez-Alonso, 2010).

Skin circulation is modulated by a variety of thermoregulatory and non thermoregulatory reflexes, including central and peripheral temperature, blood pressure control, muscle mechanoreceptor and metaboreceptor stimulation (Gonzalez-Alonso et al., 2008). At the onset of exercise in the heat there is an initial cutaneous vasoconstriction (Kenney and Johnson, 1992; Gonzalez-Alonso et al., 2008). Subsequently, increases in body temperature and activation of cutaneous vasodilation increase skin blood flow (Gonzalez-Alonso et al., 2000). This additional demand is met by a 1 - 2 L.min\(^{-1}\) increase in cardiac output, mediated by an increased heart rate and decreased perfusion of splanchnic and renal tissue (Rowell et al., 1966; Gonzalez-Alonso et al., 2000). The extent of cutaneous vasodilation in exercise and therefore skin blood flow is modulated by an elevated temperature threshold for vasodilation compared to rest and a limit to skin blood flow at a core temperature of ~ 38°C (Gonzalez-Alonso et al., 2008).

During exercise in the heat elevated plasma noradrenaline levels suggest increased sympathetic vasoconstrictor activity in active muscle vasculature to promote effective thermoregulation. However, augmented levels of vasodilator substances offset this effect
and consequently, vascular conductance in active skeletal muscle is maintained. (Crandall and Gonzalez-Alonso, 2010). As such, in the early stages of low-to-moderate intensity exercise in the heat muscle blood flow is maintained and metabolic requirements of exercise are fulfilled. Similarly, mean arterial pressure is preserved. Therefore, as perfusion of cutaneous vasculature is moderated, requirements for thermoregulation are not achieved and core temperature is increased (Gonzalez-Alonso, 2012).

Increased core temperature during exercise elicits an enhanced sudomotor response including an initial increase in number of active eccrine glands and an increase in output (Kondo et al., 2001). When exercise is extended beyond eight minutes further increases in sudomotor function are achieved by additional increases in gland output (Kondo et al., 2001). Evaporation of one litre of sweat may liberate 2.4 MJ or 680 W.hr\(^{-1}\) of heat energy (Gleeson, 1998; Kenney, 1998; Kurz, 2008; Cheung 2010). Although this process contributes to 80% of heat loss in exercise, under high heat stress and in particular high humidity, limited evaporative potential may exist and sweat gland output may be reduced due to hidromeiosis. Under such conditions sustained sudomotor activity may contribute to exercise-heat dehydration and elevated heat strain (Equation 2.4.).

When moderate-to-high intensity exercise in the heat is prolonged, elevated cardiovascular strain is observed even in euhydrated participants with ad libitum water intake. Rowell et al., (1966), reported a 150 – 200 ml reduction in central blood volume, 19 – 23 ml reduction in stroke volume, 21 – 29 b.min\(^{-1}\) increase in heart rate and a 1.1 – 1.2 L reduction in cardiac output during walking at 3.5 m.hr\(^{-1}\) on 12.5 and 15% gradients in 43.3°C compared to 25.6°C. With sweat loss in excess of 3 L.hr\(^{-1}\) reported in the heat and maximal gastric emptying rates of only 600 – 800 ml.hr\(^{-1}\) prolonged exercise in the heat without water intake will induce profound dehydration and this also significantly elevates cardiovascular strain with increases in heart rate and decreases in stroke volume and cardiac output directly related to the magnitude of dehydration (Montain and Coyle, 1992; Gonzalez-Alonso et al., 2000). Exercise to exhaustion at 61 ± 2% \(\text{VO}_{2}\)\text{\textsubscript{max}} in 35°C, 40 - 50% rh with dehydration during exercise (3.9 ± 0.3% body weight on cessation of
exercise) resulted in a 3.3 ± 0.6 l.min\(^{-1}\) decrease in cardiac output, 2.0 ± 0.6 l.min\(^{-1}\) decrease in leg blood flow, 29% decrease in skin blood flow, 28 b.min\(^{-1}\) increase in heart rate, a 40 ml reduction in stroke volume and significant reductions in systolic and diastolic blood pressure at the end of exercise in comparison to a euhydrated control (Gonzalez-Alonso et al., 1998).

Further, Gonzalez-Alonso et al., (2000) demonstrated a 6.4 ± 1.3 % decrease in stroke volume per percent of dehydration, 11 - 14 b.min\(^{-1}\) higher heart rate and decreased mean arterial blood pressure when participants performed 30 minutes of cycling at 72% VO\(_{2}\)max in hot (35°C) or cold (8°C) conditions when euhydrated or dehydrated by 1.5, 3.0 and 4.2% of body weight. Similarly, blood volume was depressed 200 - 300ml in the heat and cardiac output was reduced at the higher levels of dehydration compared to euhydration (Gonzalez-Alonso et al., 2000). Under such circumstances thermoregulatory and metabolic responses are changed. Reduced blood volume and pressure reduces ventricular filling pressure and diastolic filling time is decreased due to tachycardia (Gonzalez-Alonso et al., 2000). Consequently, stroke volume and cardiac output are reduced and skin blood flow declines leading to impaired thermoregulation, elevated core temperature and decreased exercise performance (Gonzalez-Alonso et al., 2000; Crandall and Gonzalez Alonso, 2010).

Much work has been completed on the effects of heat stress on thermoregulation during low to moderate intensity exercise, prolonged exercise, and high intensity exercise to exhaustion with and without dehydration. Less is known about the thermoregulatory dynamics associated with high intensity, intermittent-sprint exercise in the heat and the additional effect of dehydration. Although no information exists with respect to cardiac output, stroke volume and skin blood flow, evidence of elevated heart rate and reduced whole-body \(^{\text{VO}_2}\) during intermittent-sprint exercise in the heat compared to control conditions in euhydrated and hypohydrated states would indirectly suggest elevated cardiovascular strain and reduced active muscle perfusion (Maxwell et al., 1996; Morris et al., 1998; Drust et al., 2005; Sunderland et al., 2005; Maxwell et al., 2009). With respect to
metabolic demand during intermittent-sprint exercise, Racinais et al., (2007) have demonstrated a progressive muscle deoxygenation using near infra-red spectroscopy when 10 x 6 s sprints with 30 s recovery were performed in temperate conditions, suggesting a mismatch between supply and demand that contributed to elevated muscle anaerobiosis (Racinais et al., 2007). Whether this response is exacerbated in hot conditions in intermittent sprinting is unknown.

2.3.2. Climatic heat stress and Indices for assessment

Climatic heat stress\(^2\) has been defined as a “combination of environmental conditions that stress the body’s thermoregulatory system” (Buskirk, 1977). Conditions determining the level of climatic heat stress include; ambient temperature, air humidity, air movement and radiant heat (Verdaguer-Codina et al., 1993).

It is approximately 250 years since researchers recognized that the thermal stress of an environment was dependent upon other factors in addition to temperature and approximately 230 years since the first documented experiments in this field (Ellis, 1758; Blagden, 1775). Since then, numerous investigators have attempted to quantify the thermal stress of the environment using developed measures of heat stress or physiologic strain and there is evidence of over forty documented indices in the literature\(^3\) (Epstein et al., 2006). As early as 1905 Haldane proposed the wet-bulb temperature as the most appropriate measure to express heat stress (MacPherson, 1962). In an effort to quantify the additive effect of radiant heat to thermal stress the globe thermometer temperature was introduced in 1930 (Vernon, 1930). Dysfunction in specific environmental conditions leading to incorrect representation of physiologic strain demonstrated practical limitations that resulted in the abandonment of this index as a measure of thermal stress.

\(^2\) Heat stress: A combination of environmental conditions that stress the body’s thermoregulatory system.

\(^3\) A heat stress index is a single value that integrates the effect of the basic parameters in any human thermal environment such that its value will vary with the thermal strain experienced by the individual (Parsons, 2003).
soon after its inception. In 1947 the predicted four hour sweat rate (P4SR), an index based on physiological strain, was developed and considered a significant improvement on the nine previous indices presented between 1905 and 1947. In addition to key environmental factors of ambient temperature, surrounding air temperature, humidity and air movement, P4SR considered the rate of energy expenditure and the clothing worn, but was limited to indoor use only (MacPherson, 1962).

In 1957 Wet Bulb Globe Temperature (WBGT) was presented as a measure of heat stress (Yaglou and Minard, 1957). An International Standard (ISO 7243) and widely used for the determination of heat stress in military, occupational and sporting environments, WBGT combines the effect of humidity, wind, environmental temperature in the shade, sun and surface radiation to calculate the heat stress index (Verdaguer-Codina et al., 1993). Developed from research concerned with preventing the occurrence of heat injury in military recruits in Parris Island, WBGT was originally intended for use in an outdoor environment (Budd, 2008). Outdoor WBGT heat stress index was derived from wet bulb temperature (WBT), black globe temperature (BGT) and dry bulb temperature (DBT) and calculated as shown in equation 2.5;

\[
\text{Equation 2.5. Wet bulb globe temperature (outdoor)}
\]

\[
\text{WBGT} = 0.7 \times \text{WBT} + 0.2 \times \text{BGT} + 0.1 \times \text{DBT}
\]

(Verdaguer-Codina et al., 1993).

WBGT was later modified to permit determination of heat stress indoors on the assumption that in an indoor environment globe temperature approximates ambient temperature. In this context heat stress was calculated as shown in equation 2.6;
Equation 2.6. Wet bulb globe temperature (indoor)

\[ \text{WBGT} = 0.7 \times \text{WBT} + 0.3 \times \text{DBT} \]

(Verdaguer-Codina et al, 1993; Epstein et al., 2006).

Limitations of WBGT as an index of heat stress include inadequate reflection of the additional strain experienced when evaporation is limited and the omission of globe temperature when determining heat stress for an indoor environment (Budd, 2008).

In addition to WBGT, the discomfort index (DI) has been used as an index of heat stress for over forty years. Originally proposed by Thom (1959) and later modified by Sohar et al., (1963) heat stress is simply calculated from two variables (Equation 2.7.), wet bulb and dry bulb as;

Equation 2.7. The discomfort index

\[ \text{DI} = 0.5 \times \text{WBT} + 0.5 \times \text{DBT} \]

(Epstein et al., 2006).

Adopted by the Israeli Defense Forces, DI was adapted in 1999 to its current state, the modified discomfort index (MDI) to reflect improved statistical analyses. MDI is calculated as shown in equation 2.8. and has been shown to be highly correlated to WBGT (Moran et al., 1999), but does not consider key environmental factor such as solar radiation.

Equation 2.8. The modified discomfort index

\[ 0.75 \times \text{WBT} + 0.3 \times \text{DBT} \]

(Moran et al., 1999)
The WBGT, despite adoption by the US military, World Health Organisation and the American College of Sports medicine is difficult to measure in the field due to issues with measuring globe temperature. Recent technological developments have, however, led to the production of heat stress metres that can accurately quantify WBGT in outdoor and indoor surroundings, for example, the HT30 WBGT meter (EXTECH, New Hampshire). Nevertheless, previous research has demonstrated WBGT does not reflect physiologic strain (Ramnathan et al., 1973). In 1998 the physiological strain index (PSI) was introduced to permit quantification of the strain imposed by hot environments using measurements of core temperature and heart rate. PSI has been shown to accurately reflect both strain in different climatic conditions during heat stress (Moran et al., 1998a) and hydration (Moran et al., 1998b), is derived from resting core temperature ($T_{c0}$), core temperature at a given timepoint ($T_{ct}$), resting heart rate ($f_{c0}$) and heart rate at a given timepoint ($f_{ct}$) and is calculated as:

**Equation 2.9. The physiological strain index**

$$5(T_{ct} - T_{c0})(39.5 - T_{c0})^{-1} + 5 (f_{ct} - f_{c0})(180 - f_{c0})^{-1}$$

(Moran et al., 1998a)

Continued development of environmental monitoring technology that permits easier and faster measurement in field situations resulted in the development of the environmental stress index (ESI) as an alternative to WBGT in 2001 (Moran et al., 2001). ESI employs ambient temperature ($T_a$), solar radiation (SR) and relative humidity (rh) to determine heat stress as:

**Equation 2.10. The environmental stress index**

$$0.63T_a - 0.03rh + 0.002SR + 0.0054(T_a \times rh) - 0.073(0.1+SR)^{-1}$$

(Moran et al., 2001)
Research has demonstrated high correlation with the WBGT and therefore, ESI is proposed as a practical alternative to WBGT in field situations where determination of globe temperature is difficult (Moran et al., 2001).

Studies examining intermittent-sprint exercise, heat stress and the effect of different compositions of environmental factors on physiological and performance responses during intermittent-sprint exercise should match environments for overall heat stress using, for example, WBGT. However, a significant portion of the literature in this field merely reports ambient temperature with or without relative humidity, and makes no reference to any index of heat stress. Research has demonstrated composite environmental indices provide better indicators of environmental influence than individual factors alone (Zhang et al., 1992) and as such, studies comparing differing compositions of environmental factors should use an appropriate index to measure and match heat stress e.g. WBGT, the accepted international standard for the quantification of heat stress (Nevill et al., 1995; Morris et al., 1998; Morris et al., 2000; Parsons, 2006). Where this is not done, comparison between studies is made difficult and many discrepancies in findings may simply be explained by differences in WBGT employed by investigators. In addition, where investigators have considered heat stress in terms of WBGT and the impact of differing compositions of thermal factors on physiological responses, evidence can only be drawn from sub-maximal exercise and conditions have not always been equally matched for heat stress (Fox et al., 1967, Griefahn, 1997; Maughan et al., 2012). Further work is required to elucidate the impact of differing compositions of thermal factors on high-intensity intermittent-sprint exercise in the heat when environments are matched for heat stress.

2.3.3. Intermittent-sprint exercise in a hot environment

Intermittent-sprint exercise presents a significant physiological challenge to the games player and with international competition increasingly conducted in hot environments, performance in such activity is further challenged. There is greater thermal strain during
intermittent-sprint exercise in the heat compared with continuous exercise of the same average work intensity (Nevill et al., 1995). Demand for cardiac output is increased due to the elevated skin blood flow needed to maintain thermoregulatory homeostasis and the elevated metabolic requirements of exercise (Maxwell et al., 1996). Reduced perfusion of muscle and the concomitant reduction in oxygen supply to the tissue increase the reliance on anaerobic metabolism. Of the limited research on this matter no effect, a beneficial effect and a deleterious effect of heat on performance and physiological responses to intermittent-sprinting have all been reported (Falk et al., 1998; Backx et al., 2000; Linnane et al., 2004). This section will begin by considering research that has used two or more sprints of up to 30 s duration and move to a focus on work that has concerned itself with intermittent-sprint exercise observed in field-based team-sports characterized by short duration sprints (≤ 10 s) with moderate-to-long recovery periods (60–300 s), allowing near complete recovery (Girard et al., 2011). A composite bar chart illustrating the extent of performance improvement or impairment for the studies cited in this section is provided in figure 2.5.

When three, 30 s sprints were completed pre and post sixty min recovery in hot humid, hot dry and temperate conditions, no effect on performance or metabolic responses was observed (Backx et al., 2000). In contrast, using passive water immersion for 16 ± 3.2 minutes at 43°C, 30 s sprint performance was improved compared to a no immersion trial but subsequent sprint performance after 4 min recovery was unchanged (Linnane et al., 2004, Figure 2.5). A temperature mediated increase in cross bridge cycling and pedal cadence was suggested to explain the initial ergogenic effect (Linnane et al., 2004) and lack of this response in the work of Backx et al., (2000) owing to the recovery method used may explain the discrepancy in studies.

With respect to multiple sprints, when five 15 s efforts with 30 s recovery were repeated after sixty minutes of rest in hot compared to temperate conditions performance was improved, but no difference in metabolic variables was reported (Falk et al., 1998, Figure 2.5). In contrast, when 20 s sprints with 100 s recovery were completed to exhaustion on
a treadmill at 10.5% incline and 1.2 km.hr\(^{-1}\) increments in speed, cumulative sprint time was significantly lower in hot compared to control conditions possibly due to a dehydration induced increase in intramuscular H\(^+\) concentration (Maxwell et al., 1996, Figure 2.5.). Sweat rate, heart rate and rectal temperature were significantly higher post sprints in the hot trials, but plasma ammonia and lactate were not different between trials (Maxwell et al., 1996). The exhaustive nature of the protocol and the subsequent dehydration may explain the discrepancy between the work of Maxwell and colleagues (1996) and Falk et al., (1998).

Further evidence of the detrimental effect of heat on multiple sprint performance was also reported by Drust et al., (2005, Figure 2.5.). When repeated sprint bouts were interspersed with 40 minutes of intermittent exercise in 40 and 20°C mean blood glucose and noradrenaline and all measures of physiological strain were higher in 40°C while glycogen utilisation tended to be greater (P = 0.06; Drust et al., 2005). Further, during repeated sprints in the heat mean power output was lower, plasma NH\(_3\) was higher, plasma K\(^+\) and muscle H\(^+\) were lower, but muscle glycogen, CP, ATP and IMP were unchanged (Mohr et al., 2006). Decrements in performance were not explained by metabolic factors, but a trend toward a reduction in isometric handgrip force in hot compared to the control trial indicated a possible reduction in voluntary drive mediated by hyperthermia and / or elevated plasma NH\(_3\). While this work was one of the first to postulate a neuromuscular mediated fatigue during intermittent-sprint exercise, it is important to note however, that the use of isometric handgrip provides insight to the general state of CNS function, but does not provide evidence of the voluntary drive to cycling specific locomotor muscles.

When multiple sprint work is completed in the heat to exhaustion or in a repeated-sprint ability style (6 s sprints < 30 s recovery) following 40 minutes of activity, a detrimental effect of heat on performance is observed. In contrast, Almudehki et al., (2012) recently reported no effect on performance when eight, 6 s sprints were interspersed with 5 minutes recovery (Almudehki et al., 2012). Peak power output and neuromuscular
efficiency were not different in 40 compared to 24°C despite higher cardiovascular and perceptual strain in the hot trial (Almudehki et al., 2012) suggesting that the extent of recovery between multiple sprints and the metabolic consequences may have been a primary factor in reduced performance in the heat. Nevertheless, it is known when short sprints are interspersed with 2 minutes recovery performance is not reduced (Balsom et al., 1992). As such, while the work of Almudehki et al., (2012) serves to isolate and elucidate the effect of heat on intermittent-sprinting without confounding metabolic influences, the lack of difference in performance may be explained by the recovery period used. Further, the protocol did not induce marked hyperthermia in the hot trial (37.7 ± 0.4°C). Given that field-based team-sport athletes routinely complete twenty to sixty sprints per game with mean recovery of ~ 147 s, and core temperatures of 39.6 ± 0.1°C have been reported at the end of the first half of a match in 43°C, the ecological validity of this study is questionable (Spencer et al., 2005; Mohr et al., 2012). Therefore, a focus on research that uses protocols reflective of the durations and work-rest patterns of team-sports is warranted.

Research employing intermittent-sprint protocols that are exhaustive or simulate activity patterns of team-sports have demonstrated decreased performance in the heat (Morris et al., 1998, 2000; Sunderland et al., 2005; Castle et al., 2011; Figure 2.5.) In cycle-based exercise, when twenty, two minute blocks each comprising a 5 s sprint, 105 s recovery at 35% $\dot{V}O_{2max}$ and 10 s were completed in ~ 33°C peak power output was reduced (Figure 2.5.) and physiological strain was higher in the heat (Castle et al., 2011). Lack of a temperate condition in this study does, however, limit the ability to draw conclusions about the effect of heat separate from the effect of intermittent-sprint exercise.
Figure 2.5. Percentage change in sprint performance from control. PPO = peak power output; MPO = mean power output; TDR = total distance run; TRT = total run time; ST = sprint time; * = Significant difference from control.

In running-based protocols, use of the Loughborough intermittent shuttle test (LIST) to replicate the activity pattern of field-based team-sports has demonstrated a deleterious effect of heat on intermittent-sprint exercise in a number of studies using male and female athletes. Generally, when completed in hot compared to temperate conditions, total distance run by male and female athletes during the LIST is reduced (Morris et al., 1998; 2000; 2005) or tends to decline (Sunderland and Nevill, 2005). Further, although one study found no difference (Morris et al., 1998) 15 m sprint times during the LIST were reduced in hot compared to temperate conditions (Morris et al., 2000; 2005; Sunderland and Nevill, 2005). In the heat, physiological strain and RPE during the LIST were higher compared to temperate conditions (Morris et al., 2000; 2005; Sunderland and Nevill,
2005), but metabolic markers including blood lactate and plasma ammonia were not different (Morris et al., 1998; 2000; Sunderland and Nevill, 2005). A trend toward greater muscle glycogen usage in the heat and greater muscle glycogen remaining in the muscle at exhaustion in the hot trial has, however, been reported in one study coupled with greater blood lactate, glucose, serum cortisol and catecholamines (Morris et al., 2005) in contrast to previous work (Maxwell et al., 1996; Drust et al., 2005). In addition completion of the LIST in hot conditions can induce a significant decrement in hockey skill performance (Sunderland and Nevill, 2005). Finally, in studies using the LIST to examine the effect of heat on intermittent-sprint exercise a strong negative correlation has been reported (r > 0.9) for rate of rise in rectal temperature and distance run. Taken together with the consistent lack of difference in metabolic markers between trials, it has been suggested that hyperthermia mediated decrements in CNS function may explain the reduction in performance observed in these studies.

More recently, evidence from field-based studies has served to elucidate the impact of heat on intermittent activity. When twenty professional football players completed a full football match in ~ 31°C high intensity running was reduced by ~ 57 % in the final compared to the first 15 minute interval and repeated sprints over 30 m were reduced by 2.6% (Mohr et al., 2010). No difference in mean blood lactate or heart rate was observed between halves, but muscle temperature was 0.8 ± 0.2°C lower in the second half of the match. Lack of a control group in this study limits the ability to draw conclusions about whether the observed findings were mediated by the environment or the activity. In a further study, Mohr et al., (2012) addressed this issue by examining football performance in elite players during football match play at ~ 43°C compared to ~ 21°C. Total game distance and high intensity running declined by 7% and 26% respectively, compared to control. Peak sprint speed during the match was 4% higher in the hot trial, but post match sprint performance was not different (Mohr et al., 2012). Muscle glycogen tended to decline (488 ± 25 mmol.kg⁻¹ vs. 300 mmol.kg⁻¹ baseline and post-exercise dry weight respectively), but was not different between trials (Nybo et al., 2013). Similar to previous studies no difference was observed in average heart rate, plasma lactate and body weight loss (Mohr et al., 2012). In this study CNS function was assessed from voluntary activation
using the interpolated twitch technique and post exercise the reduction was similar in both trials (~1.5%) indicating that during match play central drive is not affected to a greater extent by heat. Application of findings is, however, limited by use of the interpolated twitch technique (see section 2.4.4.3.) and the self-paced nature of the match that contributed to reduced work during the hot trial possibly impacting the extent of fatigue observed.

2.3.4. Summary

When intermittent-sprint exercise similar to that observed in field-based team-sports is completed using cycling or running-based protocols in hot compared to temperature condition, the overwhelming evidence indicates that performance is decreased. Further, physiological strain is increased possibly reflecting compensation for a reduced cardiac output and decreased skin blood flow, but evidence of such physiological sequelae is lacking for intermittent-sprint sports. Although increased physiological strain may limit muscle blood flow and increase reliance on anaerobic metabolism, generally, no differences in metabolic variables are observed during intermittent-sprint exercise in the heat compared to control. This suggests intermittent-sprint exercise taxes the glycolytic system maximally and heat does not have an additive effect, indicating the cause of reduced performance in exercise of this type is not metabolic.

A number of earlier studies have demonstrated a reduced performance during intermittent-sprint exercise in the heat in the presence of marked endogenous hyperthermia and a strong negative relationship has strengthened the suggestion that hyperthermia mediated effects on CNS function contribute to the decrements in performance. Recent work has, however, provided evidence that the decrements in voluntary activation post exercise are not different in hot or temperate conditions but application of findings is possibly limited by methodological issues. Considering this, there is a need for further work examining the genesis of performance decrements during
intermittent-sprint exercise in the heat where work:rest ratios replicate those of field-based team-sports.

2.4. Fatigue

2.4.1. Overview

Fatigue is a universal phenomenon commonly observed during exercise. In this context, fatigue manifests as increased sense of effort and reduced force output soon after the onset of activity, but before task termination (Enoka and Duchateau, 2008) and can be observed as decreased power output during the exercise (Nybo and Nielsen, 2001a&c; Tucker et al., 2004). The genesis of fatigue is multifactorial but displays task dependency indicating that rather than an omnipresent mechanism fatigue may be induced by a variety of mechanisms (Enoka and Stuart, 1992). For the purposes of this review fatigue will be examined from a neuromuscular perspective.

Neuromuscular fatigue has been described as any exercise-induced reduction in the ability of a muscle or group of muscles to generate force or power whether or not the task can be sustained (Gandevia, 2001; Taylor et al., 2006; Ross et al., 2007; Taylor and Gandevia, 2008; Sidhu et al., 2009a; Millet et al., 2011). If muscle is viewed from the perspective of a motor in a system then its effective functioning will depend on its intrinsic properties and state in addition to how the muscle is ‘driven’ and how feedback affects its operation (Gandevia, 2001). As such, neuromuscular fatigue can be considered to consist of both peripheral and central aspects (Figure 2.6.).
2.4.2. Central fatigue

Central fatigue is a progressive exercise induced reduction in voluntary activation or neural drive to the muscle and is considered to comprise both spinal and supraspinal components (Gandevia, 2001; Taylor et al., 2006). Supraspinal fatigue has been described as an exercise-induced decline in force, caused due to suboptimal output from the motor cortex (Gandevia, 2001; Taylor et al., 2006).

2.4.2.1. Mechanisms of spinal fatigue

During sustained submaximal isometric contraction at 30% MVC the discharge rate of human muscle spindles (group Ia afferents) has been shown to decline up to 50% (Macefield et al., 1991). Such reduced firing rate may contribute to spinal mediated fatigue through disfacilitation of α motoneurons (Gandevia, 2001; Boyas and Guevel, 2011). More recently, however, McNeil et al., (2011) demonstrated that reduced spindle
discharge was not a contributor to reduced motoneurone excitability observed in fatigue and suggested motoneurone intrinsic property changes contributed to a reduced responsiveness to excitatory input. It is important to note however, that the data provided is for sustained isometric exercise and little is known of how fatigue can alter spindle discharge during dynamic activity.

Golgi tendon organs may also exert a role in spinal fatigue as discharge of these group Ib afferents has been suggested to decrease with the reductions in muscle force observed during maximal contractions (Taylor and Gandevia, 2008). In addition, such afferents are suggested to exert an inhibitory effect on motoneurone activity (Boyas and Guevel, 2011). Group III and IV afferents innervate free nerve endings throughout muscle and are responsive to alterations in the mechanical, biochemical and thermal state of the tissue (Gandevia, 2001). During exercise, alterations in the state of muscle augments the discharge rate of these afferents providing inhibitory input to the CNS limiting central motor drive and performance (Amann et al., 2013). The inhibitory action of such afferents on motor drive has been confirmed in high-intensity exercise by reducing sensory feedback using pharmacological blockade or augmenting inhibitory feedback (Amann and Dempsey, 2008; Amann, 2011), but there is still much debate about their role in fatigue (Taylor and Gandevia, 2008; Perrey et al., 2010a).

Renshaw cells provide autogenic inhibition to motoneurons and receive excitatory input from high threshold motoneurons in addition to descending and peripheral inputs (Gandevia, 1998, 2001). Evidence on the role of Renshaw cells in fatigue during sustained voluntary contractions is contradictory with reduced inhibition evident in sustained submaximal voluntary contractions using H-reflex methods, but increased inhibition observed with maximal efforts (Gandevia, 1998, 2001). Intrinsic properties of the motoneurone have also been identified as possible mediators of spinal fatigue. Motoneurones alter their firing rate when stimulated with intracellular or extracellular current and the pattern of discharge varies over time (Gandevia, 2001). Motoneurone
gain decreases over time depressing discharge rate and potentially contributing to fatigue (Gandevia, 1998, 2001).

2.4.2.2. Mechanisms of supraspinal fatigue

Mechanisms of supraspinal fatigue can be divided into those that result in a decrease in motor cortical output, or those that do not alter the extent of cortical output but reduce the effectiveness of descending drive (Taylor et al., 2006). This can result from decreases in excitatory input, increases in inhibitory input or changes in intrinsic motoneuron properties (Taylor et al., 2006; Taylor and Gandevia, 2008).

Short latency motor evoked potentials (MEPs), and cortical silent periods are excitatory and inhibitory responses respectively, recorded in EMG when TMS is applied over the motor cortex during an MVC. Examination of MEP characteristics e.g. amplitude and silent period duration provides evidence of both the excitatory and inhibitory responses of the corticospinal tract to exercise and has been examined primarily during sustained and repetitive maximal and submaximal voluntary efforts following sustained locomotor activity (Loscher et al., 2002; Sogaard et al., 2006; Ross et al., 2007; Smith et al., 2007; Sidhu et al, 2012a&b). MEP amplitudes have been shown to be increased with sustained MVC suggesting increased motor cortical excitability and drive to motoneurons (Sidhu et al., 2009a). Similarly, silent period duration is shown to increase with sustained MVC (Sidhu et al., 2009a). As the latter part of the silent period is thought to correspond to inhibition of voluntary descending drive by action of the intracortical inhibitory neurons any observation of an increased silent period duration may reflect increased intracortical inhibitory action contributing to supraspinal fatigue (Taylor et al., 1996, 2008).

Sidhu and colleagues (2012a) propose that observed differences in opposing corticospinal excitability responses between studies can be explained by task specificity and demand. Therefore, observations of unchanged corticospinal excitability and increased intracortical
inhibition with sustained locomotor exercise compared to brief MVCs may simply reflect greater physiological demand with sustained work. This demand may prolong group III and IV muscle afferent activity, as discussed, and provide increased feedback to the CNS at a level upstream of the motor cortical outputs to impair voluntary descending drive once again providing support for the inhibitory feedback hypothesis (Taylor et al., 2006; Sidhu et al., 2009a, 2012a).

In addition to afferent feedback, other factors that may induce supraspinal fatigue, include alterations in brain neurotransmitters, increased cerebral ammonia concentration and altered GABA levels.

2.4.2.3. The Central neurotransmitters serotonin, dopamine and noradrenaline

Neurohumoral factors and their accumulation or depletion have been implicated in fatigue during prolonged exercise in both temperate and hot environments. Originally, Romanowski and Grabiec (1974) cited the monoamine neurotransmitter serotonin (5-Hydroxytryptamine, 5-HT) as a mediator of central fatigue (Roelands and Meeusen, 2010). Newsholme and colleagues (1987) later proposed the original central fatigue hypothesis to elucidate the role of increased cerebral serotonin concentration on neurotransmission and fatigue during prolonged exercise. 5-HT regulates an array of physiological functions and states including mood, anxiety and alertness (Kroeze et al., 2002). As 5-HT cannot cross the blood brain barrier cerebral serotonergic neurons synthesise the compound from the amino acid tryptophan (TRP) and consequently, TRP transport across the blood brain barrier functions as a rate limiting step (Newsholme and Blomstrand, 2006). During prolonged exercise altered substrate utilisation elevates plasma FFA concentration promoting uncoupling of TRP from albumin and elevation of free TRP concentrations. Concomitantly, exercise-induced BCAA oxidation may decrease humoral BCAA concentrations altering the free TRP:BCAA ratio (Davis, 1995; Meeusen et al., 2006). As TRP transport across the blood brain barrier is via the same carrier utilised by the BCAA, cerebral TRP increases and elevates 5-HT production possibly resulting in
increased lethargy, loss of motivation and central drive during prolonged exercise and ultimately fatigue. However, while a theoretical basis for a role of 5-HT in fatigue exists, evidence from human studies is equivocal. Pharmacological intervention to augment 5-HT has shown no effect on time to exhaustion or time trial performance in ≥ 30°C (Strachan et al., 2004, 2005; Roelands et al., 2009), but does produce increased core and skin temperatures (Strachan et al., 2005; Roelands et al., 2009), possibly due to the hyperthermic effects of 5-HT on the pre-optic hypothalamus (Soares et al., 2007).

Dopamine and noradrenaline share a common biosynthesis pathway and control a number of biological functions / states including locomotor activity, cognition, emotion, arousal, motivation, endocrine regulation, mood and reward systems (Missale et al., 1998; Roelands and Meeusen, 2010). Depletion of these neurotransmitters has been implicated in fatigue during exercise (Davis, 1997; Watson et al., 2005) and research has indicated that dopamine manipulation has the potential to improve performance during exercise in the heat by maintenance of motivation and arousal and overriding of inhibitory signals from the CNS that would otherwise result in a hyperthermia mediated reduction in power output. (Watson et al., 2005; Roelands et al., 2008a, 2008b, 2013).

Existing research demonstrates that 5-HT concentration is increased and dopamine concentration decreased at the point of fatigue in exercise due to FFA mediated increases in free TRP coupled with increased BCAA oxidation altering the competitive transport of TRP and tyrosine, precursors to serotonin and dopamine, across the blood brain barrier (Cansev and Wurtman, 2007; Meeusen and Watson, 2007). Considered the revised central fatigue hypothesis (Davis, 1997; Meeusen and Watson, 2007), research examining the effect of an altered ratio of serotonin to dopamine on fatigue is equivocal with no effect on time to exhaustion in the heat when serotonin concentration is increased (Hobson et al., 2013), but an improvement in time to exhaustion when dopaminergic activity is enhanced (Tumilty et al., 2011).
A consequence of BCAA metabolism during exercise is glutamate production that may form glutamine or alanine, but also ammonia (Wilkinson et al., 2010). Ammonia readily crosses the blood brain barrier and increased production during exercise may result in high cerebral concentrations (Nybo et al., 2005). Elimination of excess ammonia requires the synthesis of glutamine from glutamate and ammonia and thus a high concentration of ammonia in prolonged exercise may affect both glutamate and glutamine and consequently, CNS function (Nybo et al., 2005; Wilkinson et al., 2010).

2.4.3. Peripheral fatigue

Peripheral fatigue is generally described as being “produced by changes at or distal to the neuromuscular junction” resulting in muscle fatigue, “any exercise-induced reduction in the ability of a muscle to generate force or power” (Gandevia, 2001, p. 1733). Muscle fatigue is considered to consist of both peripheral and central components, but this section will focus on the peripheral contributions to muscle fatigue.

The genesis of peripheral fatigue during exercise can be multi-factorial. From a neuromuscular perspective exercise can elicit peripheral fatigue by impairing action potential transmission along the sarcolemma, altering excitation-contraction coupling and actin-myosin interactions (Martin et al., 2010). In addition, metabolic factors, reactive oxygen species and mechanical aspects of muscle function have been implicated in peripheral fatigue (Allen et al., 2008; Place et al., 2009).

Action potential propagation across the sarcolemma generally proceeds at \( \sim 2 - 6 \text{ m.s}^{-1} \) in humans to ensure effective muscle contraction (Allen et al., 2008). Disrupted propagation, consistent with reduced excitability, can arise due to alteration in ionic balance, primarily accumulation of intracellular \( \text{Na}^+ \) and loss of muscle \( \text{K}^+ \) to the extracellular environment (Martin et al., 2010). Such perturbations would manifest as altered M wave characteristics, and if reduced sarcolemmal excitability consistent with a
decrease in action potential propagation is symptomatic of exercise induced peripheral fatigue, should present as a depression in M wave amplitude (Martin et al., 2010). M wave amplitude has, however, been shown to decrease, increase and not change in response to exhaustive endurance, repeated sprint and maximal concentric/isometric knee extensor exercise despite a reduction in force characteristics in associated MVCs and twitch responses (Martin et al., 2010; Perrey et al., 2010b; Decorte et al., 2012; Froyd et al., 2013; Girard et al., 2013a&b). It is suggested the methodology employed may affect the extent of fatigue observed. Allen et al., (2008) propose that membrane excitability is preserved in exercise through a number of mechanisms that ultimately limit the effect of $K^+$. Such factors include variation in motor unit recruitment and firing rate, sarcolemmal action potential changes, Na$^+$-K$^+$ pumping, Cl$^-$ channels, alterations in leak conductances and Ca$^{2+}$ release feedback (Allen et al., 2008). As such, the observation that M wave characteristics can be unchanged in exercise but MVC and twitch forces depressed, suggests that the genesis of peripheral fatigue lies elsewhere within the muscle fibre and membrane excitability is not a contributory factor (Allen et al., 2008; Place et al., 2009). More recently however, Sidhu et al., (2012a) demonstrated that sarcolemmal excitability may be reduced when M wave amplitude is assessed during submaximal dynamic exercise. Reductions in M wave amplitude were reported when measurements were made every 3 minutes during 30 minutes of high intensity cycling at 75% maximum workload (Sidhu et al., 2012a). Such evidence suggests that sustained isometric MVCs may have limited applicability when trying to model fatigue during activities involving dynamic contractions such as observed in exercise.

Excitation-contraction coupling has been identified as a potential contributor to peripheral fatigue during exercise (Place et al., 2009; Martin et al., 2010). Factors that may exacerbate fatigue by this mechanism could include decreased Ca$^{2+}$ release from the sarcoplasmic reticulum contributing to reduced activation of cross bridges, decreased myofibrillar Ca$^{2+}$ sensitivity and a reduction in the force produced by each cross bridge (Place et al., 2009). Such perturbations may occur from alterations in the metabolic milieu within the muscle fibre, the presence of reactive oxygen species or mechanical factors.
During exercise, concentrations of metabolites including lactate, \( \text{H}^+ \) and inorganic phosphate (\( \text{P}_i \)) increase within the muscle. Experimental evidence demonstrates lactate does not contribute to peripheral fatigue (Allen et al., 2008). Similarly, it has been demonstrated that the effect of increased \([\text{H}^+]\) and reduced pH on peripheral fatigue is limited (Allen et al., 2008; Westerblad et al., 2010; Boyas and Guevel, 2011). Low pH does not noticeably inhibit voltage sensor activation of \( \text{Ca}^{2+} \) release, nor does it impact membrane excitability (Allen et al., 2008; Westerblad et al., 2010; Boyas and Guevel, 2011). Although low pH reduces the contractile apparatus sensitivity to \( \text{Ca}^{2+} \), evidence demonstrates that the effect on contractile apparatus and \( \text{Ca}^{2+} \) release is limited and contradictory as low pH may have an enhancing effect on force development through its effect on the \( \text{Ca}^{2+} \) pump (Allen et al., 2008). Similarly, it has been demonstrated that the low pH observed at exhaustion in exercise does not impair glycolytic and glycogenolytic activity (Greenhalf et al., 1993; Bangsbo et al., 1996; Westerblad et al., 2002; Allen et al., 2008). Both in vitro and in vivo studies have, however, demonstrated that low pH may slow relaxation rate in skeletal muscle due to a reduced rate of cross-bridge cycling and may contribute to impaired performance in dynamic exercise through this mechanism (Bruton et al., 1998; Allen et al., 2008; Place et al., 2010).

At the onset of exercise degradation of phosphocreatine (PCr) by creatine kinase serves to maintain ATP concentration in skeletal muscle. As a result, the concentration of creatine and \( \text{P}_i \) is increased. Increases in \([\text{P}_i]\) may contribute to muscle fatigue through a number of mechanisms. Firstly, increased \([\text{P}_i]\) is known to reduce the number of force generating cross bridges with subsequent decreases in force production (Allen et al., 2008; Westerblad et al., 2010; Boyas and Guevel, 2011). Secondly, elevated \([\text{Pi}]\) reduces myofibrillar \( \text{Ca}^{2+} \) sensitivity through the interaction between myosin cross-bridge attachment and actin activation (Allen et al., 2008; Westerblad et al., 2010; Boyas and Guevel, 2011). Finally, by entering the sarcoplasmic reticulum during fatigue, increased \([\text{P}_i]\) may precipitate a reduction in release of free \( \text{Ca}^{2+} \) (Allen et al., 2008; Westerblad et al., 2010; Boyas and Guevel, 2011).
During intense fatigue [ATP] may decrease from 7 to 1.2 mM, but in vitro studies with skinned intact fibres demonstrate no reduction in force production unless [ATP] decreases to < 20 μM (Westerblad et al., 2002; Allen et al., 2008). In similar circumstances [Mg\(^{2+}\)] can increase from 1 - 2 mM and although not affecting force production, this can reduce Ca\(^{2+}\) sensitivity and release by the sarcoplasmic reticulum (Allen et al., 2008; Westerblad et al., 2010; Boyas and Guevel, 2011). Similarly, decreased [ATP] can slow the rate of Ca\(^{2+}\) pumping and re-uptake by the sarcoplasmic reticulum resulting in elevated resting [Ca\(^{2+}\)] conditions readily observed at fatigue (Westerblad et al., 2002; Allen et al., 2008). In addition, the sarcoplasmic reticulum Ca\(^{2+}\) release channel is strongly stimulated by ATP and inhibited by Mg\(^{2+}\) (Westerblad et al., 2002; Allen et al., 2008; Boyas and Guevel, 2011). As such, when [ATP] decreases and [Mg\(^{2+}\)] increases in exercise Ca\(^{2+}\) release is inhibited, reducing the rate of ATP usage by reducing cross-bridge cycling and Ca\(^{2+}\) uptake by the sarcoplasmic reticulum (Westerblad et al., 2002; Allen et al., 2008; Boyas and Guevel, 2011). Such a sequence of events can ultimately lead to increased muscle fatigue. Finally, skeletal muscle glycogen content has been implicated in muscle fatigue. In isolated murine muscle cell models a reduced pre-fatigue glycogen concentration has been demonstrated to result in a faster decrease of tetanic [Ca\(^{2+}\)] and force suppression during fatigue and exercise mediated glycogen depletion may induce muscle fatigue by depression of sarcoplasmic recticulum Ca\(^{2+}\) release (Allen et al., 2008).

Reactive oxygen species (ROS) are produced in skeletal muscle e.g. in mitochondria, sarcoplasmic reticulum, transverse tubules and plasma membrane at rest and in response to exercise with the primary free radicals produced in cells being superoxide and nitric oxide. While low concentrations of ROS serve a number of important functions such as regulation of cell signalling pathways and control of gene expression in cells, in large quantities they can induce cellular damage (Power and Jackson, 2008; Westerblad et al., 2010). Low levels of ROS are necessary for normal force production in skeletal muscle (Power and Jackson, 2008). With large concentrations, as observed in exercise, force production in maximal and submaximal contractions is depressed (Power and Jackson, 2008). It has been suggested that such alterations in force production are mediated by a number of factors including reduced myofibrillar Ca\(^{2+}\) sensitivity, altered
myofilament structure and function and altered cross-bridge kinetics (Power and Jackson, 2008).

From a mechanical perspective, a reduced rate of relaxation is a normal response to muscle fatigue and may impair performance in tasks requiring dynamic muscle contraction (Allen et al., 2008). Evidence from animal and human studies has demonstrated that the increased \([H^+]\), \([Pi]\) and \([ADP]\) are likely mediators that cause the reduction in relaxation rate contributing to muscle fatigue (Allen et al., 2008). Alternately, Todd et al., (2005) suggest that, with hyperthermia, relaxation rate of muscle is significantly increased requiring faster motor unit firing rates to produce fusion of force. Therefore an inability of descending voluntary drive to compensate for altered augmented muscle relaxation rate may contribute to central fatigue (Todd et al., 2005).

2.4.4. Measurement of Fatigue

2.4.4.1. Maximal voluntary contractions

Although research has begun to examine fatigue during sustained locomotor exercise (Sidhu et al., 2012a), the majority of existing research on central and peripheral fatigue during exercise has used maximal voluntary contractions (MVC) with peripheral motor nerve stimulation pre-post exercise to elucidate neuromuscular fatigue mechanisms. Brief MVCs have been frequently used to examine force production pre-post locomotor exercise, but more recently, sustained MVCs have been incorporated to provide further insight to the mechanisms mediating neuromuscular fatigue. In both modes, a progressive decline in voluntary force production is observed in the presence of neuromuscular fatigue (Bigland-Ritchie et al., 1983; Nybo and Nielsen, 2001a&c; Ross et al., 2007; Periard et al., 2011a&b; Girard et al., 2013a&b).
2.4.4.2. Evoked mechanical responses

The interpolated twitch technique is routinely used to assess central and peripheral contributions to muscular fatigue using direct supramaximal electrical stimulation of the motor nerve during and immediately following a maximal voluntary contraction (MVC) (Merton, 1954). When compared to a baseline resting potentiated twitch, supramaximal stimulation of a motor nerve in a resting muscle post MVC allows examination of evoked twitch parameters including twitch force, maximal rate of force development (MRFD), maximal rate of relaxation (MRR) and relaxation half time (RT_{0.5}) and insight to peripheral fatigue. Compared to a baseline potentiated twitch, twitch force is generally reduced post MVC demonstrating evidence of peripheral fatigue (Ross et al., 2007; Perrey et al., 2010b). Reductions in MRFD indicate decreased muscle shortening velocity and specifically, a reduced rate of cross-bridge formation. MRR and (RT_{0.5}) provide insight to muscle relaxation velocity with any decrease in these parameters indicating impairment in rate of weak to strong cross bridge binding and rate of cross bridge uncoupling, respectively (Jones, 2010).

2.4.4.3. Peripheral voluntary activation

Application of a supramaximal stimulus to a motor nerve during an MVC evokes a superimposed twitch, (SIT), an indication that voluntary drive to the muscle is not maximal (Taylor et al., 2006; Gandevia, 2001). Any increase in the size of the superimposed twitch indicates a reduction in voluntary drive suggesting reduced recruitment or firing frequency of motor units and therefore provides evidence of central fatigue. Although used for many years to assess muscular fatigue, the interpolated twitch does not permit identification of the site of central fatigue beyond proximal to the neuromuscular junction (Goodall et al., 2012a; Ross et al., 2012). Further, the interpolated twitch technique can provide an estimate of drive to muscles but is proposed not to provide an indication of descending drive to motor neurons or take into account the non-linear input-output relationship of the motor neuron pool (Herbert and Gandevia, 1999). In addition, increased isometric force, indicative of central fatigue, has been observed in intact single fibres when such an occurrence should not be possible.
(Place et al., 2008). Therefore, it is proposed that an alternate mechanism, increased \( \text{Ca}^{2+} \) concentration, contributes to the increased forced production during isometric contraction as opposed to increased central fatigue. Therefore, it is suggested the interpolated twitch technique over-estimates voluntary activation and central fatigue (Place et al., 2010).

### 2.4.4.4. Cortical voluntary activation

More recently, transcranial magnetic stimulation (TMS) applied to the motor cortex has been used to examine central fatigue and has the advantage that it permits the site of failure of voluntary drive to be localized (Sidhu et al., 2009b; Goodall et al., 2012a; Ross et al; 2012). When TMS is applied to the motor cortex during an MVC, observation of a superimposed twitch provides evidence that motor cortical output is submaximal and insufficient to drive the motoneurons optimally (Taylor et al., 2006). Again, any exercise mediated increases in the SIT induced by TMS over the motor cortex indicates decreased cortical voluntary activation, central fatigue and therefore, more specifically, the presence of supraspinal fatigue (Sidhu et al., 2009b; Goodall et al., 2012a; Ross et al., 2012). TMS has been used to assess central contributions to fatigue in a number of studies and, when used in conjunction with the interpolated twitch technique permits assessment of central and peripheral fatigue (Ross et al., 2007; Goodall et al., 2010; Goodall et al., 2012a; Ross et al., 2012). As corticospinal excitability increases during voluntary contraction and cortical stimulation at rest activates fewer motor neurons, it is necessary to estimate the resting twitch amplitude when using TMS from linear regression of the torque responses to TMS of the motor cortex during contractions (SIT) at 100, 75 and 50% MVC (Goodall et al., 2009, Ross et al., 2012). The y intercept of the regression line is then taken as the estimated resting twitch. Further, use of TMS stimulates locomotor muscle additional to the target muscle. Consequently, the target muscle should be stronger than the antagonist for valid assessment. To-date, TMS has been used to assess fatigue in a number of studies and is reliable for the assessment of supraspinal fatigue in the tibialis anterior, elbow flexors and knee extensors (Todd et al., 2003; Cacchio et al., 2009; Goodall et al., 2009; Sidhu et al., 2009b).
2.4.4.5. Evoked EMG responses

In addition to evoked mechanical responses from supramaximal stimulation of a motor nerve surface electromyography (EMG) provides insight to the electrical activity of the muscle of interest in the form of a compound muscle action potential (M-Wave). M wave characteristics including amplitude and area provide insight to sarcolemmal excitability and propagation of the action potential across the sarcolemmal membrane. Any reduction in M-Wave characteristics indicates impaired sarcolemmal excitability and conversely, when no alterations occur in M wave the site of peripheral fatigue is considered elsewhere within the muscle, for example, impaired excitation-contraction coupling or actin myosin interaction (Martin et al., 2010). In research concerned with repeated-sprint exercise, M wave amplitude has been reported to increase (Racinais et al., 2007), decrease (Perrey et al., 2010b) or not change (Girard et al., 2013a). Discrepancies in findings may be explained by the moderate reliability of M wave responses (Place et al., 2007), mode of exercise and type of muscle contraction involved whereby exercise with primarily concentric work (for example, cycling) may not impair membrane excitability but exercise with a distinct stretch shortening cycle (for example, running) might (Perrey et al., 2010b).

Direct stimulation of the motor cortex at rest or during voluntary contraction using TMS elicits a motor evoked potential (MEP) in surface EMG that permits examination of corticospinal excitability (Goodall et al., 2012b). MEP latency provides a measure of the velocity of neural impulse propagation from motor cortex to muscle and MEP area, when compared to the evoked EMG response from motor nerve stimulation, provides insight to the extent of motor unit pool recruited (Goodall et al., 2012b). When TMS is delivered during a maximal voluntary contraction a period of EMG silence follows the MEP, referred to as the cortical silent period (Goodall et al., 2012b). Inhibitory spinal mechanisms are thought to contribute to the initial portion of the cortical silent period (< 100 ms), whereas increased cortical inhibition is considered to represent the latter stages of the silent period (Inghilleri et al., 1993; Taylor and Gandevia, 2001).
2.4.4.6. Voluntary EMG responses

Voluntary EMG activity provides further insight to the manifestation of fatigue during locomotor exercise as EMG activity provides not only an indication of motor unit recruitment, but also motor unit firing frequency. During maximal voluntary efforts voluntary muscle activity can be assessed from EMG amplitude determined using the root-mean-squared (RMS) value with decreased voluntary force corresponding to decreased RMS value (EMG amplitude) as fatigue develops. Further EMG amplitudes can be normalised to the M wave so that accurate assessment of peripheral and central contributions to fatigue can be made. Previously, voluntary EMG responses have been used to indicate the existence of central and / or peripheral fatigue during cycling and running-based intermittent-sprint exercise with a decrease (Racinais et al., 2007; Rampinini et al., 2011) and no change (Perrey et al., 2010b) reported. Although a reduction in RMS EMG may be interpreted as decreased voluntary drive in the aforementioned studies, EMG activity should be interpreted with caution, as amplitude cancellation and reduced sensitivity to small modification in muscle activation compared to interpolated twitch may compromise findings (Kalmar and Cafarelli, 1999; Farina et al., 2004).

2.4.4.7. Perception of effort

During exercise, fatigue manifests as increased sense of effort and reduced force output soon after the onset of activity, but before task termination (Enoka and Duchateau, 2008). The genesis of effort perception is the subject of debate. Perception of effort has been correlated with physiological markers during exercise (Edwards et al., 1972). Further, group III and IV afferents, abundant in skeletal muscle and sensitive to physiologic, metabolic and mechanical stress, project to the sensory cortex (Craig, 2002). Consequently, it has been suggested that sense of effort is derived from peripheral afferent stimuli (Marcora, 2009). However, there is strong evidence to suggest perception of effort is strongly influenced, if not totally dependent, upon centrally generated corticofugal motor commands that give rise to corollary discharges (Enoka and Stuart, 1992). That is, perception of effort is centrally generated by neural signals from the motor
to sensory cortex (Marcora, 2009) that during exercise would reduce central drive contributing to fatigue and ultimately reduced performance (Enoka and Stuart, 1992).

2.4.5. Mechanisms of exercise induced neuromuscular fatigue in temperate environments

The extent of central and peripheral fatigue during exercise is affected by the intensity and duration of the activity, the speed of contraction and the extent to which the activity is continuously sustained (Enoka and Stuart, 1992). This section will examine peripheral and central mechanisms contributing to fatigue in intermittent-sprint exercise. As literature pertaining to the study of this concept in this mode of exercise is sparse, where relevant, evidence will be drawn from endurance and repeated-sprint exercise to elucidate possible mechanisms.

2.4.5.1. Endurance type exercise

Millet et al., (2003) examined central and peripheral fatigue in the knee extensors after a 30 km running race. Isometric force and EMG responses of the knee extensors were assessed using maximal percutaneous electrical stimulation and superimposed twitches (Millet et al., 2003). MVC and voluntary activation of the knee extensor and M wave amplitude were reduced following exercise indicating reduced central drive and altered sarcolemmal excitability. No failure of excitation contraction coupling was observed. Similarly, Ross et al., (2007) reported decreased central voluntary activation in ankle dorsiflexors and tibialis anterior after a treadmill marathon using TMS. Further, potentiated twitch and MEP amplitude (but not M wave) were significantly reduced immediately post exercise indicating suboptimal output from the motor cortex and disruption to the contractile apparatus without impairment of sarcolemmal excitability (Ross et al., 2007). Large significant decrements in peripheral voluntary activation and voluntary force has also been reported In longer distance races (24 hour treadmill run) with concomitant reductions in M wave amplitude but no evidence of failure of excitation contraction coupling. (Martin et al., 2010)
In the first study to use TMS to assess voluntary activation of the knee extensors after prolonged cycling exercise, Sidhu et al., (2009a) demonstrated decreased MVC force, significant decrement in cortical voluntary activation and resting twitch amplitude after exercise. Knee extensor fatigue post exercise was attributed to central and peripheral factors with 60% of the initial reduction in force owing to failure of the motor cortex to drive optimally the knee extensors, possibly mediated by afferent feedback (Sidhu et al., 2009a). With MVC and single, paired potentiated and interpolated magnetic stimulations of the femoral nerve Decorte et al., (2012) demonstrated that mechanical responses to magnetic stimulation were decreased mostly in the first half of cycling while voluntary activation decreases were reported towards the end of exercise only. It was concluded that peripheral fatigue develops early in constant load cycle exercise owing to afferent sensing, which is compensated by additional motor drive and central fatigue is associated with task failure (Decorte et al., 2012).

Taken together, the evidence demonstrates that central fatigue is primarily responsible for the decrement in force immediately following prolonged running and cycling exercise. Where TMS has been used, evidence suggests altered motor cortical output contributes to this decrement and is supported by evidence from cycling based protocols (Sidhu et al., 2009b). Consistent with the inhibitory feedback hypothesis (Amann, 2011; Amann et al., 2011, 2013) afferent signaling from group III and IV afferents sensitive to homeostatic disruption in cytokines, extracellular K+ and other metabolites of fatigue have been implicated in the reduction in central drive in this type of exercise (Millet et al., 2003, Sidhu et al., 2009b). Running duration appears to contribute to the extent of central fatigue (Martin et al., 2010), but the extent of elevation gain and loss may also alter the fatigue profile as significant peripheral fatigue through failure of excitation-contraction coupling has been observed in a mountain ultramarathon (Millet et al., 2011).
2.4.5.2. Intermittent-sprint type exercise

Central and peripheral fatigue following 10 x 6 s repeated cycle sprints with 30 s recovery has been examined in a temperate environment (Racinais et al., 2007). Isometric force and responses to supramaximal stimulation immediately before and after exercise were examined during brief MVCs, in addition to EMG recording during exercise (Racinais et al., 2007). Completion of exercise produced a significant decrement in voluntary force. Potentiated twitch was reduced 9% and while indicating the presence of peripheral fatigue this decrement was not significant. Further, M wave amplitude increased post exercise (+13.7%) indicating no disruption to sarcolemmal excitability or action potential transmission. Voluntary activation was, however, significantly reduced (-3%) post exercise as was the EMG:M wave ratio and PPO across sprints (Racinais et al., 2007). In addition, a progressive peripheral muscle deoxygenation was observed and it was concluded failure of neural drive mediated by group III and IV afferents in response to peripheral muscle deoxygenation was responsible for the reduced performance (Racinais et al., 2007). Such an observation would be consistent with the central governor model of fatigue that suggests during exercise, regulation of effort (motor unit recruitment) to ensure task completion is achieved by continuous afferent sensing that could include group III and IV afferents (Figure 2.7, Noakes, 2012). Further, the conclusion of this study is consistent with the tenet of the inhibitory feedback model of fatigue (Amann, 2011; Amann et al., 2013, Figure 2.8), that suggests motor drive is modulated by feedback from group III and IV peripheral afferents. Amann (2011), however, also suggests the existence of an individual critical threshold or sensory tolerance limit of peripheral locomotor fatigue that determines the extent of peripheral fatigue that is tolerated and the extent of inhibitory feedback to the CNS. Whether this threshold is achieved with the work:rest activity pattern of intermittent-sprint sports is currently unknown. Similarly, in the current study (Racinais et al., 2007) decrements in voluntary activation were observed with completion of only ten 6 s sprints and it is unknown how, in field-based team-sports, where up to sixty sprints can be completed in a match, the extent or pattern of fatigue is altered or how the extent of recovery affects these parameters.
Figure 2.7. The central governor model of exercise regulation (Noakes, 2012).

Figure 2.8. Schematic representation of the inhibitory feedback model (Amann, 2011).
Using an identical protocol to Racinais et al., (2007), Mendez-Villanueva, (2008) demonstrated with EMG monitoring during exercise a reduction in EMG RMS activity (14.6%) across 6 s sprints. As a concomitant reduction in peak power output of 24.6% occurred, the authors inferred from the EMG data that impaired repeated-sprint ability was associated with progressive inhibition of motor unit recruitment and/or motor unit firing rate. Further, when ten 6 s sprints were interspersed with 30 s recovery and followed six minutes later by five 6 s sprints again with 30 s recovery, EMG amplitude (recorded during exercise) decreased significantly across the first ten sprints and remained depressed in the final five sprints (Mendez-Villanueva et al., 2012). It was concluded that afferent sensing related to control of PCr metabolism may have contributed to suboptimal motor unit activity, as inferred from decreased EMG amplitude, that resulted in an inability to maintain power output during repeated sprint exercise. While such findings would again appear to align with the concepts of a central governor and inhibitory feedback hypotheses, it is important to note that no direct measure of central activation was performed in either of the aforementioned studies (Mendez-Villanueva et al., 2008, 2012) and there are limitations associated with inferring activation from EMG activity as previously described (2.4.4.6). As such, the ability to elucidate mechanisms of fatigue in repeated sprinting is constrained.

In running based intermittent type exercise, completion of 12 x 40 m sprints with 30 s recovery produced evidence of central and peripheral fatigue when MVCs, interpolated twitch, high and low frequency stimulations and EMG data was assessed pre-post exercise in temperate conditions (Perrey et al., 2010b). In contrast to Racinais et al., (2007) M wave amplitude, but not duration, was depressed suggesting impaired action potential synaptic transmission and sarcolemmal excitability with repeated running sprints. In addition, responses to low frequency stimulations suggested the presence of low frequency fatigue indicating potential failure of excitation-contraction coupling in intermittent type exercise which may indicate an effect of the eccentric muscle activity inherent in running but not cycling exercise modes (Perrey et al., 2010b). A depressed voluntary activation (-3%) immediately post exercise implied impaired central neural drive, but EMG RMS normalised to M wave was not altered (Perrey et al., 2010b). At a
spinal level the $H_{max}/M_{max}$ ratio, demonstrating reflex excitability of the motoneuron pool was unchanged. The authors concluded that fatigue in repeated running sprints was primarily due to peripheral factors, including sarcolemmal excitability and excitation-contraction coupling (Perrey et al., 2010b).

In a more ecologically valid study, Rampinini et al., (2011) examined neuromuscular fatigue in twenty male professional footballers pre-post a simulated football match. Before and immediately following a ninety minute match players completed brief MVCs with interpolated twitch, high and low frequency stimulations and EMG data recorded (Rampinini et al., 2011). Immediately post exercise, sprint performance and MVC were significantly depressed. M wave characteristics were unchanged, however, the ratio of RMS: M wave amplitude was significantly reduced. As voluntary activation and EMG activity were also significantly reduced it was suggested the decreased MVC post exercise could partly be attributed to central fatigue. Similar to Perrey et al., (2010b) low frequency fatigue was also observed indicating a peripheral effect on performance, specifically, alterations in excitation-contraction coupling (Rampinini et al., 2011).

The only study to-date to examine neuromuscular fatigue in intermittent sprint exercise using TMS was conducted by Girard et al., (2013a). TMS and femoral nerve stimulation was performed during brief (5 s) and sustained (30 s) MVCs pre and post 10 x 6 s sprints with 30 s recovery on a cycle ergometer followed six minutes later by five, 6 s sprints with 30 s recovery in twelve male participants (Girard et al., 2013a) in a temperate environment. In brief MVCs MEP amplitudes, cortical voluntary activation and cortical silent period were not different pre-post exercise (Girard et al., 2013a). In sustained contractions cortical voluntary activation was reduced pre and post exercise but exercise mediated a greater reduction. M wave amplitude was decreased pre-post exercise in sustained MVC but was unchanged in rest and brief MVCs (Girard et al., 2013a). Post exercise, the potentiated twitch was reduced while also showing decreased contraction and relaxation rates (Girard et al., 2013a). It was concluded that the responsiveness of corticospinal neurons was not impaired by repeated cycle sprints and peripheral factors.
were primarily involved in the exercise-mediated impairment in neuromuscular function (Girard et al., 2013a).

2.4.6. Mechanisms of Exercise Induced Fatigue in Hot Environments

Previously, impairments in voluntary activation, assessed using the interpolated twitch technique, have been reported in brief MVCs with passive hyperthermia to induce a core temperature of $\sim 39.5^\circ$C (Morrison et al., 2004; Thomas et al., 2006, Figure 2.9.). Similarly, with modest hyperthermia of $\sim 38.5^\circ$C a reduction in voluntary activation using TMS in sustained 2 minute MVCs has been reported (Todd et al., 2005). The impairment in voluntary activation with hyperthermia has been correlated with cerebral blood flow at rest and after exercise in the heat (Nybo and Nielsen, 2001b; Ross et al., 2012). In hypoxia, decreased cerebral blood flow exacerbates the decline in cerebral oxygenation and contributes to a two-fold greater decrease in cortical voluntary activation after exercise despite near identical levels of muscle fatigue (Goodall et al., 2012a). As such, given the decreased cerebral blood flow observed at rest and in exercise in heat stress and the subsequent potential decrease in cerebral oxygenation, the physiological conditions exist for a greater central fatigue during exercise in the heat and decreased central neural drive in hyperthermia. Consequently, the impact of hyperthermia on fatigue has been investigated in a number of studies.
2.4.6.1. Endurance type exercise

The impact of hyperthermia on central and peripheral fatigue in endurance type exercise has been the subject of investigation in a number of studies (Nybo and Nielsen, 2001a; Periard et al., 2011a&b). Nybo and Nielsen (2001a) reported that 40°C heat stress induced significant decrements in voluntary activation following exercise to exhaustion at 60% \( \dot{V}O_{2\text{max}} \) in 40°C. Consistent with the concept of a critical limiting temperature (Nielsen et al., 1993; Gonzalez-Alonso et al., 1999) the authors attributed the decrement in central drive to the exercise-induced core (oesophageal) temperature of 40.0 ± 0.1°C (Nybo and Nielsen, 2001a). In contrast, Periard et al., (2011a) demonstrated evidence of central and peripheral fatigue in sustained MVCs with percutaneous tetanic stimulation following 40 km cycle time trials in both hot and temperate conditions. The decline in voluntary activation was calculated to represent \( \sim 20\% \) of the decrease in mean force in both trials and it was concluded that impairment of force production in 40 km cycle time trial exercise was localised (no change in hand grip MVC), mediated primarily by peripheral fatigue with no additional effect of heat stress (Periard et al., 2011a). No EMG or evoked
twitch data were reported in this study and the possible mechanism of peripheral fatigue could not be elucidated. Similar findings were, however, reported by Periard et al (2011b) and it was speculated that excess cardiovascular strain associated with cycle exercise in the heat contributed to the observed fatigue. Such a suggestion is supported by Ely et al., (2009), who posit that excessive cardiovascular and thermal strain better explain fatigue in the heat than a limiting core temperature and have demonstrated that, in studies supporting the concept of a limiting core temperature, exhaustion has always coincided with excessive cardiovascular strain that likely compromises performance due to the competitive metabolic and thermoregulatory demands on cardiac output (Ely et al., 2009; Cheuvront et al., 2010). Further, during exercise in the heat it has been shown that performance can be maintained when cardiovascular strain is low (Ely et al., 2009, 2010; Cheuvront et al., 2010).

Finally, Racinais and Girard, (2012) examined fatigue in incremental cycling exercise preceded by submaximal cycling in hot (40°C) and control (24°C) conditions using TMS and percutaneous stimulations. Termination of exercise in the heat occurred at a lower peripheral fatigue and despite reductions in MVC and M wave that would suggest impaired membrane excitability and action potential transmission in the plantar flexors compared to knee extensors in the heat, most of the effects of heat exposure and exercise induced fatigue were independent of each other (Racinais and Girard, 2012). Although EMG activity was lower in the heat at exhaustion, MEP amplitudes normalised to M wave were maintained and the authors concluded that neuromuscular fatigue was unlikely to explain earlier exercise cessation in the heat (Racinais and Girard, 2012). Interestingly, core temperature at exhaustion in the heat was only 38.9°C and it is tempting to speculate whether this attenuated responses.

2.4.6.2. Intermittent-sprint type exercise

Duffield et al., (2009) examined the effect of heat stress on repeated cycling sprints in seven male and six female team-sports athletes. In either 22°C or 33°C (40% rh)
participants completed a 30 minute exercise protocol comprising a 20 s maximal sprint every 5 minutes separated by submaximal cycling at 100 watts with MVCs and evoked twitches performed before and immediately following exercise. No differences in peak power output were reported between conditions. MVC and evoked twitch was significantly reduced immediately post exercise in both trials, but was not significantly different between hot and control conditions (Duffield et al., 2009).

Similarly, Almudehki et al., (2012) reported no significant difference in peak power output when ten males performed 35 minutes of cycle exercise comprising 8 x 6 s sprints interspersed with 1 minute of passive recovery followed by 4 minutes of constant load pedalling in 40°C, 40% rh or 24°C, 20% rh. Although physiological and perceptual measures were higher in the hot trial, EMG RMS and neuromuscular efficiency (power:RMS ratio) were not different between trials (Almudehki et al., 2012). It was concluded that heat stress does not impact performance or neuromuscular factors during intermittent-sprint cycling although observed lack of change may have been due to the low core temperatures evoked by the protocol (~ 37.7°C). Similarly, when recreationally active males performed 10 x 6 s sprints followed six minutes later by 5 x 6 s maximal sprints with 30 s recovery in 24°C or 35°C, no difference was observed between conditions in strength, rate of force development, and voluntary activation, although power output was increased in the hot trial (Girard et al., 2013b). It is important to note, however, that in the work of Duffield et al., (2009) and Almudehki et al., (2012) the protocols used do not replicate the work:rest ratios of field-based team-sports. As such, relatively long recovery periods were employed, limiting the extent of hyperthermia induced. Similarly, in the work of Girard et al., (2012b) the mean core temperature in exercise was only 38°C in the heat and 37.7°C in the temperate trial. As such, the data is unlikely reflective of core temperatures that would be expected in a field-based setting over durations reflective of field-based team sports and therefore, the observations from such studies are of limited application.
Nybo et al., (2013) in an ecologically valid design, examined neuromuscular function in seventeen semi-professional football players completing a football match in 43°C and 21°C. MVCs and interpolated twitch of the plantar flexors were performed pre-post exercise in both conditions. Exercise impaired force generation and voluntary activation in both conditions, but there was no difference between trials (Nybo et al., 2013). It was concluded that heat stress does not provide an additional load that exacerbates central and peripheral fatigue (Nybo et al., 2013). In the current study, core temperature was reported as 39.6°C in the hot match and muscle temperature was reported between 39.9°C - 41.1°C. As such, the extent of hyperthermia was marked and sufficient to mediate fatigue and may have accounted for the small, but significant change in voluntary activation in both conditions (average - 1.5%). It is important to note that in this study total game distance declined by 7% and high intensity running by 26% in the hot compared to temperate trial. As such, given the self-regulated nature of the match it is tempting to suggest that afferent sensing may have contributed to a down regulation of motor unit activity to ensure task completion (Amann, 2011; Noakes, 2012; Amann et al., 2013) and may have contributed to findings. Similarly, it is unknown whether such a value is reflective of a true change or merely variation inherent in the measurement method.

2.4.7. Summary

Fatigue may be defined as “a reduction in the maximal force-generating capacity of a muscle” (Gandevia, 1992) and is frequently referred to as peripheral or central in origin. When undertaken in a hot compared to temperate environment, exercise performance across a spectrum of disciplines is reduced. The genesis of fatigue remains unclear, but is accepted to have both central and peripheral components the extent of which is dependent on a number of variables, including duration and intensity of exercise, endogenous and exogenous heat load, cardiovascular function, neuro-humoural factors and brain function. A large body of literature exists on the genesis of fatigue in endurance type activity and a number of hypotheses are proposed to explain its occurrence. In intermittent sprint type exercise however, a distinct lack of literature limits our understanding of central and peripheral fatigue. Further, existing fatigue hypotheses have
not been applied to explain fatigue in intermittent-sprint exercise. For example, Amann, (2011) and Amann et al., (2013) propose existence of an individual critical threshold or sensory tolerance limit of peripheral locomotor fatigue that determines the extent of peripheral fatigue that is tolerated and the extent of inhibitory feedback to the CNS. Whether the intermittent nature of field-based team sport and the inherent work:rest ratios allow such a threshold to be exceeded is unknown. Further, whether heat stress would increase the likelihood that this threshold would be surpassed in intermittent activity is also unknown. More recent work has started to provide insight but again, this has tended to rely on interpolated twitch techniques which limit conclusions on central fatigue. Only one study to-date has examined fatigue in intermittent-sprint exercise using TMS and while this serves to provide excellent insight it was conducted in a temperate environment using a protocol that did not replicate the duration or work:rest ratio of field-based team-sports. As such, evidence is still lacking on how hyperthermia modulates central and peripheral fatigue during intermittent-sprint exercise that replicates field-based team sports, particularly under conditions of heat stress. Current work suggests that fatigue during intermittent-sprint exercise, rather than being mediated by neural drive, is predominantly mediated by peripheral factors, including PCr supply and oxygen content and metabolites e.g. H⁺ and Pᵢ (Bishop, 2012). However, much of the work providing this evidence is limited in ecological validity and further research is required.
2.5. Heat Acclimation

Heat acclimation / acclimatisation\(^4\) is frequently used to minimise the effect of excessive heat stress on physiological strain and performance during competition in a hot environment. Different approaches are used to evoke heat acclimation, but the effectiveness of a protocol is generally judged by its ability to confer classic physiological responses. While studies differ in their interpretation of these (for example, Wyndham et al., 1976; Barnett and Maughan, 1993; Buono et al., 2009; Amorim et al., 2011) and therefore, the effectiveness of their protocol, classic markers of heat acclimation include decreased heart rate and core temperature in rest and exercise, increased sweat rate and increased plasma volume. This section of the review will commence with an overview of methods of heat acclimation and will examine adaptation to heat acclimation. It will then consider the efficacy of existing heat acclimation protocols against classic criteria for a heat acclimated phenotype but, will also consider additional criteria of reduced skin temperature, decreased perception of effort / thermal sensation or comfort and improved physical performance that are reported in the literature (for example, Pandolf et al., 1977; Horowitz and Kodesh, 2010). Finally, this section will examine possible alternative methods for heat acclimation. Data for this section has been derived from literature searches of databases (Web of Science, PubMed and Sport Discus) and existing reviews on heat acclimation. Studies presented in tables 2.3 - 2.6. are limited to humans with a minimum of four participants and evidence on at least three markers of heat acclimation.

2.5.1. Heat acclimation methods and protocols

Many strategies have been used to elicit and maximise adaptive responses to heat stress. Protocols for heat acclimation have included passive methods, or more frequently, a combination of long or short duration, intermittent or continuous, moderate or high

\(^4\) Heat acclimation refers to the physiological adaptations to heat brought about by exposure to a controlled laboratory environment, while heat acclimatization refers to the same adaptations brought about by exposure to a naturally hot climate. Both methods result in similar physiological adaptations (Armstrong and Maresh, 1991) and therefore, the term heat acclimation will be used to encompass responses. Where a study has specifically used heat acclimatisation this will be identified.
intensity exercise for a fixed duration, or for a target core temperature. Despite the myriad of protocols, regimes can be classified as one of three types; self-regulated exercise, constant work rate or isothermal strain (Taylor, 2000; Garrett et al., 2011).

2.5.1.1. Self-regulated protocols

In self-regulated acclimation protocols, exercise intensity is participant-regulated based on fitness (Taylor, 2000; Garrett et al., 2011). Consequently, potential for premature cessation of exercise due to hyperthermia is minimised. Self-regulated protocols are, however, of limited effectiveness from a research perspective as the intensity of exercise and hence endogenous heat production can vary between participants during a session (Taylor, 2000; Garrett et al., 2011). As such, this type of protocol may be more suitable in a practical setting (Taylor et al., 2000; Garrett et al., 2011) than for understanding the true mechanisms that underpin adaptation to repeated heat exposures.

2.5.1.2. Constant work rate protocols

Constant work rate protocols that require participants to exercise at a fixed intensity over a specific number of days represent the traditional model of heat acclimation and are the most frequently used (Taylor, 2000; Garrett et al., 2011). A large body of research has been generated providing evidence as to the effectiveness of traditional acclimation in eliciting adaptations to the heat and to optimize response it is recommended exercise for 60 - 100 minutes per day is completed in conditions that simulate the expected environment, at intensities of greater than 40% VO_{2max} for 10 - 14 days (Mitchell et al., 1976; Sawka et al., 1985; Armstrong and Maresh, 1991; Maughan and Shirreffs, 2004). For the purpose of this review, protocols involving ≥ 60 minutes per day at ≥ 40% VO_{2max} for 10 - 14+ days will hereafter be referred to as traditional (Table 2.3.). Where protocols have used similar intensities, but reduced number of days they will be considered medium to short-term traditional heat acclimation (Table 2.4.).
#### 2.5.1.3. Isothermal strain / controlled hyperthermia protocols

Isothermal strain / controlled hyperthermia / thermal clamp protocols were developed in the 1960s by Fox and colleagues on behalf of the Medical Research Council (Turk and Thomas, 1975). Isothermal strain models of heat acclimation are based on the premise that attainment and maintenance of an elevated core temperature above the sweating threshold is central to effective heat acclimation (Taylor et al., 1997; Taylor, 2000). As such, this method of heat acclimation may offer more complete adaptation to the heat by elevating all participants to a pre-determined ‘critical’ core temperature (~38.5°C) and maintaining it by modifying workload throughout the session (Taylor, 2000; Garrett et al., 2011). Such a model of heat acclimation has the potential to maximise adaptive responses (Table 2.5.), because as the athlete adapts across the protocol greater work is required to achieve the required core temperature, thus maximising heat strain for the duration of the protocol. Research on isothermal protocols is limited however, and although Garrett et al., (2012) demonstrate the efficacy of short term isothermal acclimation for highly trained athletes, little is known about the effect of the required increase workload on the training load of athletes prior to competition and, for example the subsequent immune response. Further from an individual perspective it is unknown how individuals of differing fitness or age cope with the extent of work required to achieve / sustain a core temperature of 38.5°C.

#### 2.5.2. Adaptation to heat acclimation

Heat acclimation protocols typically result in a number of adaptations within the human body that serve to reduce physiological strain, increase the ability to exercise in a hot environment and lessen the chance of heat illness (Armstrong and Maresh, 1991, Figure 2.10.). Research has demonstrated that the rate of adaptation varies for different physiological markers of heat acclimation typically in a biphasic pattern (Wyndham et al., 1976; Horowitz and Kodesh, 2010, Figure 2.10.). Further, while it is proposed that adaptations are more or less complete within 7 - 14 days, it is suggested 66 - 75% of physiological adjustments are seen in 4 - 6 days (Pandolf, 1998; Maughan and Shirreffs,
2004) and physical fitness may contribute to inter-individual variation in response time (Pandolf et al., 1977). The extent of adaptation may be altered by the environment in which the heat acclimation takes place. Hot humid environments are suggested to promote a greater eccrine gland adaptation (Armstrong and Maresh, 1991). Hot dry environments have been suggested to confer enhanced responses including earlier onset of sweating and lower skin temperature that promotes enhanced circulatory stability due to a decreased requirement for skin blood flow (Shvartz et al., 1973). Griefahn (1997) argues, however, when environments are matched for heat stress there is no difference in acclimation response.

<table>
<thead>
<tr>
<th>Adaptation</th>
<th>Days of Heat acclimatisation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Rate decrease</td>
<td></td>
</tr>
<tr>
<td>Plasma volume expansion</td>
<td></td>
</tr>
<tr>
<td>Rectal temperature decrease</td>
<td></td>
</tr>
<tr>
<td>Perceived Exertion Decrease</td>
<td></td>
</tr>
<tr>
<td>Sweat Na⁺ and Cl⁻ decrease*</td>
<td></td>
</tr>
<tr>
<td>Sweat Rate increase</td>
<td></td>
</tr>
<tr>
<td>Renal Na⁺ and Cl⁻ decrease</td>
<td></td>
</tr>
</tbody>
</table>

*While consuming a low NaCl diet

Figure 2.10. Adaptations to traditional heat acclimation (Armstrong and Maresh, 1991).

2.5.2.1. Molecular and cellular responses to heat acclimation

To achieve the heat acclimated phenotype, re-programming of gene expression must occur. Consistent with the observed biphasic systemic adaptations to heat acclimation (Figure 2.10.), evidence at a molecular level, albeit in muroid models, also supports a biphasic altered gene expression in response to chronic sub-lethal heat exposure (Horowitz et al., 2004). As such, it has been proposed the molecular response to heat acclimation constitutes a defence strategy comprising two stages, an immediate transient stage with a focus to maintain DNA integrity and a sustained stage where signalling networks aligned to cytoprotection are enhanced (Horowitz et al., 2004). Therefore,
Horowitz et al., (1998, 2002, 2004, 2010) view heat acclimation as a two component model comprising short and long term components, whereby the primary function of the process is to widen the dynamic thermoregulatory range by reductions in body temperatures and temperature thresholds and an elevation of the upper temperature that can be experienced before heat injury (Figure 2.11.).

**Figure 2.11.** A conceptual model of heat acclimation (Horowitz et al., 2002).

Horowitz et al., (1998, 2002, 2004, 2010) propose that the initial stage of heat acclimation is mediated by the enhanced activity of the autonomic nervous system (1) promoting over-activation of heat defence mechanisms (2) to compensate for insufficiently adapted systemic responses. The molecular basis for this is observed in the upregulation of genes linked with DNA maintenance and repair, but also the upregulation of gene coding for voltage gate ion channels, ion pumps, hormones, transmitter receptors and cellular messengers (3) (Horowitz and Kodesh, 2010). Further, as heat acclimation progresses it is
suggested upregulation of signalling networks and gene transcription for cytoprotective mechanisms is enhanced as systemic adaptation is achieved (Horowitz and Kodesh, 2010). In addition, with long term acclimation there is downregulation of genes associated with energy metabolism, food intake and cellular disturbance and a return to preacclimation levels of genes encoding for enhanced nervous system activity (4) (Horowitz and Kodesh, 2010).

As described, the ability to acquire the heat acclimated phenotype through heat exposure is a chronic adaptation requiring gene reprogramming (Horowitz and Kodesh, 2010). Although not studied in respect to heat acclimation, the ACE gene, given the importance of this gene in the renin-angiotensin pathway and fluid regulation, may also be important in heat acclimation and tolerance. Heled et al., (2004) examined the ACE gene and polymorphisms in fifty eight participants during a two hour heat test and demonstrated that thermoregulatory capacity was consistently better in those with the I+ allele compared to DD suggesting that this allele may confer better heat tolerance.

The ability of humans to tolerate heat stress is multifactorial. However, a common, acute and transient reaction to heat stress is the heat shock response. The heat shock response is a cellular adaptation that confers thermotolerance in the cell, the ability to survive a potentially lethal heat stress due to cellular adaptation caused by prior exposure to a single severe, non-lethal heat exposure (Moseley, 1997). Thermotolerance is mediated by heat shock proteins.

Heat shock proteins (HSPs) are a highly conserved group of proteins classified according to their molecular weight (in kiloDaltons, for example, HSP70) and function into five groups (Moseley, 1997; Schmitt et al., 2007). HSPs operate at both an intracellular and extracellular level completing a range of functions in mammalian cells, including cytoprotection, apoptosis mediation, neuroprotection and molecular chaperoning of antigenic peptides (Moseley, 1997; Horowitz et al., 2002; Schmitt et al., 2007; Horowitz...
and Kodesh, 2010). Essentially, HSPs function as molecular chaperones assisting with protein folding and refolding after denaturation and therefore, function to conserve protein homeostasis (Kresfelder et al., 2006; Schmitt et al., 2007). Cell HSP concentration is augmented in response to insult such as hyperthermic, oxidative or exercise stress and upregulation occurs within minutes at a cellular level, but full thermotolerance is expressed ~ 24 hours post exposure with activity maintained for a number of days (Horowitz, 1998; Maloyan et al., 1999; Horowitz and Kodesh, 2010; Magalhaes et al., 2010; Taylor et al., 2011).

Originally, thermotolerance, an acute cellular response to heat stress was considered separate to heat acclimation, a systemic adaptation conferred by chronic exposure to sub-lethal heat stress resulting in the acclimated phenotype (Kueenen et al., 2011). However, evidence that HSPs were augmented by exposure to physiologically relevant temperatures and attenuated a heat-induced permeability of the epithelial monolayer conferring protection against endotoxaemia and cytokine production suggested that HSP and particularly HSP70 could play a role in conferring heat acclimation mediated tolerance in a multicellular system (Moseley, 1997). Consequently, the role of HSP70 and its inducible form HSP72, considered the most responsive to heat stress, in acclimation has received much attention and contrasting findings have been reported. In a muroid model Maloyan et al., (1999) demonstrated that heat acclimation resulted in a larger resting concentration of HSP72 and an accelerated transcription of the HSP72 gene making the HSP system better able to respond to heat stress. HSP production was attenuated in the early days of acclimation however, ruling out a protective effect of HSP in heat acclimation (Maloyan et al., 1999). Kresfelder et al., (2006) reported depressed basal levels of extracellular HSP70 and increased ability to induce HSP70 in combination with a specific HSP70 genotype only in individuals who were able to acclimate. Similarly, Marshall et al., (2006) reported reduced resting extracellular HSP72 in the first two days of an acclimation programme and speculated this may be necessary for improved cellular stress tolerance. Yamada et al., (2007) demonstrated sustained elevation of intracellular HSP72 from day six to ten of a heat acclimation protocol and concluded such a response may have contributed to improved heat tolerance and reduced risk of heat injury.
Similarly, McClung et al., (2008) demonstrated augmented intracellular HSP72 and inducibility of HSP72 after 10 days of heat acclimation. In 2010, Magalhaes et al., demonstrated that isothermal acclimation induces increased basal levels of intracellular HSP and during subsequent exercise in the heat acclimation inhibits the exercise-induced increase in intracellular and extracellular HSP72. With respect to research focussed on intermittent-sprint exercise, Castle et al., (2011) demonstrated that 10 days of traditional acclimation increased resting plasma HSP concentration and improved performance during 40 minutes of intermittent sprinting. Although the aforementioned provides evidence for a role of HSP70/72 in heat acclimation it is important to note that the heat shock response may be invoked by a number of stressors including, for example exercise and glycogen depletion. Therefore, as control groups were not used in the aforementioned studies it is difficult to disentangle the individual effects of different stressors in an exercise-heat acclimation protocol. That said, compelling evidence for a common mechanism for thermotolerance and acclimation and therefore a role of HSPs in acclimation is provided by Kuennen et al., (2011). Eight males completed a 7 day isothermal exercise-heat acclimation with either placebo or quercetin, a heat shock response inhibitor and were assessed for markers of thermotolerance including gastrointestinal (GI) permeability and leukocyte HSP70 content (Kuennen et al., 2011). On completion of acclimation GI permeability remained elevated, HSP70 was not increased and body temperature was not reduced in comparison to the placebo group. It was concluded that use of a heat shock inhibitor prevents both cellular (thermotolerance) and systemic (acclimation) adaptations but the authors also acknowledged the antioxidant effect of quercetin that may mean any observed lack of change was due to an antioxidant mediated reduction in cellular stress with heat insult (Kuennen et al., 2011).

2.5.2.2. Thermoregulatory adjustments to heat acclimation

A primary thermoregulatory adaptation observed in the heat acclimated phenotype is a decreased resting and exercise core temperature (Armstrong and Maresh, 1991). This adaptation is frequently observed with both traditional and isothermal heat acclimation protocols. Yet, where intermittent exercise is used to induce adaptation the response is
less clear with no change in exercise core temperature (Sunderland et al., 2003), a reduction in exercise but not resting core temperature (Sunderland et al., 2008; Brade et al., 2013) and no change in either (Petersen et al., 2010). In the instance of the cited literature such discrepancy may reflect the short term nature of the protocols used. Generally, the magnitude of the reduction in resting rectal temperature with heat acclimation is suggested to be in the region of 0.3°C - 0.5°C and it is proposed that this reduction accounts for between 37 - 50% of the observed reduction in end exercise rectal temperature (Buono et al., 1998; Kampmann et al., 2008). Evidence suggests a lowering of the hypothalamic thermoregulatory set point and a reduction in heat storage via improved heat loss mechanisms, in conjunction with cardiovascular adjustments, is responsible for this response (Garden et al., 1966; Buono et al., 1998, Armstrong 1998). More recently, research has suggested levels of prostaglandin E2, cyclooxygenase and orexin, key thermoregulatory molecules that mediate hyperpyrexia, are attenuated after heat acclimation and therefore, it is suggested they play a role in mediating the decreased resting core temperature (Shin et al., 2013). Kampmann et al., (2008) also demonstrated that training in a neutral environment reduced resting core temperature and therefore, speculate that during acclimation in moderate heat stress the intensity of exercise is an important mediator of the reduced core temperature.

Evidence suggests that skin blood flow is increased following heat acclimation at a given internal temperature (Fox et al., 1963; Roberts et al., 1977), but maximal skin blood flow is not altered (Lorenzo and Minson, 2010). Originally, it was suggested that this adaptation was due to a reduction in the threshold for cutaneous vasodilation mediated by a central mechanism (Nadel et al., 1976; Yamazaki and Hamasaki, 2003). More recent research has, however, demonstrated a peripheral control mechanism may also contribute (Lorenzo and Minson, 2010). Lorenzo and Minson (2010) demonstrated this peripheral aspect through an increased cutaneous vascular conductance and skin blood flow to a local stimulus by microdialysis with acetylcholine. It was suggested that heat acclimation may increase cutaneous vascular conductance by increased muscarine receptor number, decreased sensitivity to cholinesterase activity or alterations in the
pathway of vasodilation in smooth muscle or endothelial cells (Lorenzo and Minson, 2010).

A reduction in skin temperature during both endurance and intermittent-sprint exercise in hot and temperate conditions has been reported after traditional, isothermal and intermittent-exercise acclimation (Shvartz et al., 1973; Fein et al., 1975; Nielsen et al., 1993; Regan et al., 1996; Nielsen et al., 1997; Buono et al., 2009; Lorenzo et al., 2010; Brade et al., 2013). Depressed skin temperature may augment the core:skin temperature gradient increasing heat dissipation that contributes to a lower core temperature and a reduced skin blood flow in exercise post acclimation (Regan et al., 1996; Lorenzo et al., 2010). It is proposed a reduction in the hypothalamic setpoint for sweating onset coupled with an increased sweat rate and increased evaporation contribute to the lower skin temperature post acclimation (Shvartz et al., 1973; Lorenzo and Minson, 2010).

Heat acclimation induces an increase in whole body sweat rate and this has been shown during exercise in hot and temperate environments in traditional and isothermal protocols (Nadel et al., 1974; Nielsen et al., 1993; Patterson et al., 2004; Machado-Moreira et al., 2005; Buono et al., 2009). With intermittent exercise acclimation protocols sweat rate is increased (Brade et al., 2013), but is also reported to not change (Sunderland et al., 2008) possibly owing to the training state of participants. Adaptation to sweat rate arises through lowering of the zero point of the central nervous system for sweating, i.e., a central neutrally mediated adaptation (Nadel et al., 1974). In addition, enhanced thermosensitivity, i.e., a peripheral adaptation, contributes including eccrine gland hypertrophy, increased cholinergic sensitivity of the gland and increased peri-glandular concentrations of acetylcholine (Buono et al., 2009). Further, heat acclimation augments capacity of all sweat glands towards maximal flow rates (Patterson et al., 2004). Originally, it was suggested enhanced sudomotor function was a slow response occurring in the latter days of heat acclimation and requiring up to fourteen days to achieve complete adaptation (Armstrong and Maresh, 1991). More recent research, however, has demonstrated sudomotor adaptation from approximately day 3 - 6 of heat acclimation,
but more complete adaptation e.g. improved sensitivity requiring up to fourteen days (Patterson et al., 2004; Buono et al., 2009). Further, recent research contends that early observations of slow sudomotor adaptation may merely reflect the type of protocol used and demonstrate that sudomotor function can be enhanced within six days using an isothermal model of heat acclimation (Patterson et al., 2004).

In addition to an increased whole body sweat rate, altered sweat composition through the retention of Na\(^+\) and Cl\(^-\) is reported with heat acclimation (Kirby et al., 1986; Buono et al., 2007; Chinevere et al., 2008). It is suggested enhanced retention of Na\(^+\) is mediated through the action of plasma renin and aldosterone (Kirby et al., 1986; Chinevere et al., 2008). In response to fluid loss and a decrease in systemic blood pressure, renin is produced in the juxtaglomerular cells of the kidneys. This enzyme catalyses the conversion of angiotensin formed in the liver to angiotensin I (Gard, 1998). During blood transit through the alveolar capillaries angiotensin I is converted to angiotensin II by angiotensin converting enzyme. Together with its metabolite angiotensin III, angiotensin II acts on receptor cells in the zona glomerulosa to induce the synthesis of aldosterone (Gard, 1998). Aldosterone acts on target cells in the distal tubule of the kidneys and sweat glands to increase their reabsorption of Na\(^+\). By stimulating return of Na\(^+\) to the blood, aldosterone prevents depletion of Na\(^+\) from the body. The Na\(^+\) reabsorption leads to reabsorption of Cl\(^-\) and HCO\(_3^-\) and retention of water, thus contributing towards improved cardiovascular stability and reduced thermal strain.

2.5.2.3. Endocrine and metabolic adjustments to heat acclimation

The effect of heat acclimation on the fluid regulatory hormone aldosterone has also been examined. Research concerning hormonal responses to acclimation / acclimatization has produced conflicting results. Bonner et al., (1976) reported no significant difference in aldosterone level after thirteen days passive heat acclimation using the hot bath technique (41°C H\(_2\)O in 40°C chamber). Armstrong et al., (1989) also demonstrated no reduction in aldosterone level from days one to ten of an intense acclimation protocol.
(68% VO$_{2\text{max}}$, 41.2 ± 0.5°C, 39.0% ± 1.7% rh). Similarly, Francesconi et al., (1983) suggested acclimation to heat (10 d. treadmill @ 28-35% VO$_{2\text{max}}$, 2 x 50 min bouts alternate days @ 35°C 79% rh and 49°C 20% rh) does not consistently affect responses of plasma aldosterone, particularly when subjects were euhydrated. Garrett et al., (2009, 2012) supports this as short-term heat acclimation in moderately trained males did not increase aldosterone over day 1 - 5 but in highly trained athletes resting aldosterone was increased when short-term heat acclimation was accompanied by dehydration. Other research has observed an initial increase in aldosterone levels in the early days of a heat acclimation protocol followed by attenuated levels in the latter stages (Kirby et al., 1986, Francesconi et al., 1993). It is thought this response may be due to the plasma volume expansion accompanying acclimation (Francesconi et al., 1983). A primary function of aldosterone in the initial stages of a heat acclimation programme is to reduce the loss of Na$^+$ due to elevated sweat rates. Reabsorption of Na$^+$ leads to reabsorption of Cl$^-$ and HCO$_3^-$ and retention of water, thus contributing towards improved cardiovascular stability and reduced thermal strain. In combination with antidiuretic hormone, aldosterone is important in the control of plasma volume expansion and thus the biphasic pattern of aldosterone expression may be due to the plasma volume expansion accompanying acclimation (Francesconi et al., 1983; Garret et al., 2011). Evidence to support this is demonstrated by research using spironolactone to inhibit aldosterone that observed a restricted plasma volume expansion in participants from which it was concluded 40% of plasma volume expansion could be explained by aldosterone (Luetkemeier et al., 1994).

Other endocrinological responses to heat acclimation include altered cortisol concentration. With exposure to heat stress during the initial days of a heat acclimation programme, concentrations of plasma cortisol are increased (Armstrong, 1998). Cortisol is suggested to reflect the degree of physiological strain experienced and heat tolerance during heat stress or during a particular heat acclimation protocol. As an acclimation protocol progresses and greater cardiovascular and thermoregulatory stability is achieved, concentrations of cortisol are attenuated (Pepper, 1985; Armstrong et al., 1989). In contrast, Garrett et al., (2009) and Sunderland et al., (2008) have reported no change in cortisol in response to short-term heat acclimation.
Metabolic adaptations to heat acclimation include altered fuel utilisation, with research demonstrating decreased muscle glycogenolysis and lactate formation post acclimation (Kirwan et al., 1987). The mechanism responsible for this alteration in substrate utilisation is the subject of debate, with redistribution of blood flow, increased hepatic glycogenolysis, increased plasma volume, altered fibre type recruitment and decreased cathecholamine concentration possibly owing to hypervolaemia identified as possible factors (Sawka et al., 1983a; Febbraio, et al., 1994; Young et al., 1985).

2.5.2.4. Cardiovascular adjustments to heat acclimation

Other physiological adaptations to heat acclimation include plasma volume expansion of which 40% can be attributed to the thermal stimulus and 60% to exercise related factors (Convertino et al., 1980). A plethora of investigations using both traditional and isothermal acclimation have demonstrated an initial plasma volume expansion in response to heat acclimation (Senay et al., 1976; Kirby et al., 1986; Armstrong et al., 1989; Nielsen et al., 1993; Aoyagi et al., 1995; Nielsen, 1998; Garrett et al., 2012). Further, while plasma volume response to intermittent exercise acclimation protocols has not been examined, one study has reported an expansion in plasma volume using normal football training and natural acclimatization (Racinais et al., 2012). Generally, plasma volume expansion is attenuated in the latter stages of an acclimation protocol, but it is argued this response may merely reflect the protocol employed as the reduction in strain over time of constant-rate protocols may produce this response pattern (Patterson et al., 2004). Further, when isothermal acclimation is used plasma volume expansion can remain for up to twenty two days (Patterson et al., 2004). A protein influx to vascular compartments increasing colloid osmotic pressure and subsequent fluid influx may be responsible for plasma volume expansion (Nielsen et al., 1993, 1997), but an electrolyte retention mechanism has also been proposed (Wendt et al., 2007).

Accompanying an increased plasma volume with heat acclimation is a decrease in resting and exercise heart rate observed in response to traditional, isothermal and intermittent
exercise type heat acclimation. Such reductions are thought to be mediated primarily through the vagal pathway without a change in autonomic control (Yamazaki and Hamasaki, 2003). In addition, it has been proposed there is autonomic nervous system habituation with resultant redirection of cardiac output to the skin capillary beds and active muscle (Armstrong and Maresh, 1991). More recently, it has been suggested that a rapid and significantly reduced norepinephrine concentration during exercise in the heat that may indicate reduced sympathetic nervous system activity may mediate the reduction in heart rate observed in heat acclimation (Hodge et al., 2013). Taken together, the aforementioned acclimation induced changes serve to reduce the cardiovascular strain.

2.5.2.5. Perceptual adjustments to heat acclimation

Perception of effort and thermal sensation are improved in response to traditional, isothermal, and non-traditional methods of heat acclimation and this is observed in endurance-based and intermittent-sprint exercise (Pandolf, 1977; Regan et al., 1996; Molloy et al., 2004; Sunderland et al., 2008; Castle et al., 2011; Burk et al., 2012). The mechanisms responsible are not well understood but thermal sensation is correlated with skin temperature and therefore, acclimation induced reduction in this variable may contribute to reduced thermal sensation (Kamon et al., 1974). RPE has previously been correlated to physiological variables such as heart rate, ventilation and blood lactate and therefore, reductions in physiological strain with heat acclimation may mediate the reduction in this measure (Edwards et al., 1972; Armstrong and Maresh, 1991). Recent work, however, indicates afferent feedback from the heart and lungs does not contribute significantly to the perception of effort during exercise (Marcora, 2009). As such, alterations in central factors with heat acclimation e.g. neural pathways and dopamine may be involved (Marcora et al., 2009).
2.5.3. Overview of studies using traditional, medium to short-term traditional and isothermal strain acclimation

Table 2.3. Traditional heat acclimation in humans.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Participants</th>
<th>Protocol</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Robinson et al., (1943)*</td>
<td>5M</td>
<td>10-23 d, 1-1.5 hr, treadmill 3.5 mph, 4 – 5.6% grade. 40°C, 23% rh.</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Lind &amp; Bass (1963)*</td>
<td>16M</td>
<td>9 d, 50 – 200 min.d⁻¹, treadmill 3.5 mph 0% grade.</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Garden et al., (1966)</td>
<td>38M</td>
<td>10 d, (1, 1.6 or 2 hr.d⁻¹) 36.6°C db, 32.2°C wb.</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nadel et al., (1974)</td>
<td>6M</td>
<td>10 d, 1 hr.d⁻¹ @ 50% VO₂max, 45°C DH or 36°C HH.</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mitchell et al.,</td>
<td>4M</td>
<td>10 d, 4 hr.d⁻¹ @ 40 – 50% VO₂max, 45°C db, 32°C wb.</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Senay &amp; Kok, (1977)</td>
<td>5M</td>
<td>8 d, 4 hr.d⁻¹, stool stepping = 35W, 33°C db, 32°C wb.</td>
<td>-</td>
<td>-</td>
<td>X</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Avellini et al., (1980)</td>
<td>4M, 4F</td>
<td>10 d, 2 hr.d⁻¹, 36°C db, 32°C wb.</td>
<td>X</td>
<td>X</td>
<td>✓</td>
<td>X</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Shapiro et al., (1981)</td>
<td>8M</td>
<td>10 d, 120 min.d⁻¹, treadmill @ 1.34 m.s⁻¹, 40°C, 30% rh. (summer and Winter)</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Horstman &amp; Christensen, (1982)</td>
<td>6M, 4F</td>
<td>11 d, 2 hr.d⁻¹ @ 40%, VO₂max, 45°C db, 23% wb.</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>Sawka et al., (1983b)</td>
<td>6M, 6F</td>
<td>10 d, 100 min.d⁻¹, 49°C, 20% rh &amp; 35°C, 79% rh alternated.</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>X</td>
<td>X</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sawka et al., (1985)</td>
<td>13M</td>
<td>9 d, 2 hr.d⁻¹, 49°C, 20% rh treadmill @1.52 m.s⁻¹, 2-6% grade</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Kirby et al., (1986)</td>
<td>10M</td>
<td>10 d, 2 hr.d⁻¹, 45% VO₂max, 40°C db, 45% rh.</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Griefahn et al., (1997)</td>
<td>6M, 2F</td>
<td>15 d, 100 min.d⁻¹, Warm humid, hot dry or radiant heat (WBGT = 33.4 – 33.6°C)</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>X</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cheung &amp; McLellan (1998)</td>
<td>15M (MT + HT)</td>
<td>10 d, 1 hr.d⁻¹, 40°C, 30% rh</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>X</td>
<td>-</td>
<td>-</td>
<td>X</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Shido et al., (1999)</td>
<td>10M, 2F</td>
<td>10 d, 4 hr.d⁻¹, resting in 46°C, 20% rh.</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>X</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Yamada et al., (2007)</td>
<td>10M, 2F</td>
<td>10 d, 100 min.d⁻¹, 56% VO₂max, 42.5°C, 27.9% rh</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>✓</td>
</tr>
<tr>
<td>Chinevere et al., (2008)</td>
<td>8M</td>
<td>10 d, 100 min.d⁻¹, 45°C, 20% rh.</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>X</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Kampmann et al., (2008)</td>
<td>8M, 2F</td>
<td>15 d, 2 hr.d⁻¹, dry, humid and radiant heat (WBGT = 33.5°C)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Epstein et al., (2010)</td>
<td>12M</td>
<td>12 d, 120 min.d⁻¹, 40°C. 40% rh.</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lorenzo et al., (2010a&amp;b)</td>
<td>10M, 2F</td>
<td>10 d, 90 min, @ 50% VO₂max, 40°C, 30% rh.</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>✓</td>
</tr>
<tr>
<td>Amorim et al.,</td>
<td>7M, 2F</td>
<td>10 d, 100 min.d⁻¹ @ 56% VO₂peak,</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
(2011) 
Castle et al., (2011) 8M 
10 d, 1 hr.d⁻¹, 50% VO₂peak.

42°C, 30% rh,

1 = resting HR decreased; 2 = resting Tcore decreased; 3 = exercise HR decreased; 4 = exercise Tcore decreased; 5 = Tsk decreased; 6 = increased Sweat rate; 7 = increased Plasma volume; 8 = improved thermal comfort/sensation/RPE; 9 = improved exercise performance or duration; ✓ = criteria achieved; X = criteria not achieved; = not reported or measured; M = males; F = females; MT = moderately trained; HT = highly trained; AC = acclimation group; CT = control; TR = training
Tre = rectal temperature; Tau = aural temperature; Tac = aural canal temperature; WB = wet bulb; DB = dry bulb; WBGT = wet bulb globe temperature; A = results based on effect size only.

Table 2.4. Medium to short-term traditional heat acclimation in humans.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Participants</th>
<th>Protocol</th>
<th>Heat acclimation criteria (see footer for detail)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shvartz et al., (1973)</td>
<td>17M (HW; HD, TR)</td>
<td>6 d, 60-90 min.d⁻¹, stepping in 37°C, 20% rh or 50°C, 20% rh.</td>
<td>✓ ✓ ✓ ✓ ✓ - - -</td>
</tr>
<tr>
<td>Finberg et al., (1977)</td>
<td>8M (acc n = 4)</td>
<td>7 d, 90 min.d⁻¹ @ 50°C, HR 150-170 b.min⁻¹ at end of session.</td>
<td>- - ✓ ✓ ✓ - - -</td>
</tr>
<tr>
<td>Pandolf et al., (1977)</td>
<td>24M</td>
<td>9 d, 110 min.d⁻¹, 49°C, 20% rh</td>
<td>- - ✓ ✓ ✓ X - - -</td>
</tr>
<tr>
<td>Frye &amp; Kamon, (1981)</td>
<td>4M, 4F</td>
<td>8-9 d, 2 hr.d⁻¹ 48°C db, 25°C wb.</td>
<td>- - ✓ X ✓ ✓ - - -</td>
</tr>
<tr>
<td>King et al., (1985)</td>
<td>10M</td>
<td>8 d, 90 min.d⁻¹, 55% VO₂max, 39.7°C, 31% rh.</td>
<td>- - ✓ ✓ - - ✓ - ✓</td>
</tr>
<tr>
<td>Young et al., (1985)</td>
<td>13M</td>
<td>9 d, 2 hr.d⁻¹ @ 40 – 50% VO₂max, 49°C, 20% rh.</td>
<td>- - ✓ ✓ - - - - -</td>
</tr>
<tr>
<td>Kirwan et al., (1987)</td>
<td>8M</td>
<td>8 d, 90 min.d⁻¹, 50% VO₂max, 39.6°C, 29.2% rh.</td>
<td>- - ✓ ✓ - - - - -</td>
</tr>
<tr>
<td>Aoyagi et al., (1995)</td>
<td>9M</td>
<td>6 d, 60 min.d⁻¹, 40°C, 30% rh</td>
<td>- - ✓ ✓ ✓ ✓ - - -</td>
</tr>
<tr>
<td>Febbraio et al., (1994)</td>
<td>13M</td>
<td>7 d, 90 min.d⁻¹, 50% VO₂max, 40°C, 20% rh. Cycle ergometer.</td>
<td>- - ✓ ✓ - - - - -</td>
</tr>
<tr>
<td>Buono et al., (1998)</td>
<td>9M</td>
<td>7 d, 2 hr.d⁻¹ 35°C, 75% rh.</td>
<td>- ✓ - ✓ - - - - -</td>
</tr>
<tr>
<td>Yamazaki and Hamasaki, (2003)</td>
<td>8M, 2F</td>
<td>6 d, 80 min.d⁻¹(4x20 min) @ 50% VO₂max, 36°C, 50% rh.</td>
<td>- - ✓ ✓ - - - - -</td>
</tr>
<tr>
<td>Machado Moreira et al., (2005)</td>
<td>6M</td>
<td>9 d, 1 hr.d⁻¹, 50% VO₂max, 40°C, 32% rh.</td>
<td>✓ ✓ ✓ ✓ ✓ ✓ ✓ x</td>
</tr>
<tr>
<td>Kresfelder et al., (2006)</td>
<td>5 M, 17F</td>
<td>4 d, 1 hr.d⁻¹, stepping @ 35 – 70 W, 28°C WBGT</td>
<td>- - - ✓ - - - - -</td>
</tr>
<tr>
<td>Buono et al., (2009)</td>
<td>13M</td>
<td>8 days, 2 hr.d⁻¹, 100 min</td>
<td>- - ✓ ✓ - - - - -</td>
</tr>
<tr>
<td>Fujii et al., (2011)</td>
<td>21M; AC(10)</td>
<td>6 d, 80 min.d⁻¹, 50% VO₂peak, 37°C.</td>
<td>✓ ✓ ✓ ✓ X - ✓ - -</td>
</tr>
</tbody>
</table>
Table 2.5. Isothermal strain / controlled hyperthermia heat acclimation in humans.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Participants</th>
<th>Protocol</th>
<th>Heat acclimation criteria (see footer for detail)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fox et al., (1963)</td>
<td>9M</td>
<td>12-24 d, 3h.d⁻¹ passive hot H2O bath so Tcore = 37.3 - 38.5°C</td>
<td>1 = resting HR decreased; 2 = resting Tcore decreased; 3 = exercise HR decreased; 4 = exercise Tcore decreased; 5 = Tsk decreased; 6 = increased Sweat rate; 7 = increased Plasma volume; 8 = improved thermal comfort/sensation/RPE; 9 = improved exercise performance or duration; ✓ = criteria achieved; x = criteria not achieved; − = not reported or measured; M = males; F = females; MT = moderately trained; HT = highly trained; AC = acclimation group; CT = control; TR = training</td>
</tr>
<tr>
<td>Fox et al., (1967)</td>
<td>12M</td>
<td>12 d, 2 hr.d⁻¹, passive Tsk to 38.2°C</td>
<td>- - ✓ ✓ ✓ ✓ - - - - -</td>
</tr>
<tr>
<td>Henane &amp; Valatx, (1973)</td>
<td>9M</td>
<td>9 d, 180 min.d⁻¹, 55°C DB, 40°C WB + 35°C</td>
<td>- - ✓ ✓ ✓ ✓ - - - - -</td>
</tr>
<tr>
<td>Turk and Worsley, (1974)</td>
<td>51M</td>
<td>5 d, 2 hr.d⁻¹, bench stepping in 40 – 50°C WBGT to elevate Tbody so Tcore = 38.8°C then exercise and rest in 36°C WBGT to maintain target Tbody; Fox et al., (1980)</td>
<td>- - ✓ ✓ ✓ ✓ - - - - -</td>
</tr>
<tr>
<td>Bonner et al., (1976)</td>
<td>5 M</td>
<td>13 d, 1 hr.d⁻¹, H2O bath @ 41°C in a chamber at 40°C db 28°C wb so Tcore = 38.5°C</td>
<td>✓ ✓ ✓ ✓ - - - - - -</td>
</tr>
<tr>
<td>Convertino et al., (1980)</td>
<td>8M</td>
<td>8 d, 2 hr.d⁻², passive acclimation @ 42°C 93% rh to achieve Tcore = 38.5°C</td>
<td>- - ✓ ✓ ✓ ✓ ✓ ✓ - - - - - -</td>
</tr>
<tr>
<td>Harrison et al., (1981)</td>
<td>6M</td>
<td>11 d, 70 min.d⁻¹, 30 min H2O bath + 40 min cycle ergometer at 40°C db 20% wb so Tsk at 38.3 ± 0.3°C</td>
<td>✓ ✓ ✓ ✓ X ✓ - - - - - -</td>
</tr>
<tr>
<td>Havenith and Middendorf, (1986)</td>
<td>4M</td>
<td>7 d, 2 h.d⁻², 40°C, 20% rh, work + rest so Tcore = 38.3°C</td>
<td>✓ ✓ ✓ ✓ ✓ X - - - - - -</td>
</tr>
<tr>
<td>Regan et al., (1996)</td>
<td>14M</td>
<td>10 d, 1 hr.d⁻¹, in 38.2°C, 39.7% rh. Vary workload so Tsk = 38°C</td>
<td>- - ✓ ✓ ✓ ✓ - - - - - -</td>
</tr>
<tr>
<td>Cotter et al., (1997)</td>
<td>8M</td>
<td>6 d, 70 min.d⁻² in 39.5°C, 59.2% rh so Tsk = 1.4°C &gt; rest.</td>
<td>- - ✓ ✓ ✓ ✓ - - - - - -</td>
</tr>
<tr>
<td>Patterson et al., (2004)</td>
<td>11M</td>
<td>16 d in 3 wks, 90 min.d⁻¹ in 40°C 60% rh. 30 min + 60 min so Tsk = 38.5°C</td>
<td>✓ ✓ ✓ ✓ ✓ - - - - - -</td>
</tr>
<tr>
<td>Weller et al., (2007)</td>
<td>16M</td>
<td>10 d, 110 min.d⁻¹, 46°C, 17.9% rh 60 min + 40 min so Tsk = 38.5°C</td>
<td>- - ✓ ✓ ✓ ✓ - - - - - -</td>
</tr>
<tr>
<td>Garrett et al., (2009)</td>
<td>10M MT</td>
<td>5 d, 90 min.d⁻¹, 40°C, 60% rh to elevate and maintain Tsk = 38.5°C</td>
<td>- - ✓ ✓ ✓ ✓ - - - - - -</td>
</tr>
<tr>
<td>Magalhaes</td>
<td>9M</td>
<td>11 d, 1 hr.d⁻¹, 30 min + then 30 min so Tsk = 38.5°C</td>
<td>✓ ✓ ✓ ✓ ✓ - - - - - -</td>
</tr>
</tbody>
</table>
et al., (2010) 1°C above rest

| Heat acclimation criteria (see footer for detail) |
|---|---|---|---|---|---|---|---|---|---|
|   |   |   |   |   |   |   |   |   |

<table>
<thead>
<tr>
<th>Reference</th>
<th>Participants</th>
<th>Protocol</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kuennen et al., (2011)</td>
<td>8M</td>
<td>7 d, 100 min.d⁻¹, 46°C 20% rh so T₉₀ ≥ 39°C</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

1=resting HR decreased; 2 = resting T_{core} decreased; 3 = exercise HR decreased; 4 = resting T_{core} decreased; 5 = T_{sk} decreased; 6 = increased sweat rate; 7 = increased Plasma Volume 8 = improved thermal comfort/sensation/RPE; 9 = improved exercise performance or duration; ✓ = criteria achieved; X = criteria not achieved; - = not reported or measured; M = males; F = females; MT = moderately trained; HT = highly trained; T₉₀ = rectal temperature; T_{au} = aural temperature; T_{ac} = aural canal temperature; WB = wet bulb; DB = dry bulb; WBGT = wet bulb globe temperature.

2.5.3.1. Efficacy of traditional heat acclimation protocols

Since the initial work of Robinson et al., (1943) documented the adaptive responses to 60 - 90 minutes of treadmill walking over 10 - 23 days in dry heat and Lind and Bass (1963) identified what was interpreted as ‘complete acclimatisation’ owing to plateaus in heart rate and rectal temperature after 9 - 10 days of treadmill walking for 100 min.d⁻¹ in - 49°C, traditional heat acclimation methods have been used in many investigations. Of the studies cited in this category, traditional protocols, when judged against the aforementioned criteria (section 2.5.) demonstrate the ability to consistently evoke reductions in exercise heart rate and core temperature (Table 2.3.). Further, mean sweat rate is improved by traditional acclimation, but the effect on thermal comfort / perception of effort is less clear as many studies do not readily report this criterion in their findings, although previous work suggests a decrease in this variable (Armstrong and Maresh, 1991). In addition, although performance improvement, for example through increased time to exhaustion or increased power output, is potentially a key aim of acclimation, few traditional acclimation studies have included this as a criterion measure, preferring instead to focus on physiological responses to fixed heat stress tests that have limited application to the performance setting. Where true measures of performance are included (Lorenzo et al., 2010) a positive effect is observed. Few studies have examined the benefit of traditional acclimation to intermittent-sprint performance, but Castle et al., (2011) reported an improvement in peak power output with a ten day protocol in males.
(Table 2.3.). For the criteria considered where evidence in table 2.3. suggests traditional acclimation did not induce a change, for example, Senay and Kok, (1976) or Cheung and McLellan, (1998), the exercise mode and intensity may explain findings. As such, considering the evidence in table 2.3. traditional protocols appear effective at eliciting classic acclimation responses but may not achieve maximal adaptation as the heat strain declines in subsequent exposures (Taylor, 2000).

2.5.3.2. Efficacy of medium to short-term traditional heat acclimation protocols

The requirement to complete ≥10 days of exercise-heat stress to induce acclimation may be untenable in occupational and athletic settings. Consequently, the efficacy of medium to short-term traditional heat acclimation (MSTHA) has received much consideration (Table 2.4.). With respect to the research cited, studies in this category have used exercise modes including cycling, treadmill walking and bench stepping for four (Kresfelder et al., 2006) to nine days (7 ± 1.5 d) (for example, Pandolf et al., 1977) for 1-2 hours (1.6 ± 0.32 hrs) at 25 - 55% VO2max (46 ± 8%). While evidence demonstrates MSTHA consistently evokes adaptation in exercise heart rate, core temperature, Tsk and sudomotor function (Table 2.4.) and is therefore, an effective method of heat acclimation, lack of examination of key criteria in some studies (e.g. Kresfelder et al., 2006) does limit the ability to draw firm conclusions as to the efficacy of very short, traditional heat acclimation protocols. Few studies have considered performance as a key criterion with such protocols, but King et al., (1985) demonstrated maintenance of total work during a 45 s sprint following a six hour heat stress test but a decrease in this variable in an un-acclimated group. Conversely, Machado-Moreira et al., (2005) found no difference in maximal power output or total exercise time during graded exercise after heat acclimation. No studies have examined the effect of MSTHA on intermittent-sprint exercise performance and therefore, no conclusion can be drawn on this aspect.
2.5.3.3. Efficacy of isothermal strain heat acclimation protocols

Comparatively few studies have used isothermal strain to elicit heat acclimation (Table 2.5.). Fox and colleagues (1964, 1967) first applied this model in army volunteers to examine the effect of repeated local heating and hot wet / hot dry exposure on local sweat response using a long-duration protocol. Participants completed twelve to fifteen, two hour acclimation sessions. Core temperature was elevated by exposure to a hot, moist air stream or hot room and thin vapour barrier suits were used to maintain core temperature at 37.9°C (Fox et al., 1964) or 38.3°C (Fox et al., 1967). Classic heat acclimation responses were observed. Of the studies cited in table 2.5, a further six used long duration protocols (Bonner et al., 1976; Harrison et al., 1981; Regan et al., 1986; Patterson et al., 2004; Weller et al., 2007; Magalhaes et al., 2010) and the majority evoked physiological adaptations consistent with the criteria for a heat acclimation protocol to be deemed effective. With Magalhaes (2010), the comparatively shorter session duration combined with allocation of half of the session to elevating core may have contributed to the lack of change in key markers during exercise.

Prolonged isothermal strain protocols (≥ 10 d) demonstrate effectiveness for heat acclimation. Medium to short duration protocols (≤ 8 d) provide further insight into the efficacy of this method and demonstrate similar positive responses. Of the studies cited in table 2.5., seven used isothermal strain protocols ≤ 8 d and demonstrated some or all of the classic heat acclimation criteria judging them effective methods to induced adaptation to the heat. The work of Garrett et al., (2012), also demonstrates this method is effective for inducing physiological adaptation in highly trained athletes. Further, isothermal strain protocols can improve performance in moderate and highly trained individuals in both time to exhaustion and time trial modes (Garrett et al., 2009, 2012, 2014).

The isothermal strain model of heat acclimation has potential advantages over other regimes, yet comparatively few research investigations have employed this method.
Although isothermal strain serves to maintain heat stress across the protocol and therefore, potentially maximises adaptations owing to the requirement to complete more work to achieve a pre-determined core temperature, the extent of performance benefit beyond more traditional methods is unclear. The work of Garrett et al., (2009, 2012, 2014) provides insight that for moderately trained males and highly trained endurance athletes short-term isothermal strain may be beneficial for performance, but more work is required to compare the validity of isothermal strain against other models of heat acclimation to demonstrate a criterion standard for heat acclimation. In addition, the appropriateness of isothermal strain for other athletes, for example, games players is unknown as no research has examined whether this method may promote over-reaching in an athlete at a point in the training cycle when a taper is normal.

2.5.4. Alternate heat acclimation methods

Although the body of literature is increasing there is a dearth of information on the efficacy of alternate heat acclimation protocols. Existing work focuses primarily on the impact of altered exercise intensity and duration on heat acclimation, or the effect of continuous versus intermittent protocols on acclimation (Fein et al., 1975; Houmard et al., 1990; Aoyagi et al., 1995; Gill et al., 2001; Sunderland et al., 2003; O’ Brien et al., 2004; Sunderland et al., 2008; Petersen et al., 2010, Table 2.6.). Such investigations have produced conflicting results. In addition, research in this field has used isotype protocols that require exercise at a fixed percentage of heart rate across the protocol as adaptation is achieved (Nielsen et al., 1993, 1997; Burk et al., 2012) and intermittent-sprint exercise to elicit a heat acclimated phenotype (Sunderland et al., 2008; Petersen et al., 2010; Brade et al., 2013).

Table 2.6. Alternate heat acclimation in humans.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Participants</th>
<th>Protocol</th>
<th>Heat acclimation criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fein et al.</td>
<td>12F (2 groups)</td>
<td>HA every 3\textsuperscript{rd} d for 10 sessions. 100 min.d\textsuperscript{-1}, 46.5°C db, 24.5°C wb. OR</td>
<td>- - ✔ ✔ ✔ ✔ - - ✔</td>
</tr>
</tbody>
</table>
10 d consecutive heat acclimation

<table>
<thead>
<tr>
<th>Reference</th>
<th>Participants</th>
<th>Protocol</th>
<th>Heat acclimation criteria (see footer for detail)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nadel et al., (1976)</td>
<td>5M</td>
<td>10 d, 60 min.d⁻¹ 50% VO₂max, 36°C, 35 Torr.</td>
<td>1 - 2 - 4 - 7 - - - - -</td>
</tr>
<tr>
<td>Armstrong et al., (1989)</td>
<td>13M</td>
<td>8 d, 100 min.d⁻¹, self-paced (d2-d7) @ 68% VO₂max, 41°C, 39% rh.</td>
<td>- - - - - - - - -</td>
</tr>
<tr>
<td>Houmard et al., (1990)</td>
<td>9M</td>
<td>Exp 1: 7 d 1 hr.d⁻¹, 50% VO₂max Exp 2: 7 d 30-35 min.d⁻¹, 75% VO₂max</td>
<td>- - - - X X - - -</td>
</tr>
<tr>
<td>Barnett &amp; Maughan (1993)</td>
<td>5M</td>
<td>1 d.wk⁻¹ x 4 wks 60 min.d⁻¹,55% VO₂max Day 1 = 22°C 67% rh, day 2-4 = 34.6°C, 60% rh</td>
<td>- - X X X X - - -</td>
</tr>
<tr>
<td>Nielsen et al., (1993)</td>
<td>8M</td>
<td>9 – 12 d to exhaustion, 60% VO₂max, 40°C, 10% rh.</td>
<td>- - - - - - - - -</td>
</tr>
<tr>
<td>Nielsen et al., (1997)</td>
<td>12M</td>
<td>8 – 13 d to exhaustion, 45% VO₂max, 35°C, 87% rh</td>
<td>- - - - - - - - -</td>
</tr>
<tr>
<td>Gill et al., (2001)</td>
<td>14M (consecutive gp = 7, intermittent gp = 7)</td>
<td>10 d 30 min.d⁻¹ (consecutive or over 3 weeks, 70% VO₂peak in 38°C, 70% rh.</td>
<td>- - - - - - - - -</td>
</tr>
<tr>
<td>Sunderland et al., (2003)</td>
<td>8F</td>
<td>4 sessions in 10 d, 30 min.d⁻¹, LIST (2 sets), 30°C</td>
<td>- - X X - - - - X</td>
</tr>
<tr>
<td>O’Brien et al., (2004)</td>
<td>7M</td>
<td>10 d 60 min.d⁻¹ at 50% VO₂max, OR D1 &amp; 10 as above + 8 d @ 85% VO₂max for 15 min.d⁻¹</td>
<td>- - - - - - - - -</td>
</tr>
<tr>
<td>Sunderland et al., (2008)</td>
<td>17F (3 gps; AC(6)); TR(6) CT(6))</td>
<td>4 sessions in 10 d, 30 min.session⁻¹; LIST (2 sets but 3 sets in d3&amp;4), 30°C, 24% rh</td>
<td>- - X - - - - X</td>
</tr>
<tr>
<td>Molloy et al., (2004)</td>
<td>17M</td>
<td>14 d, 30 min.d⁻¹, 70% VO₂max, 35°C, 35% rh</td>
<td>- - - - X X X - -</td>
</tr>
<tr>
<td>Skurvydas and Brazaitis (2010)</td>
<td>7M, 6F</td>
<td>7d over 2 weeks, 45 min.d⁻¹, passive heating, half-body in H₂O bath @ 44°C.</td>
<td>X - - - - - - - -</td>
</tr>
<tr>
<td>Petersen et al., (2010)</td>
<td>12M (AC(6); CG (6))</td>
<td>4 d 30 – 45 min.d⁻¹, 30°C, 61% rh Intermittent activity.</td>
<td>- - - X X - - - -</td>
</tr>
<tr>
<td>Daanen et al., (2011)</td>
<td>15M</td>
<td>9 d @ 26°C WBGT + 3 d @ 32°C WBGT, 2 hr.d⁻¹, 45% VO₂max for 60 min + incremental test.</td>
<td>- - - - - X - -</td>
</tr>
<tr>
<td>Burk et al., (2012)</td>
<td>21M</td>
<td>10 d, 110 min.d⁻¹ 55% VO₂peak for 5 d and 60% VO₂peak for 5 d, 42°C, 18% rh.</td>
<td>- - - - - - - - -</td>
</tr>
<tr>
<td>Garrett et al., (2012)</td>
<td>8M HT</td>
<td>5 d, 90 min.d⁻¹, 40°C, 60% rh so Tre = 38.5°C</td>
<td>X X - - - X</td>
</tr>
<tr>
<td>Brade et al., (2013)</td>
<td>10M</td>
<td>5 d, 3 min @ 80% PPO + 1 min rest x 8 (d1 = 32 min, d4 = 48)</td>
<td>- - - - - - - - X</td>
</tr>
<tr>
<td>Racinais et al., (2012)</td>
<td>19M</td>
<td>6 d heat acclimatisation in 38 – 43°C, 12-30% rh.</td>
<td>- - - - - X X X X</td>
</tr>
<tr>
<td>Shin et al., (2013)</td>
<td>9M</td>
<td>10 d (alternate), over 3 weeks 30 min.session⁻¹. Half-body immersion, H₂O bath @ 42°C.</td>
<td>- - - - - - - - -</td>
</tr>
<tr>
<td>Costa et al., (2014)</td>
<td>6M</td>
<td>Treadmill, 2 hr @ 60% VO₂max 3 d @ 30°C, 3 d at 35°C.</td>
<td>X X - - - -</td>
</tr>
<tr>
<td>Garrett et al., (2014)</td>
<td>9 M</td>
<td>5 d, 90 min.d⁻¹, in 39.5°C, 60% rh to maintain Trec @ 38.5°C</td>
<td>X X X X X X X</td>
</tr>
</tbody>
</table>

1=resting HR decreased; 2 = resting Tcore decreased; 3 = exercise HR decreased; 4 = exercise Tcore decreased; 5 = Tsk decreased; 6 = increased Sweat rate; 7 = increased Plasma volume; 8 =

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improved thermal comfort/sensation / RPE; √ = criteria achieved; X = criteria not achieved; - = not reported or measured; M = males; F = females; MT = moderately trained; HT = highly trained; AC = acclimation group; CT = control; TR = training.

2.5.4.1. Daily versus alternate day heat acclimation

Fein et al., (1975) acclimated twelve women using either a daily or intermittent (every third day) exposure pattern in 32.2°C (Table 2.6) and no significant differences between groups in key markers of acclimation were reported at the end of the protocols. Both protocols induced classic acclimation responses and it was concluded the pattern of exposure had no discernable effect on the rate of heat acclimation (Fein et al., 1975). Similar positive benefits of alternate day heat acclimation were reported by Shin et al., (2013). In contrast, Gill et al., (2001) demonstrated minimal heat adaptation with intermittent heat exposure in fourteen competitive rowers when ten sessions were completed over three weeks and it was proposed daily heat exposure is the most effective acclimation strategy (Gill et al., 2001). Such discordance may be explained by session duration in the work of Gill et al., (2001) as 30 minutes may represent insufficient time to elevate and maintain core temperature. Alternately, use of competitive rowers may have contributed to such findings as high levels of aerobic fitness confer many adaptations similar to heat acclimation (Pandolf, 1977), thus reducing the potential for further pronounced adaptation with heat acclimation.

2.5.4.2. Low and high intensity acclimation

In trained males, running at approximately 75% VO₂max for 30 - 35 min.d⁻¹ for seven days evoked reductions in exercise heart rate and T_re consistent with the heat acclimated phenotype and not different from those observed with a more traditional protocol (Houmard et al., 1990). In contrast, cycling at 50% VO₂max for 60 minutes on day 1 and 10 and at 85% VO₂max for 15 minutes on days 2 - 9 failed to evoke classic heat adaptation and it was suggested a traditional protocol should be used in preference to a high intensity
heat acclimation protocol during exercise in the heat as it resulted in improved thermoregulation (O’Brien et al., 2004). Such discrepancy between findings may be explained by training state and fitness level of participants as this variable is not reported by O’Brien et al., (2004), and as reported level of physical fitness can influence heat acclimation rate (Pandolf, 1977). Further the study design may have been a confounding variable as the participants completed both conditions separated only by four weeks (O’Brien et al., 2004). Previous studies have testified as to the longevity of adaptations in key markers of heat acclimation after eighteen days (Pandolf et al., 1977) and a degree of heat acclimation may have resided in the participants at the beginning of the modified protocol trial. More recent work, however, refutes this point (Garrett et al., 2009). In addition, it could be postulated that 15 minutes represents insufficient stimulus regardless of exercise intensity. Pandolf et al., (1988) in Taylor et al., (1997) support this notion as it is suggested volume of training appears to be more critical than intensity. Taylor et al., (1997) also suggest the greater the $T_{\text{core}}$ elevation the greater the heat adaptation. Thus, it could be postulated the 15 minutes acclimation period for the high intensity acclimation protocol in this study presented insufficient time for a suitable rise in $T_{\text{core}}$, or an insufficient time period at an elevated $T_{\text{core}}$.

### 2.5.4.3. Isotype acclimation

Classic isothermal strain protocols use fixed core temperature to elicit heat acclimation responses. Nielsen et al., (1993, 1997) used a similar forcing function to maintain strain but achieved this through isocardiac strain (heart rate 120-130 b.min$^{-1}$) during exercise to exhaustion. Using this approach many of the classic physiological responses to heat acclimation are achieved and in addition, time to exhaustion is markedly improved (Nielsen et al., 1993, 1997) demonstrating the efficacy of this method.

### 2.5.4.4. Intermittent exercise type acclimation

Recent intermittent-exercise type acclimation protocols use repeated patterns of high and low intensity efforts that simulate work:rest ratios of field-based team-sports or
repeated short efforts interspersed with short recoveries (Sunderland et al., 2003, 2008; Petersen et al., 2010; Brade et al., 2013). As such, protocols are designed to improve specificity of heat acclimation for field-based team-sport athletes with a view to improving performance in this type of activity. Their efficacy will be considered in section 2.5.5.

2.5.4.5. Dehydration heat acclimation

Aldosterone and antidiuretic hormone act to conserve fluid and are important mediators of plasma volume expansion (Garrett et al., 2011). Aldosterone secretion from the zona glomerulosa is controlled primarily by the renin angiotensin system that is activated in response to decreased blood volume and systemic pressure, but also plasma [K⁺] and [Na⁺] (Gard, 1998). Similarly, antidiuretic hormone is secreted from the posterior pituitary in response to increased plasma osmolarity and [Na⁺] (Gard, 1998). Exercise heat stress induced hypohydration mediates an enhanced upregulation of these fluid regulatory hormones that may promote enhanced cardiovascular stability in response to heat acclimation (Kenefick et al., 2007; Garrett et al., 2011). Previously, Garrett et al., (2014) tested this hypothesis using short term isothermal strain heat acclimation with ten moderately trained males. In a cross over design using a five week washout with euhydration and dehydration, short-term isothermal heat acclimation induced improved cardiovascular and performance responses that were generally enhanced by dehydration (Garrett et al., 2014). Further, although lacking a control group, Garrett et al., (2012) also demonstrated meaningful physiological and performance gains in highly-trained athletes with short-term dehydration acclimation. Such evidence supports the suggestion that dehydration heat acclimation may be an appropriate model to enhance heat adaptation.

2.5.5. Heat acclimation and intermittent-sprint exercise

The effect of heat acclimation on intermittent-sprint exercise performance has received limited attention. The studies considering this issue have employed different protocols with varying results (Table 2.6.). Recent evidence suggests that heat acclimation can
improve intermittent sprinting in the heat from both a physiological and performance perspective (Castle et al., 2011). When eight moderately trained males completed ten days of heat acclimation, 60 min·d⁻¹ at 50% VO₂peak in 33°C, 50% rh significant reductions were observed in resting rectal temperature, heart rate, RPE and thermal sensation by day three (Castle et al., 2011). Plasma volume was also significantly increased by day five of acclimation. With respect to intermittent-sprint cycling performance over 40 minutes in 33°C, 50% rh, peak power output was significantly increased by ~ 2% (Castle et al., 2011).

The work of Castle et al., (2011) demonstrates a beneficial effect of heat acclimation for intermittent sprint exercise completed in the heat. However, the protocol used was constant work rate and required ten days. While this may demonstrate performance improvement for moderately trained participants the duration and lack of specificity, as previously suggested, may make this method unsuitable for athletes that are well-trained.

Circumventing this limitation, Sunderland et al., (2003, 2008) have demonstrated a beneficial effect with shorter duration, intermittent type heat acclimation protocols designed (a) to reflect the intermittent nature of team-sports, thereby improving specificity and (b) to improve performance in match play. Sunderland et al., (2003) examined the impact of four, short heat acclimation sessions of 30 minutes completed within ten days on field hockey skill performance in eight well-trained, unacclimatized female hockey players. Participants completed two sets of the Loughborough intermittent shuttle test (LIST) in 30°C and although few classic adaptatory responses were observed, thermal comfort and hockey skill performance were improved (Sunderland et al., 2003).

Similarly, Sunderland et al., (2008) examined the effect of four heat acclimation sessions of 30 - 45 minutes at 30°C, 24% rh on the ability of seventeen, well trained (Control group VO₂max; 49.1 ml.kg.min⁻¹; Training group VO₂max; 49.3 ml.kg.min⁻¹; Acclimation group VO₂max 49.7 ml.kg.min⁻¹) female games players to perform high intensity intermittent running in the heat using the LIST. In comparison to the control and moderate training groups the high intensity heat acclimation group exhibited a 33% improvement in distance run in the LIST post acclimation (Sunderland et al., 2008). Post acclimation a lower rectal temperature and rate of rise in rectal temperature were observed, but no change in sweat response was observed, possibly due to the training status of the group.
In addition an improved thermal comfort was reported indicating the efficacy of this method in inducing heat acclimation (Sunderland et al., 2008). From a performance perspective, 15 m sprint time was unaffected but distance run was significantly increased (Sunderland et al., 2008). With a lower rectal temperature, improved thermal comfort and improved performance it was concluded four 30 - 45 minute sessions of intermittent exercise induced heat acclimation in female games players and provided performance benefits during intermittent exercise in the heat.

Similarly, in cricketers, the use of a 4 day intermittent sprint cycling protocol produced partial heat acclimation. Petersen et al., (2010) randomly assigned twelve male cricketers to acclimation and control groups (n = 6) and participants completed four consecutive days of sprint-based acclimation in 30°C, 60% rh (Table 2.6.). On completion of the protocol performance in a cricket specific running test was not altered, but this test was conducted in a temperate environment (Petersen et al., 2010). Effect sizes for key acclimation markers suggested partial acclimation had been achieved (Petersen et al., 2010). Brade et al., (2013) used a five day protocol involving repeated 3 minute efforts at 80% maximum power with 1 minute recovery to examine the effect of acclimation (and pre-cooling) on repeated-sprint performance in the heat in ten males. Participants completed 32 minutes of activity in session one building to 48 minutes in the last session. No significant differences were observed in key markers of acclimation, but effect size suggested a meaningful effect (Brade et al., 2013). Similarly, intermittent-sprint performance in the heat was not different pre and post acclimation.

The work of Petersen et al., (2010) and Brade et al., (2013), previously cited, suggest that, although some psychophysiological benefit may be gained from short-term acclimation when intermittent sprint exercise is conducted in the heat, performance is not improved. The work of Sunderland, (2003, 2008) partly counters this by demonstrating improvements in hockey skill performance and distance run after four acclimation sessions of the LIST in the heat, but it is important to note that 15 m sprint time and decline in sprint time was not improved. Consistent with Nevill et al., (1995) who
identified greater thermal strain during intermittent compared with continuous exercise, Sunderland et al., (2008) demonstrated rectal temperatures of 39.3°C within 30 minutes of intermittent exercise in the heat. As such, intermittent acclimation protocols would appear an appropriate stimulus to evoke threshold core temperatures necessary to elicit heat adaptation, but with short duration protocols in particular, the adaptation appears incomplete. From the evidence provided with regard to physiological responses to heat acclimation in the works of Petersen et al., (2010) and Brade et al., (2013) it is unlikely that complete adaptation was achieved and thus the efficacy of short duration intermittent exercise type acclimation protocols in evoking the heat acclimated phenotype is not yet clear.

2.5.6. Issues associated with current heat acclimation methods

The desire to understand and improve heat tolerance in occupational and endurance based sporting contexts has contributed to a large body of heat acclimation literature with over five hundred research studies published on humans since the first studies of Robinson et al., (1943). Consequently, much of the research has explored heat acclimation from this perspective and unsurprisingly, posits use of moderate intensity, relatively long duration, prolonged protocols as the most effective method of heat acclimation (Armstrong and Maresh, 1991; Maughan and Shirreffs, 2004). Such methods may, however, prove sub-optimal or compromise performance in other sports for a number of reasons.

Firstly, since the initial seminal work of Lind and Bass (1963) acquisition of complete heat acclimation has been based on attainment of a plateau in key markers of heat acclimation including heart rate and core temperature. This belief has persisted through the research on heat acclimation (Pandolf, 1977; Young et al., 1985; Buono et al., 1998), yet may essentially be incorrect. The general adaptation syndrome (Selye, 1946) demonstrates that in response to stress the body eventually responds through supercompensation to a new elevated level. Further, the model indicates that lack of additional stimulus results in
a plateau or decay of adaptations. As such, observation of a plateau in constant work rate protocols after initial adaptation may reflect the lack of additional strain across the protocol and not achievement of optimal or maximal heat acclimation responses and recent work supports this notion (Nielsen, 1993; Patterson et al., 2004). Therefore, traditional heat acclimation protocols may not maximise heat acclimation responses.

Secondly, Nevill et al., (1995), identified the potential for greater thermal strain on the body during intermittent exercise in comparison to continuous of the same average work intensity. Therefore, use of traditional protocols with athletes involved in high intensity intermittent-sprint activity, as displayed in some team sports may provide a sub-optimal stimulus and consequently, sub-maximal acclimatory responses. Isothermal models maintain strain, but the necessity to increase work rate to achieve and maintain a critical core temperature may evoke over-reaching in the athlete when tapering and a reduction in training load is desired. In addition, whether isothermal models confer extra benefits compared to traditional heat acclimation methods is unclear and the efficacy of this method for games players is unclear. With respect to specificity, the emergence of intermittent exercise type acclimation protocol addresses this issue for games players. While there is evidence of some beneficial effect of such methods most are short-term and further work must be done to elucidate their benefit to team-sport. Further due to the work-rest ratios incorporated in intermittent heat acclimation, the stimulus may be insufficient to evoke maximal adaptation and the physiological stress placed on the athlete by having to repeat this exercise may be untenable prior to competition requiring greater recovery periods between sessions.

Finally, conventional heat acclimation practice essentially involves the application of ‘one size fits all’ prolonged, moderate intensity heat acclimation protocols regardless of the individual. A review of the literature, however, suggests that such an approach may compromise the ability to achieve maximal heat adaptation as there is much evidence of participants failing to complete prescribed sessions in the initial days of a heat acclimation programme as a consequence of the abrupt exposure to high heat stress.
(Garden, 1966, Wyndham et al., 1976; Horstman et al., 1982, Febbraio, 1994,). Such an approach clearly limits heat stress exposure and therefore, as mentioned, may evoke sub-maximal adaptation as maintaining a critical core temperature for extended periods is considered key to effective heat acclimation (Taylor et al., 1997). It is known that the ability to adapt to the heat differs between individuals due to genotypic and phenotypic traits (Heled et al., 2004; Kresfelder et al., 2006). As such, in specific athletic groups, it could be proposed, non-traditional heat acclimation protocols may be more effective and warranted to optimise individual responses and exercise performance.

2.5.7. A progressive model of heat acclimation

As evidenced throughout this review, a myriad of acclimation protocols have been used to investigate human adaptation to heat stress. While the protocols differ in intensity, duration and scheduling, a common denominator is the targeting of heat strain. Existing protocols essentially use a heat strain model of acclimation whereby a desired strain is evoked by application of exercise intensities that will elicit sufficient endogenous heat load to provoke adaptation. In the case of isothermal strain protocols, desired strain is clearly defined as a specific threshold temperature. For traditional protocols the strain is less well defined. As identified throughout this literature review, a number of potential issues arise with acclimation protocols that are strain-orientated. This includes inability to complete prescribed sessions due to high levels of exercise-heat stress in the initial stages of acclimation and reductions in strain across the protocol as adaptations occur. To counter such limitations, it is proposed that a stress orientated model, whereby external variables, for example, temperature or humidity or exercise, are manipulated in a progressive manner across the protocol, may prove as, if not more effective than existing strain-orientated approaches. Three recent studies demonstrate support for this concept. Daanen et al., (2011) manipulated heat stress when acclimating fifteen participants by exposure to 26°C WBGT for nine days followed by 32°C WBGT for three days. Classic acclimation responses were reported in the initial nine days of acclimation and a further reduction in resting rectal temperature was reported after the additional three days in the elevated temperature suggesting an additional beneficial effect (Daanen et al., 2011).
Exercise measures of heart rate and rectal temperature were not, however, altered by the additional three days but it was suggested a longer period of exposure to the elevated heat stress may prove beneficial (Daanen et al., 2011). Burk et al., (2012) also adopted a stress-orientated model by manipulating exercise intensity from 55% to 60% $\text{VO}_{2\text{peak}}$ during a ten day acclimation protocol. Although classic acclimatory responses were observed, the research was not concerned with optimising heat acclimation. Therefore, no reference was made to the design of the protocol and no control group was involved making it difficult to draw conclusions about the efficacy of the method used. Finally, Costa et al., (2014) demonstrated an additional benefit with heat acclimation that adopted a stress orientated approach. When six male ultra endurance athletes completed three two hour treadmill runs at 60% $\text{VO}_{2\text{max}}$ in an environmental chamber at 30°C followed by three at 35°C additional improvements in thermal comfort and physiological strain were reported (Costa et al., 2014).

Comparative evidence from intermittent hypoxic exposure also provides support for potential benefit of a progressive heat acclimation strategy. Hamlin and Hellemans, (2007) exposed twenty-two multi-sport endurance athletes to 90 minutes of intermittent normobaric hypoxic exposure at rest versus 90 minutes of placebo exposure at rest for 15 days over a 3-week period. Oxygen in the hypoxic gas decreased from 13% in week one to 10% by week three. Results showed 3-km run time decreased by 1.7% 2 days after, and by 2.3% 17 days after the last hypoxic episode in the training relative to the placebo group. In addition, relative to the placebo group, the training group increased reticulocyte count 2 days (23.5%) and 12 days post-exposure.

Considering the above, a progressive heat stress orientated model of heat acclimation that sequentially manipulates environmental stress across the duration of a protocol to maintain the stimulus for adaptation may be beneficial for team-sports where a high level of thermal strain is reported.
2.6. Literature Review Summary

When intermittent-sprint exercise that replicates the work-rest ratios and activity patterns of field-based team-sports is conducted in a hot environment, physiological and performance responses are negatively affected. The origin of the fatigue contributing to the reduction in performance in exercise of this type is not well understood. While peripheral and central factors have been proposed further investigation is warranted. Heat acclimation is known to reduce the deleterious effect of heat stress on intermittent exercise. However, traditional ‘one size fits all’ protocols are limited in their application to exercise of this type. Traditional protocols that use a fixed exercise intensity may not optimize responses as strain is reduced across the programme. Isothermal strain models that target a critical core temperature may provide too great an additional training load prior to competition and may not be appropriate for less heat tolerant individuals. Few studies have investigated specific heat acclimation protocols for intermittent exercise, especially that modify the external heat stress. Further research is needed to develop a suitable acclimation protocol for games players performing high-intensity, intermittent-sprint exercise in the heat.

2.7. Studies, Aims and Hypotheses

2.7.1. Chapter 4 Study 1

**Title:** Peak power output provides the most reliable measure of performance in prolonged intermittent-sprint cycling.

**Aim:** To determine the reliability of an intermittent-sprint cycling protocol and to determine the efficacy of one practice session on main trials.
Hypotheses:

Primary: The Cycling Intermittent-Sprint Protocol (CISP) would be a reliable measure of intermittent-sprint exercise performance.

Secondary: One practice trial would be sufficient to minimize learning effects on peak and mean power output.

2.7.2. Chapter 5 Study 2

Title: The influence of hot humid and hot dry environments on intermittent-sprint exercise performance.

Aim: To determine the effect of a hot humid compared to a hot dry environment, matched for heat stress, on intermittent-sprint exercise performance.

Hypotheses:

Primary: A hot-humid environment would produce greater physiological strain compared to a hot dry environment.

Secondary: Intermittent-sprint exercise performance would be reduced in a hot humid compared to a hot dry environment.

2.7.3. Chapter 6 Study 3

Title: Physiological and perceptual responses to progressive heat acclimation compared to traditional and effect on intermittent-sprint exercise in a hot environment.

Aim: To ascertain the effect of a progressive heat acclimation protocol versus a traditional protocol on high intensity, intermittent-sprint exercise in the heat in games players.
Hypotheses:

Primary: There would be no difference in the physiological responses to heat acclimation between progressive and traditional acclimation.

Secondary: Progressive acclimation would ameliorate the reduction in intermittent-sprint performance in the heat to the same extent as traditional acclimation.

2.7.4. Chapter 7 Study 4

Title: The effect of progressive heat acclimation on fatigue following intermittent-sprint exercise in the heat.

Aim: To examine the effect of progressive heat acclimation on neuromuscular function and intermittent-sprint exercise in the heat using transcranial magnetic stimulation (TMS) and femoral nerve stimulation (FNS).

Hypothesis: Progressive heat acclimation would reduce central and peripheral fatigue pre-post intermittent-sprint exercise in the heat compared to control as a result of reduced physiological strain.
CHAPTER III. GENERAL METHODS

3.1. Introduction

The materials and methods of the studies presented in this thesis are described in detail in the following twelve sections. If additional materials or methods were used, they are described in detail within the relevant experimental chapter.

3.2. Health and Safety

All investigations reported in this thesis were approved by the University of Brighton Research Ethics and Governance Committee and conducted in accordance with the guidance outlined in the Declaration of Helsinki (2008). Written informed consent was obtained from all participants prior to the start of the study after explanation of the demands, risks and benefits of the study and the right to withdraw at any time. Biological materials and waste were handled and disposed of with regard to relevant guidelines and all experimentation was carried out in line with University of Brighton standard operating procedures and risk assessments for their laboratories.

Criteria for termination of testing during investigations included; participant request, attainment of the Ethics and Governance Committee approved maximum permissible core temperature of 39.7°C and evidence of signs of heat illness including syncope, exhaustion, disorientation, nausea and vomiting.

On completion of testing and post-test measures, participants were monitored and provided fluids. Participants were permitted to leave the environmental physiology laboratory when core temperature had returned to within 0.5°C of the baseline measure obtained prior to testing.
3.3. Participants

Participants who volunteered to participate in the studies presented, comprised physically active male and female games players between the ages of 18 - 30 years. Prior to all testing the purpose, demands, risks and benefits of each study were explained to the participants. Participants were informed of their right to withdraw from the study at any time. Written informed consent and medical history were obtained from each participant prior to testing. Any participant displaying a medical condition that contraindicated participation in maximal intermittent-sprint exercise was excluded from the investigation. Where participants were required to be matched for investigation (study 3 & 4), a short questionnaire pertaining to physiological measures, sporting background, training status and experience of the Cycling Intermittent-Sprint Protocol (CISP) was completed and anthropometric measures obtained to determine body surface area using the equations of Dubois and Dubois (1916).

3.4. Pre-trial Diet and Exercise Standardisation

In all studies, participants were required to refrain from strenuous activity and abstain from any form of caffeine or alcohol in the 24 hours preceding testing. Furthermore, participants were required to write down everything eaten the day (24 hours) before their first main trial so that diet could be replicated before subsequent main trials. During study three and four, participants were provided with dietary logs to record in detail dietary intake to limit the effect of altered calorific or fluid intake on the acclimation process and intermittent-sprint exercise in the heat.

To limit the effect of hypohydration, participants in all studies were required to drink water frequently in the 24 hours before main trials. Prior to arriving at the laboratory for main trials participants were also instructed to consume their last meal three hours previous and to drink 0.5 litres of water 1 - 1.5 hours before testing. On arrival to the laboratory participant’s hydration status was assessed prior to main trials using urine specific gravity and urine osmolality (section 3.8.). On completion of main trials in study
two, three and four participants were instructed to drink 150% of body mass lost from exercise-heat induced dehydration (Maughan & Leiper, 1995) over a 2 hour period, drink 0.5 litres of water half an hour before going to bed and consume 0.5 litres of water in the morning.

To limit the influence of circadian rhythms on sprint performance and body temperature (Winget et al., 1985; Hill et al., 1992), all trials were completed at the same time of day ± 2 hours for each participant. For all main trials testing was completed in shorts, t-shirt and running shoes.

3.5. Measurement of Performance in Intermittent-Sprint Cycling

3.5.1. Cycle ergometer and SRM calibration

For the studies presented in this thesis assessment of intermittent-sprint performance was completed using one of two modified cycle ergometers (Monark Ergomedic 620, Monark Ergomedic 874E, Varberg, Sweden) fitted with SRM powermeters (620 = SRM: Scientific model, Julick, Germany; 874E = SRM: FSA Gossamer Standard, Julick Germany). Power output during each trial was recorded continuously by the SRM powermeter at a rate of 50 Hz and stored to a powercontrol (SRM Powercontrol V, VI or VII). All recorded data was analysed using SRM software (SRM version 6.42.06). Peak power output (PPO, W) was determined from the highest recorded power output during each sprint. Mean power output (MPO, W) was the highest 3 s power output from the 5 s sprint to counter frictional and other factors experienced when starting sprints from a stationary position (Winter and Fowler, 2009). External work done (J) for each sprint was calculated as mean power output multiplied by duration (3 s) (Castle et al., 2011).
Cycle ergometers and SRM systems were calibrated prior to all main trials. For the Monark Ergomedic 620 with Scientific SRM system, calibration required the participant to reverse pedal to activate the signal to the powercontrol. Once achieved, the cranks were placed in a horizontal position and no force was applied through the pedals. The zero offset for the system was then determined by pressing the mode and set buttons on the powercontrol. When the zero offset value stabilised the new zero offset was selected by pressing set on the powercontrol. With the Monark Ergomedic 874E and FSA Gossamer standard SRM system forward pressure was required on the pedals to activate the powercontrol. Setting of the new zero offset was then achieved as described for the Monark Ergomedic 620.

Figure 3.1. Modified Monark ergomedic 874 E cycle ergometer with SRM power cranks.
3.6. Preliminary Visit and CISP Practice

Prior to main trials, each participant completed a preliminary visit to accustom themselves with the equipment and procedures involved. During this visit anthropometric data including height, body mass and percentage body fat were determined. Height was recorded to within 0.1 cm in the Frankfurt plane using a Detecto beam scale (Detecto, USA) and body mass was measured to within 0.1 kg using a standard laboratory scales (SECA, UK). Percent body fat was determined from four sites (Tricep, Bicep, Suprailium, Subscapular) using skinfold calipers (Harpenden Instruments, West Sussex, UK) as described by Durnin and Womersley (1974) and the equations of Siri (1961).

On completion of baseline measures, lactate threshold and peak aerobic capacity $\text{VO}_{2}\text{peak}$ were determined from cycle ergometer exercise using a standard graded exercise laboratory test. This test required participants to cycle on a modified cycle ergometer at 80 revolutions per minute (r.min$^{-1}$) until volitional exhaustion, using an incremental exercise procedure comprising 3 minute stages. The test began at a pre-determined
workload of 95 W and this was increased 25 W every three minutes until lactate threshold had been achieved, determined as the first intensity at which there was a sustained increase in blood lactate concentration above rest (Bourdon, 2000). From this point, workload was increased by 25 W every minute until exhaustion. Whole blood fingertip samples were taken in the last 30 s of each 3 minute stage for lactate analysis (= 25 µL; YSI 2300 Plus, Yellow Springs Instruments, Ohio USA). Gas analysis was undertaken using open circuit spirometry for ~45 s during each three minute stage and every minute thereafter during exercise to determine oxygen consumption. Typical error of measurement (TEM) was 2.1% (95% CI: 1.6 - 6.3) for VO$_2$ when eight samples were obtained on two consecutive days from one participant during steady state exercise (60 W). Within day variation for VO$_2$ was 2.2%. Heart rate (Polar FS1, Kempele, Finland) and RPE (Borg Scale) were recorded in the last 10 s of each stage throughout the test. Expired air was analysed for volume, oxygen, carbon dioxide and temperature using a Servomex Xentra 4100 gas analyser (Crowborough, UK; CV, VO$_2$ = 1.5%; VCO$_2$ = 1.9%) calibrated with a two point calibration against nitrogen and a gas mixture of known O$_2$ and CO$_2$ concentration (BOC, UK) prior to each test.

Following a 25 ± 5 minute recovery period, CISP familiarisation was undertaken by all participants. This included completion of the standardised CISP warm up and one quarter of the CISP protocol (10 minutes). The warm up involved five minutes of exercise at 80 r.min$^{-1}$ against a resistance of 1.2 kg (95 W) followed by two 30 s rest periods interspersed with two, 30 s bouts of higher intensity cycling at 100 r.min$^{-1}$ (120 W, Figure 3.3.).

![Figure 3.3. Schematic of standardised CISP warm-up.](image)
On completion of the CISP warm up participants completed one quarter of the CISP protocol equating to five, two minute blocks of exercise as described.

3.7. The Cycling Intermittent-Sprint Protocol (CISP)

The cycling intermittent-sprint protocol required completion of twenty, 2 minute blocks of activity. Each block comprised 10 s passive rest, 5 s sprinting against a resistance of 7.5% of body mass and 105 s of recovery cycling (80 r.min\(^{-1}\)) at 35% of \(\dot{V}O_2\) peak (Castle et al., 2006). Power output for recovery was determined from linear regression of power output against \(\dot{V}O_2\) derived from the lactate threshold \(\dot{V}O_2\) peak test conducted in the preliminary visit. The resistance required to elicit the calculated power output during 105 s of active recovery at 35% \(\dot{V}O_2\) peak was determined from the following equation:

**Equation 3.1 Calculation of power output**

\[
\text{Power Output (W)} = \text{mass x } 9.81 \times 6.02 \times r.s^{-1}
\]

(Williams and James, 2001)

Where m is the mass on the weight pan required to elicit a given power output; 9.81 is the acceleration due to gravity; 6.02 is the horizontal distance covered by the flywheel for each revolution of the pedals and \(r.s^{-1}\) is revolutions per second (Williams and James, 2001).
Power output during each trial was recorded continuously by the SRM powermeter at a rate of 50 Hz and stored to a powercontrol (SRM Powercontrol V, VI or VII). All recorded data was analysed using SRM software (SRM version 6.42.06). PPO was determined from the highest recorded power output during each sprint. MPO was the highest 3 s power output from the 5 s sprint to counter frictional and other factors experienced when starting sprints from a stationary position (Winter and Fowler, 2009). External work done (J) for each sprint was calculated as mean power output multiplied by duration (3 s) (Castle et al., 2011).

During all preliminary testing, familiarisation and all CISPs, participants completed exercise on one of two modified cycle ergometers. Ergometer one was a Monark 620 Ergomedic, Sweden, fitted with SRM power cranks (SRM; scientific model, Julick, Germany). Ergometer two was a Monark Ergomedic 874E fitted with SRM power cranks (SRM; FSA Gossamer standard, Julick, Germany). During testing participants were allocated a specific ergometer which was then used by the participant for all trials to eliminate any possible differences in power output due to seat position, ergometer angles.
and SRM crank position. Data was downloaded to a personal computer and analysed using SRM software (version 6.42.06). Thermoregulatory, perceptual and heart rate measures were recorded at 1 minute into each two minute block of active recovery. Every fourth two minute block of the CISP (sprints 4, 8, 12, 16 and 20) arterialised whole blood samples from a hyperemised finger were collected and gas sampled for ~ 45 s during the active recovery.

3.8. Experimental Procedures

On arrival to the laboratory prior to all main trials, participants provided a urine sample for assessment of hydration status by urine specific gravity ($U_{sg}$) and urine osmolality ($U_{osm}$, Armstrong et al., 1994a) using a hand refractometer (Uricon NE, Atago Co. LTD, Tokyo, Japan) and hand held pocket osmometer (Vitech Scientific Ltd, Japan), respectively. If participants were not well hydrated, evidenced by a $U_{sg}$ of ≥ 1.013 or $U_{osm}$ of ≥ 442 mOsm.kg$^{-1}$ (Armstrong et al., 1994a) participants were instructed to consume extra fluid. Prior to the commencement of testing, participants were then required to provide a second sample to determine if a well hydrated state was achieved, $U_{sg}$ < 1.013, $U_{osm}$ < 442 mOsm.kg$^{-1}$ (Armstrong et al., 1994a). Nude body mass was then determined (GFK150, AEAdam, UK).

A fingertip blood sample was then obtained for resting blood lactate (= 25 μL; YSI 2300 Plus, Yellow Springs Instruments, Ohio USA). No fluid intake was permitted in any trial and post exercise body mass was measured to determine fluid rehydration quantity.

3.9. Environmental Conditions

In the study presented in chapter four of this thesis and in all other control trials testing was conducted under normal ambient conditions (17 - 23°C) in the human performance laboratories of the School of Sport and Service Management. For the study presented in chapter five, an environmental chamber (TISS, UK; range -20°C to +50°C, 20 - 95% ± 1%
relative humidity (rh)) in the laboratories (Figure 3.5.) was used to control temperature at either 33°C, 50% rh or 40°C, 33% rh to replicate hot humid and hot dry conditions. In chapters six and seven all intermittent-sprint exercise was performed in 33°C, 50% rh.

Web bulb globe temperature (WBGT), the accepted international standard (ISO 7243) for the quantification of heat stress (Parsons, 2006) was used to determine heat stress in the studies presented in this thesis. Originally introduced by Yaglou and Minard (1957), WBGT combines the effect of humidity, wind, environmental temperature in the shade, sun and surface radiation to calculate the heat stress index when in an outdoor environment. WBGT was later modified to permit determination of heat stress indoors on the assumption that in an indoor environment globe temperature approximates ambient temperature. In this context heat stress for the studies presented was calculated from equation 3.2;

**Equation 3.2. Wet bulb globe temperature (indoor)**

\[
\text{Indoor heat stress} = (0.7 \times \text{Wet bulb temperature}) + (0.3 \times \text{dry bulb temperature})
\]

(Verdaguer-Codina et al., 1993; Epstein et al., 2006)

Where wet bulb temperature quantifies the effect of relative humidity and dry bulb temperature gives the environmental temperature in the shade.
Figure 3.5. Environmental chamber at the School of Sport and Service Management human performance laboratories.

3.10. Physiological Measurements

3.10.1. Heart rate

During all trials, resting heart rate was recorded after 15 minutes rest. During the CISP heart rate was recorded at 1 minute into each two minute block of the CISP during the active recovery phase using short distance telemetry (Polar FS1 Kempele, Finland).

3.10.2. Metabolic heat production

Metabolic heat production ($M$) during intermittent-sprint exercise was calculated using equation 3.3:
Equation 3.3. Metabolic heat production

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

(Jay and Kenny, 2007)

Where \( \dot{V}O_2 \) is the volume of oxygen \( (L.min^{-1}) \) and RER is the respiratory exchange ratio (Jay and Kenny, 2007).

3.10.3. Physiological strain index

Physiological strain index during heat acclimation, training and intermittent-sprint exercise was calculated using equation 3.4. and quantified as shown in figure 3.6.

Equation 3.4. Physiological strain index

\[ \text{PSI} = 5(T_{re \ t} - T_{re \ 0})^* (39.5 - T_{re \ 0})^1 + 5 (HR_{\ t} - HR_{\ 0})^* (180 - HR_{\ 0})^1 \]

(Moran et al., 1998a)

Where \( T_{re \ t} \) and \( T_{re \ 0} \) is the rectal temperature at any time point and at rest, respectively and \( HR_t \) and \( HR_0 \) is the heart rate at any time point and at rest.
Figure 3.6. The Physiological strain index (PSI) (Moran et al., 1998a).

3.10.4. Thermoregulatory measurements

Rectal temperature ($T_{re}$) was measured using a general-purpose probe (Ref 4491H, Henley Medical supplies, Ltd. Hertfordshire, UK) attached to a data meter (Libra Medical, Reading, UK) to provide a continuous readout. Temperature was measured at a depth of 10 cm past the anal sphincter with a micropore tape bung applied to the probe prior to insertion to ensure correct depth and prevent the probe from withdrawing during sprint efforts. During all CISP trials $T_{re}$ was recorded at 1 minute into each two minute block during active recovery to an accuracy of $\pm 0.1^\circ$C to allow the returning blood to perfuse the probe area eliminating the delayed response time associated with this measure of core temperature (Armstrong et al., 1994b).

Skin temperature ($T_{sk}$) was measured using a data logger (Squirrel Meter Logger, Grant Instruments, Cambridge, UK) and skin thermistors attached to the right hand side of the body at the following sites; pectoralis major muscle belly; lateral head of tricep brachii, rectus femoris muscle belly and lateral head of the gastrocnemius (Ramanathan, 1964). Skin thermistors were attached using porous heavy-duty zinc oxide tape (Cramer Products...
Inc., Kansas, USA) on the skin sites. Resting thermoregulatory measures were obtained after a 15 minute stabilisation period. Mean skin temperature \((T_{sk})\) was calculated from equation 3.5. where \(T_{chest}\), \(T_{arm}\), \(T_{thigh}\) and \(T_{calf}\) represent the temperature at the specific measurement site.

**Equation 3.5. Mean skin temperature \((T_{sk})\)**

\[
T_{sk} = 0.3 (T_{chest}+T_{arm}) + 0.2 (T_{thigh}+T_{calf})
\]

(Ramanathan, 1964)

The temperature of the body was determined from equation 3.6. where \(T_{body}\) is the weighted mean of \(T_{re}\) and \(T_{sk}\) and 0.4 is a constant to account for the underestimation of core temperature change.

**Equation 3.6. Body temperature \((T_{body})\)**

\[
T_{body} = 0.8(T_{re}) + 0.2(T_{sk}) + 0.4
\]

(Jay and Kenney, 2007)

Body heat content (kJ) was derived from equation 3.7. where \((T_{body})\) is body temperature, \(m\) is the mass of the body (kg) and 3.47 is the specific heat capacity of body tissues (kJ.kg\(^{-1}\).°C\(^{-1}\))

**Equation 3.7. Body heat content (BHC)**

\[
BHC = T_{body} \times Mass_{body} \times 3.47 \text{ kJ.kg}^{-1}.\text{°C}^{-1}
\]

(Jay and Kenney, 2007)
3.10.5. Weight loss and sweat rate

Weight loss to estimate non-urine fluid loss and hydration volumes was determined from nude body mass measured pre and post exercise to 0.01 kg (GFK150, AEAdam, UK). Sweat rate was determined in litres.hr$^{-1}$ from pre-post mass change. Respiratory water loss during the CISP in the conditions presented in this thesis was calculated using the equation;

$$m_e = 0.019 \times \dot{V}O_2 \times (44-P_a)$$

(Maughan et al., 2007)

Where $m_e$ is evaporative water loss from the respiratory tract in g.min$^{-1}$, $\dot{V}O_2$ is the oxygen uptake in the CISP in L.min$^{-1}$ and $P_a$ is the ambient water vapour pressure in mmHg. Respiratory water loss for the environmental conditions presented in this thesis (20°C 50%rh, 33°C 80%rh, 40°C 20%rh) was estimated at ~ 0.4 ml.min$^{-1}$ - 1.3 ml.min$^{-1}$. Consequently, during the 47 minutes of the CISP (7 min warm up + 40 minutes main protocol) the estimated respiratory water loss was 18.8 ml - 61 ml and therefore, was not considered significant.

3.10.6. Perception of effort and thermal sensation

At one minute into each two minute block of all CISP trials and every five minutes in acclimation and training sessions participants were required to provide a rating of effort, and thermal sensation. Perception of effort (RPE) was determined using the Borg scale (Borg, 1970) with RPE ranging from 6 (very, very light) to 20 (very, very hard). Thermal sensation ($T_{sen}$) was measured using the thermal sensation scale (Toner et al., 1986) that rated responses in increments of 0.5 from 0.0 (unbearably cold) through 4.0 (comfortable) to 8.0 (unbearably hot).
3.11. Collection, Treatment and Analysis of Blood Samples

Blood lactate was determined from arterialised fingertip capillary samples obtained at pre-determined times. During sampling the fingertip (index or middle finger) was cleaned with an alcohol wipe, dried with a tissue and punctured using a Softclix Pro lancet (Roche Diagnostics, Lewes, UK). To prevent sample contamination or erythrolysis the first drop of blood was wiped away with a tissue and the sample obtained from free flowing blood. Blood (∼ 25 µL) was collected into lithium heparin coated microvette tubes (CB300 µL, Sarstedt, Germany) and analysed for lactate using an automated analyser (YSI 2300 Plus, Yellow Springs Instruments, Ohio USA). The YSI 2300 Plus contains a membrane comprising a lactate specific enzyme layer. As lactate enters the enzyme layer it is oxidised producing hydrogen peroxide according to the reaction:

\[
\text{L-Lactate} + \text{O}_2 \xrightarrow{\text{L-Lactate Oxidase}} \text{H}_2\text{O}_2 + \text{Pyruvate}
\]

The hydrogen peroxide then passes to a platinum electrode where it is oxidised and the extent of the resulting current is proportional to the concentration of lactate (YSI, Ohio USA). TEM for lactate determined from eight samples of 5 mM standard on two consecutive days was 0.9% (95% CI: 0.6 - 2.4) and within day variation was 2.2%.

On day 1, 6 and 12 of acclimation and training a 20 ml venous blood sample for analysis of aldosterone and cortisol was obtained pre and post exercise by venipuncture of an antecubital vein. Pre exercise sampling was preceded by a 20 minute stabilization period and all sampling was obtained with participants in a seated position to minimise the effect of posture related hemodynamic change on blood hormone concentrations (Segar and Moore, 1968; Rowell, 1993). Post exercise sampling was conducted within 5 minutes of exercise cessation for all participants to minimize the effect of time on blood hormone concentrations and all samples were obtained between 7.30 and 9.30am to standardize for hormonal periodicity (Hurwitz et al., 2004). Blood was drawn using a 21 gauge (Sigma Aldrich Co. St Louis, Mo. USA) needle and 20 ml syringe (BD Plastipak, BD, Drogheda, Ireland) and transferred to 5 ml EDTA tubes (Sarstedt, Germany). Samples (3 x 5 ml) were
immediately centrifuged at 2000 rpm for 10 minutes at 4°C and the plasma pipetted to 2 ml Eppendorf Microcentrifuge Safe-Lock Tubes (Sigma Aldrich Co. St Louis MO, USA) before being frozen at -86°C for later analysis.

Blood for determination of haemoglobin (Hb) was collected in duplicate hemocue microcuvettes (Angelholm, Sweden) and analysed in a βhb photometer (HemoCue, Sheffield) calibrated before each test session using a control cuvette (128 g.L⁻¹). TEM for eight samples on two consecutive days for the same participant was 3.5% (95% CI: 2.3 - 9.2) and within day variation was 3%. Hb was determined from hemolysis of erythrocytes by sodiumdeoxycholate and the conversion of hb to methhb by sodiumnitrite with absorbance measured at two wavelengths to compensate for turbidity.

Blood for determination of Haemocrit (Hct) was collected in triplicate into lithium heparin coated glass capillary tubes and centrifuged (Hawksley haematospin 1300, Hawksley, Lancing, Sussex, UK) at 1300 rpm for 2 minutes. Hct was measured using a micro haematocrit reader (Hawksley, Lancing, Sussex, UK). TEM for Hct from eight samples collected on two consecutive days from the same participant was 2.3% (95% CI: 1.3 - 5.1) and within day variation was 1.8%. Initial plasma volume in study three and four was calculated from body mass using the equation of Sawka et al., (1992).

**Equation 3.9. Calculation of plasma volume**

\[
PV = 0.042 \times \text{lean body mass (kg)} + 0.576
\]

(Sawka et al., 1992)

Plasma volume on day six and twelve was then calculated by correcting the initial value for the change in plasma volume according to the method of Dill and Costill (1974) where \( PV_A \) was the plasma volume after and \( PV_B \) the plasma volume before.
Equation 3.10. Calculation of plasma volume change

\[ \Delta P = 100 \frac{(P_V^A - P_V^B)}{P_V^B} \]

(Dill & Costill, 1974)


3.12.1. Aldosterone

3.12.1.1. Principle of the test

The principle of the aldosterone assay was based on competitive binding. Competition occurred between an unlabelled antigen (present in calibrators, control and patient samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. The washing and decanting procedures removed unbound materials. After the washing step, the enzyme substrate was added. The enzymatic reaction was terminated by addition of the stopping solution. The absorbance was measured on a microtitre plate reader.

The intensity of the colour formed was inversely proportional to the concentration of aldosterone in the sample. A set of calibrators was used to plot a standard curve from which the amount of aldosterone in patient samples and controls was determined.

3.12.1.2. Performance characteristics

The sensitivity of the assay was 10 pg.ml\(^{-1}\). Recovery rate for samples with a concentration of 96.3 - 412 pg.ml\(^{-1}\) was 92.1 - 123.7%. Within assay variability (CV%) for samples ranging from 18.79 - 507.22 pg.ml\(^{-1}\) was reported as 4.1 - 10.4% and between assay variability (CV%) for samples of concentration 18.36 - 505.77 pg.ml\(^{-1}\) was 9.4 - 9.7%.
3.12.1.3. Reagents

Rabbit Anti Aldosterone Polyclonal Antibody Coated Microwell Plate (96 wells)
Aldosterone Horse Radish Peroxidase (HRP) conjugate (300μl/vial)
Aldosterone calibrators (Concentration range 0 pg.ml⁻¹ - 1000 pg.ml⁻¹)
Control (0.6ml/vial of aldosterone in a human serum based buffer)
Wash buffer concentrate (50 ml)
Assay Buffer (Protein based 15ml)
TMB Substrate (tetramethylbenzidine and hydrogen peroxide 16ml)
Stop Solution (6ml 1 M sulphuric acid)

3.12.1.4. Procedure

1. Working solutions of the aldosterone HRP conjugate and buffer were prepared.
2. Reagents were removed from the fridge and samples from the - 85°C freezer and allowed to reach room temperature before use.
3. 50 μl of each standard, control and plasma sample was pipetted into plate wells in duplicate and 100 μl of the conjugate working solution was added to each well.
4. The plate was incubated on a plate shaker at ≈ 200 r.min⁻¹ for 1 hour at room temperature and wells were then washed 3 times with 300 μl of diluted wash buffer per well and the plate tapped firmly against absorbent paper to ensure that it was dry.
5. 150 μl of TMB substrate was pipetted into each well at timed intervals and incubated on a plate shaker for 15 - 20 minutes at room temperature.
6. 50 μl of stop solution was pipetted into each well at the same timed intervals and the plate was read on a microwell plate reader within 20 minutes after addition of the stopping solution.
3.12.2. Cortisol

3.12.2.1. Principle of the test

The cortisol assay used was an ELISA based on the principle of competitive binding. Wells on the test plate were coated with a monoclonal antibody specific to an antigenic site on the cortisol molecule. Cortisol in a plasma sample competed with a cortisol horseradish peroxidase conjugate for binding to the antibody. The amount of bound peroxidase conjugate was inversely proportional to the cortisol concentration in the plasma sample and the intensity of colour, after addition of a substrate solution, was proportional to the sample cortisol concentration.

3.12.2.2. Performance characteristics

The range of the assay was from 0 - 800 ng.ml\(^{-1}\) and sensitivity 2.5 ng.ml\(^{-1}\). Recovery rate for samples with a concentration of 200 - 400 ng.ml\(^{-1}\) was 86 - 111%. Within assay variability (CV%) for samples ranging from 43 - 404 ng.ml\(^{-1}\) was reported as 5.6 - 8.1% and between assay variability (CV%) for samples of concentration 55 - 361 ng.ml\(^{-1}\) was 6.5 - 7.7%.

3.12.2.3. Reagents

96 well plate coated with monoclonal antibody
Standard (7 x 1 ml vials concentration range 0 - 800 ng.ml\(^{-1}\))
Enzyme conjugate (25 ml)
Substrate solution (14 ml - TMB)
Stop Solution (14 ml 0.5 M sulphuric acid)
Wash solution (30 ml)
3.12.2.4. Procedure

1. Reagents were removed from the fridge and allowed to reach room temperature.
2. Plasma samples were removed from the -85°C freezer, allowed to thaw and inverted several times prior to testing.
3. 20 µl of standard, control and sample and 200 µl enzyme conjugate was added to each microtitre well.
4. Solution in wells was mixed thoroughly for 10 seconds, incubated at room temperature for 60 minutes and then shaken out and the wells rinsed three times with diluted wash solution (400 µl per well).
5. Residual droplets remaining after the washing procedure were removed by striking on the microtitre well plate onto absorbent paper.
6. 100 µl of substrate solution was added to each well that was then incubated for 15 minutes at room temperature.
7. 100 µl of stop solution was added to each well and optical density was determined at 450 ± 10 nm within 10 minutes of adding the stop solution.

3.13. Statistical Analyses

Descriptive and inferential statistics were determined using the Statistical Package for the Social Sciences (SPSS version 18.0 and 20.0). Data were checked for normality and sphericity and where sphericity was violated the Huynh-Feldt correction was applied. For correlated data two-way repeated measures ANOVA (condition*time) with Bonferroni correction was applied with only pairwise comparisons made. Uncorrelated data from main experimental trials was also analysed using either three way mixed ANOVA (regime*day*time) with repeated measures on two factors (day*time) or two-way mixed ANOVA (regime*day) with repeated measures on one factor (day). For analysis of perceptual measures across time in CISP trials a Friedman’s ANOVA was performed with significance identified using a Wilcoxon matched pairs test. Pearson product moment correlation was performed to establish correlation coefficients (r). Effect sizes were estimated using partial Eta squared ($\eta^2_p$) where 0.2 represented a ‘small’ effect size, 0.5 a ‘medium’ effect size and 0.8 a ‘large’ effect size (Nakagawa & Cuthill, 2007). Power
analyses were performed using commercially available G*Power software (version 3.0.10) (Faul et al., 2007). Data presented in study chapters is expressed as mean ± standard deviation and significance was accepted at $P < 0.05$. Additional statistical procedures required are explained in the appropriate study chapter.
CHAPTER IV. RELIABILITY OF THE CYCLING INTERMITTENT-SPRINT PROTOCOL

4.1. Abstract

The aims of this study were to determine the reliability of an intermittent-sprint cycling protocol and to determine the efficacy of one practice session on main trials. Eleven men, moderately trained team-sport athletes, completed three visits to the laboratory involving a graded-exercise test and practice session and two trials of a Cycling Intermittent Sprint Protocol separated by three days. Data for practice and main trials were analysed using typical error of measurement, intra-class correlation and least products regression to determine reliability. Typical error of measurement (expressed as a coefficient of variation) and intra-class correlation for peak power output from all twenty sprints for trial 1 and trial 2 was 2.9 ± 12.8% (95% confidence interval: 2.0 – 5.0%) and 0.96 (95% confidence interval: 0.85 – 0.99), respectively. Typical errors of measurement and intra-class correlation for mean power output for all twenty sprints for trials 1 and 2 was 4.2 ± 11.9% (95% confidence interval: 2.9 – 7.4%) and 0.90 (95% confidence interval: 0.66 – 0.97), respectively. Results suggest peak power output provides a more reliable measure than mean power output. Findings indicate the Cycling Intermittent Sprint Protocol provides a reliable tool for assessing intermittent sprint performance in a test-retest research paradigm.

4.2. Introduction

Team-sports such as rugby union, hockey and football are characterised by periods of intermittent high-intensity activity, interspersed with longer spells of recovery and lower intensity activity (Williams, 1990). In such sports, athletes typically complete twenty to sixty sprints over 0 - 20 m per match with mean sprint durations of less than 3 s and mean recovery times between sprints of 2 minutes (Spencer et al., 2004, 2005; Roberts et al.,
2008; Di Salvo et al., 2009). The contribution of sprinting to the total activity profile in field-based sports is therefore small. Nevertheless, sprinting frequently precedes decisive moments in play and as such, can be considered critical to the outcome of a match (Reilly, 1997; Spencer et al., 2004). Consequently, several performance-based protocols have been devised to examine the physiological and metabolic demands of sprinting in field-based sports, including the Bangsbo Sprint Test (Bangsbo, 1994), the Loughborough Intermittent Shuttle Test (Nicholas et al., 2000) and the Soccer Specific Test of Prolonged Repeated Sprint Ability (Oliver et al., 2007).

The majority of these protocols are running-based and use durations, intensities, and work-rest ratios developed from time-motion analyses of team-sports. Typically, in running-based protocols, athletes are required to complete 6 – 21 sprints of 20 – 40 m interspersed with 15 – 100 s of recovery (Nicholas et al., 2000; Oliver et al., 2007; Gabbett, 2010). Such protocols have good reliability, construct validity and specificity. However, they are not conducive to sensitive measurement of complex physiological and metabolic variables necessary to develop understanding of the demands of high-intensity activity (McGawley & Bishop, 2006). Cycling-based protocols that focus on assessment of repeated-sprint ability (Spencer et al., 2005; McGawley & Bishop, 2006; Mendez-Villanueva et al., 2008), have been shown as a valid measure of sprint performance in match play (Bishop et al., 2001). Yet, such protocols typically comprise 5 - 10, 6 s sprints interspersed with 24 - 30 s of recovery (Bishop et al., 2001; McGawley & Bishop, 2006; Mendez-Villanueva et al., 2008) and therefore, do not replicate intermittent-sprint activity over durations that occur in many team-sports.

Accurate assessment of reliability should consider systematic factors such as a learning effect attributable to a lack of practice (Phillips et al., 2004). In multiple sprint-cycling based activity, learning effects occur (Capriotti et al., 1999; McGawley & Bishop, 2006). In contrast to existing literature that identifies the need for two practice sessions when repeated-sprint exercise is performed on a cycle ergometer (Capriotti et al., 1999; McGawley & Bishop, 2006), learning effects are minimised during the Cycling Intermittent
Sprint Protocol (CISP) by completion of one practice session comprising a quarter of the protocol prior to the first main trial (Castle, et al., 2011). The CISP examines the physiological responses to intermittent-sprint exercise. However, whether a single practice is sufficient to negate learning is currently unknown.

The CISP has been used to examine effects of pre-cooling and heat acclimation on intermittent-sprint performance and has demonstrated 2 - 4% improvements in peak power output compared with control (P < 0.05) (Castle, et al., 2006, 2011). However, the reliability of the protocol has not been extensively determined (Castle, 2011). Running-based tests designed to assess prolonged repeated-sprint ability have reported typical errors of measurement for peak power output of up to 7.9% (95% confidence interval: 5.8 - 14.4) (Oliver et al., 2007). In addition, cycle ergometer based protocols have reported random errors in repeated trials of 2 - 4% (Paton & Hopkins, 2001). As such, it is difficult to determine if changes reported in previous studies using the CISP represent worthwhile interventional change or variation inherent in the test. Therefore, the primary purpose of this study was to determine the reliability of the Cycling Intermittent-Sprint Protocol using a group of moderately trained team-sport athletes. A secondary purpose was to assess the usefulness of a five sprint practice session on the main protocol.

4.3. Methods

4.3.1. Participants

Eleven men, moderately trained team-sport athletes (mean ± S.D.: age 23 ± 2.4 years, stature 178.5 ± 5.9 cm, body mass 82.3 ± 8.4 kg, sum of skinfolds, 36.7 ± 11.2 mm, peak oxygen consumption (VO_{2peak}), 42.6 ± 4.8 ml.kg^{-1}.min^{-1}) who competed in team-sports three to five times per week gave written informed consent to participate. The study was approved by the University Research Ethics and Governance Committee and conducted in accordance with the Declaration of Helsinki (2008). Before testing participants completed
a medical questionnaire and were requested to follow standard pre-trial preparation guidelines for diet, exercise and hydration (section 3.4.).

4.3.2. Experimental design

![Experimental Design Diagram]

**Figure 4.1.** Schematic of experimental design with measurements.

Each participant was required to visit the laboratory on three separate occasions including a preliminary session and two main trials. All main trials were completed at the same time of day to minimize the influence of circadian variation (Winget et al., 1985; Hill et al., 1992). During the preliminary visit anthropometric data including height, body mass and percentage body fat were determined (section 3.6.) and participants completed an incremental lactate-threshold-to-VO$_2$peak test on a modified cycle ergometer (Monark 620 Ergomedic, Varberg, Sweden) fitted with power cranks (SRM: Scientific model, Julick, Germany) (section 3.5. & 3.6.). Finally, participants completed a CISP familiarisation. During the incremental test, blood was obtained in the final 30 s of each stage and analysed with an automated analyser for lactate and glucose concentration (section 3.6. & 3.11.) (YSI 2300 Plus, Yellow Springs Instruments, Ohio, USA) calibrated prior to each session using the manufacturers 5 mM standard (YSI 2427; coefficient of variation (CV) = 2.2%). When lactate threshold was achieved (section 3.6.) no further blood samples were taken and intensity was increased by 25 W every minute until volitional exhaustion.
Oxygen uptake was recorded using open-circuit spirometry and expired air was collected for approximately 45 s during the final minute of each stage (Section 3.6.). Heart rate was determined in the final 10 s of each stage (section 3.6.). After 25 ± 5 minutes recovery a CISP practice was completed that required participation in the standardised CISP warm-up and five 2 minute blocks of the protocol (section 3.6.).

On the second and third visits participants completed the CISP (section 3.7.) (Castle et al., 2006), hereafter referred to as trial 1 and trial 2. Following the standardised warm-up, participants performed the protocol on a modified cycle ergometer (Monark 620 Ergomedic, Varberg, Sweden) fitted with power cranks (SRM, Scientific model, Julick, Germany) with data during exercise recorded at 50 Hz and analysed for peak power output (PPO), mean power output (MPO) and work done using SRM software (version 6.42.06; section 3.7.). To determine the effectiveness of one practice session before main trials to counteract learning effects data from the five practice sprints were analysed against the first five sprints of trial one and two.

4.3.3. Statistical analysis

Data were assessed for normality and sphericity and adjusted where necessary using the Huynh-Feldt method. PPO and MPO were compared using a fully within-groups factorial ANOVA (condition*time). Data were assessed for heteroscedasticity using plots of log transformed data and reliability measures. Typical errors of measurement, calculated from the standard deviation of the mean difference for each pair of trials using the formula typical error of measurement = SD_{diff}/\sqrt{2}$ (Hopkins, 2000a) and expressed as a mean coefficient of variation, and intra-class correlation were calculated from log transformation. Intra-class correlations were determined using a reliability spreadsheet (available at newstats.org/xrely.xls) as used by Laursen et al., (2007). 95% confidence limits were determined for typical errors of measurement and intra-class correlations using the methods described by Hopkins (2000b, 2007). Ordinary least products regression, where both outcome measures were compared with an assumption of error
in each, was used to determine fixed and proportional bias in peak power and mean power output between trials (Ludbrook, 1997). Using this method, estimates of the intercept \(a'\) and slope \(b'\) of the least products regression line \(E = a' + b'x\) were calculated and compared to 95% confidence intervals (determined from bootstrapping) for \(a'\) and \(b'\) to assess bias. Fixed bias was deemed present when the 95% confidence interval for \(a'\) did not span zero. Proportional bias was deemed present when the 95% confidence interval for \(b'\) did not include one. Sprints were also grouped into four phases, each phase being the mean of five sprints, to improve visual and analytical clarification. All data was analysed using SPSS (version 18.0) and reported as mean ± standard deviation. Statistical significance was accepted at the level of \(P < 0.05\). Effect sizes were calculated as described in section 3.13.

4.4. Results

There were no differences between trials 1 and 2 for PPO across all 20 sprints \((p = 0.396, \eta_p^2 = 0.073)\). Similarly, there were no differences in PPO between trial 1 and trial 2 when grouping the sprints into four phases \((p = 0.406, \eta_p^2 = 0.070)\). MPO across all 20 sprints did not differ between trials 1 and 2 \((p = 0.820, \eta_p^2 = 0.005)\). Grouping the sprints into four phases also showed no difference between trials 1 and 2 \((p = 0.820, \eta_p^2 = 0.005)\). PPO and MPO for the practice period were not different from the first five sprints of trials 1 or 2 (Table 4.1., \(P > 0.05, \eta_p^2 = 0.058\)). Typical errors of measurement and intra-class correlations for practice versus trial 1 were 2.6 ± 9.6% (1.8 - 4.7%) and 0.94 and for practice versus trial 2, 2.5 ± 7.2% (1.7 - 4.7%) and 0.95 respectively. PPO demonstrated less variability than MPO throughout the protocol (mean of sprints 1 - 20), despite both showing strong correlations (Table 4.2.).
Table 4.1. Peak power output (W) and mean power output (W) for sprints 1 - 5 in practice, trial 1 and trial 2.

<table>
<thead>
<tr>
<th>Sprint</th>
<th>Practice</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Practice</th>
<th>Trial 1</th>
<th>Trial 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1199 ± 88</td>
<td>1192 ± 75</td>
<td>1216 ± 104</td>
<td>1145 ± 88</td>
<td>1133 ± 73</td>
<td>1148 ± 77</td>
</tr>
<tr>
<td>2</td>
<td>1201 ± 105</td>
<td>1172 ± 101</td>
<td>1193 ± 110</td>
<td>1129 ± 103</td>
<td>1102 ± 99</td>
<td>1126 ± 93</td>
</tr>
<tr>
<td>3</td>
<td>1199 ± 115</td>
<td>1192 ± 107</td>
<td>1196 ± 130</td>
<td>1139 ± 117</td>
<td>1124 ± 99</td>
<td>1126 ± 93</td>
</tr>
<tr>
<td>4</td>
<td>1184 ± 130</td>
<td>1190 ± 128</td>
<td>1190 ± 120</td>
<td>1133 ± 134</td>
<td>1121 ± 113</td>
<td>1117 ± 108</td>
</tr>
<tr>
<td>5</td>
<td>1180 ± 133</td>
<td>1176 ± 107</td>
<td>1177 ± 107</td>
<td>1105 ± 108</td>
<td>1117 ± 108</td>
<td>1106 ± 93</td>
</tr>
<tr>
<td>Mean (±)</td>
<td>1193 ± 114</td>
<td>1184 ± 102</td>
<td>1194 ± 114</td>
<td>1130 ± 119</td>
<td>1119 ± 100</td>
<td>1124 ± 97</td>
</tr>
</tbody>
</table>

Data are mean ± S.D., n = 11.

Table 4.2. Peak power output (W), mean power output (W), work done (J) typical errors of measurement and intra-class correlations for 20 sprints, trial 1 and trial 2.

<table>
<thead>
<tr>
<th></th>
<th>Peak power output (W)</th>
<th>Mean power output (W)</th>
<th>Work done (J)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial 1</td>
<td>1174 ± 132</td>
<td>1104 ± 129</td>
<td>3312 ± 387</td>
</tr>
<tr>
<td>Trial 2</td>
<td>1187 ± 145</td>
<td>1100 ± 112</td>
<td>3300 ± 336</td>
</tr>
<tr>
<td>Difference</td>
<td>-13 ± 13</td>
<td>4 ± 17</td>
<td>12 ± 51</td>
</tr>
<tr>
<td>TEM (CV 95%)</td>
<td>2.9 ± 12.8 (2.0-5.0)</td>
<td>4.2 ± 11.9 (2.9-7.4)</td>
<td>4.2 ± 11.9 (3.1-7)</td>
</tr>
<tr>
<td>ICC (r value)</td>
<td>0.96 (0.85-0.99)</td>
<td>0.90 (0.66-0.97)</td>
<td>0.90 (0.66-0.97)</td>
</tr>
</tbody>
</table>

Data are mean ± S.D., n = 11. TEM: typical error of the measure. CV: coefficient of variation, at a 95% confidence interval (CI). ICC: intra-class correlation.

For grouped sprints (mean of five sprints), PPO in phases 1 and 4 had smaller typical errors of measurement (3.4 ± 10.9% and 3.3 ± 14.1%, respectively) than phases 2 and 3 (3.5 ± 12.8% and 3.9 ± 14.1%, respectively), (Table 4.3.). This also occurred for MPO. However, typical errors of measurement were greater for MPO compared to peak power output in phases two, three and four (Table 4.3.). Intra-class correlation for PPO in grouped sprints between trials 1 and 2 remained above r = 0.92 throughout each phase, however this was not the case for MPO in phase 2 and phase 3 (r = 0.85).
Table 4.3. Peak power output (W), mean power output (W), work done (J), typical errors of measurement and intra-class correlations for each phase (Phase 1 = sprints blocks 1-5, Phase 2 = 6-10, Phase 3 = 11-15 and Phase 4 = 16-20), trial 1 and trial 2.

<table>
<thead>
<tr>
<th></th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
<th>Phase 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trial 1</strong></td>
<td>1203 ± 111</td>
<td>1178 ± 129</td>
<td>1160 ± 153</td>
<td>1152 ± 140</td>
</tr>
<tr>
<td><strong>Trial 2</strong></td>
<td>1221 ± 138</td>
<td>1189 ± 148</td>
<td>1166 ± 147</td>
<td>1170 ± 157</td>
</tr>
<tr>
<td><strong>TEM</strong></td>
<td>3.4 ± 10.9 (2.3-6.0)</td>
<td>3.5 ± 12.8 (2.4-6.2)</td>
<td>3.9 ± 14.1 (2.7-6.9)</td>
<td>3.3 ± 14.1 (2.3-5.8)</td>
</tr>
<tr>
<td><strong>ICC</strong></td>
<td>0.92 (0.73-0.98)</td>
<td>0.94 (0.78-0.98)</td>
<td>0.94 (0.78-0.98)</td>
<td>0.95 (0.84-0.99)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
<th>Phase 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trial 1</strong></td>
<td>1134 ± 105</td>
<td>1104 ± 133</td>
<td>1093 ± 151</td>
<td>1086 ± 135</td>
</tr>
<tr>
<td><strong>Trial 2</strong></td>
<td>1138 ± 99</td>
<td>1101 ± 115</td>
<td>1081 ± 119</td>
<td>1080 ± 124</td>
</tr>
<tr>
<td><strong>TEM</strong></td>
<td>3.0 ± 9.0 (2.1-5.4)</td>
<td>5.1 ± 12.3 (3.6-9.2)</td>
<td>5.7 ± 13.7 (3.9-10.2)</td>
<td>4.3 ± 13.1 (3.0-7.6)</td>
</tr>
<tr>
<td><strong>ICC</strong></td>
<td>0.92 (0.73-0.98)</td>
<td>0.85 (0.54-0.96)</td>
<td>0.85 (0.53-0.96)</td>
<td>0.91 (0.7-0.97)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
<th>Phase 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trial 1</strong></td>
<td>3402 ± 315</td>
<td>3312 ± 399</td>
<td>3279 ± 453</td>
<td>3258 ± 405</td>
</tr>
<tr>
<td><strong>Trial 2</strong></td>
<td>3416 ± 297</td>
<td>3303 ± 345</td>
<td>3243 ± 357</td>
<td>3240 ± 372</td>
</tr>
<tr>
<td><strong>TEM (CV)</strong></td>
<td>2.9 ± 9.7 (2.2-4.7)</td>
<td>5.1 ± 12.3 (3.8-8.3)</td>
<td>5.8 ± 13.8 (4.3-9.4)</td>
<td>4.2 ± 13.1 (3.1-6.8)</td>
</tr>
<tr>
<td><strong>ICC</strong></td>
<td>0.92 (0.79-0.97)</td>
<td>0.85 (0.61-0.95)</td>
<td>0.84 (0.59-0.94)</td>
<td>0.91 (0.76-0.97)</td>
</tr>
</tbody>
</table>

Data are mean ± S.D., n = 11. TEM: typical error of the measure. CV: coefficient of variation, at a 95% confidence interval (CI). ICC: intra-class correlation.

PPO and MPO were normally distributed for trial 1 and 2. Although PPO tended to be higher in trial 2 than trial 1 (Figure 4.2.) no fixed bias was observed (a’ = -158.2 W; 95% confidence interval (CI) = -449.6 - 133.2 W). Similarly, no proportional bias was observed between trial 1 and 2 for PPO (b’ = 1.15; 95% CI = 0.90 - 1.39). MPO demonstrated fixed bias between trial one and trial two (Figure 4.3.) (a’ = -295.0 W; 95% CI = -534.9 - -55.12 W) and proportional bias of ~ 20% (b’ = 1.26; 95% CI = 1.05 - 1.48).
Figure 4.2. Scatterplot of peak power output (W) in trial 1 and trial 2. Solid line: Least products regression line $E(\text{trial } 2) = -158.2 + 1.15(\text{trial } 1)$. Dotted line: $45^\circ$ line of identity.
The purpose of this study was to determine the reliability of the Cycling Intermittent-Sprint Protocol. The main finding of this study was that the protocol provided a reliable measure of high-intensity intermittent-exercise in team-sport athletes. Further, one practice session is sufficient to minimise learning effects on PPO and MPO.

The reliability of the protocol was assessed from two trials separated by three days. The typical error of measurement scores across all 20 sprints for PPO and MPO for the two trials suggested a low level of within participant variability. The same conclusions can be
drawn for PPO when sprints were grouped into four phases. For MPO, the variation between each typical error of measurement for each phase suggested greater within participant variability in the middle two phases.

No other studies to date have examined the reliability of the CISP. Compared with protocols that have examined reliability of repeated-sprint activity on non-motorised treadmills, the typical errors of measurement (expressed as a coefficient of variation) reported in this study are lower. Oliver et al., (2007) reported a typical error of 7.9% (95% confidence interval: 5.8 - 14.4%) for peak power and 5.9% (4.3 - 10.2%) for mean power in the soccer specific intermittent-sprint test when performed with youth soccer players. Such discrepancy between typical errors for peak and mean power in our study and that of Oliver et al., (2007) may be explained by the mode of assessment of repeated sprint ability as the reliability of power output from non-motorised treadmill running is poor (Tong et al., 2001; Oliver et al., 2007). In addition, when compared to the random error reported in repeated trials for cycle ergometers (Paton & Hopkins, 2001) the values reported for mean and peak power in this study compare favourably. Therefore, when assessing worthwhile interventional change using peak and mean power output, cycle ergometer-based exercise may enable more accurate assessment of performance. However, researchers must also consider that, for team-sport athletes whose primary mean of locomotion is running, cycle exercise may be unfamiliar and lack specificity contributing to greater variability and reduced sensitivity when monitoring interventional change. Thus, careful consideration must also be given to the mode of exercise used.

A commonly used cycling test to assess repeated-sprint ability in team-sport athletes is the 5 x 6 s maximal sprint test (Bishop et al., 2004; McGawley & Bishop, 2006). McGawley & Bishop (2006) reported typical error scores for peak power output in the first sprint between trial 1 and 2 of 25 W (95% confidence interval: 17 - 48 W). In the current study typical error in the first sprint between trial 1 and 2 was approximately 48 W (33 - 84 W). Expressed as a percentage of maximal peak power output, typical error of measurement is similar in both studies (McGawley sprint 1: ≈ 2.8%, present study sprint 1: 2.5%),
suggesting the CISP compares favourably. Similarly, intra-class correlations reported in our study compare favourably with those in other studies of intermittent-sprint exercise involving treadmill running and overland sprinting (Lemming et al., 2004; Laursen et al., 2007; Gabbett, 2010).

Differences between trial 1 and 2 such as fixed and proportional bias are revealed with ordinary least product regression analysis. This analysis of peak power for trial 1 vs. 2 revealed no significant fixed or proportional bias. However, for mean power output least products regression revealed a significant fixed bias of \(-295\) W and a proportional bias of \(-20\%\) \((b’ = 1.26)\). This discrepancy may be explained by the higher PPO observed in trial 2 contributing to greater variation in MPO compared to trial 1 and suggests that PPO is a more reliable measure of performance.

The present study showed greater within-participant variability for average mean power output in grouped sprints in phases 2 and 3. Laursen et al., (2007) suggest greater variability in time to exhaustion tests was related to participant boredom or lack of motivation. Although not a time to exhaustion test, the CISP’s duration and frequent need for participants to perform maximal efforts make it conceivable that such variables contributed to the greater variability found in phase 2 and 3 for mean power output, despite consistent verbal encouragement throughout. The fixed-paced portion of the protocol does not allow self-selection of exercise intensity as participants are required to maintain a pre-determined recovery intensity. However, during the 5 s sprint, although instructed to be all out, participants may have self-paced. Consequently, participants may be adopting a pacing strategy to allow completion of the protocol thus reflecting greater variability in average mean power output in the middle portion of the protocol, particularly as research shows the occurrence of pacing in anticipation of the number of sprints within a trial (Billaut et al., 2011).
A practice or learning effect is defined as any systematic change in the performance scores during the performance of a novel exercise task distinct from any experimental intervention (Watt et al., 2002). In a meta-analysis of thirty tests of maximal-intensity exercise, Hopkins et al., (2001) identified a practice effect between first and second experimental trials and recommended at least one practice trial should precede formal testing. Presently, the CISP involves one practice of five sprints prior to main trials. An important finding of the current study was that no difference (P > 0.05, $\eta_p^2 < 0.058$) existed between practice and the first five sprints in either trials 1 or 2 as evidenced in previous work (Castle, 2006). Although the large standard deviation values may have prevented detection of any difference, previous investigations that have used the CISP have reported similar standard deviation values and identified significant differences between trials (Castle et al., 2006; Castle et al., 2011). Similarly, when percentage change is considered, PPO declined by 0.69% in trial 1 (95% confidence interval - 2.05 - 0.65%) and increased by 0.83% in trial 2 (- 0.35 - 2.1%). There was a decline of 0.96% (- 2.57 - 0.64%) for MPO in trial one and an increase of 0.36% (- 1.09 - 1.81%) in trial two. Taken together, this lack of a substantial change between trials indicates minimal practice effect between familiarisation and subsequent trials (Hopkins et al., 2001). Therefore, it is plausible to suggest that one practice trial consisting of five sprints is sufficient to obtain a reliable measure of intermittent-sprint exercise performance.

In contrast, Capriotti et al., (1999) reported two practice sessions identical to the tests were required for satisfactory reliability when participants unaccustomed to multiple sprint exercise performed 10 x 7 s sprints with 30 s recovery. Similarly, McGawley & Bishop, (2006) reported one practice trial was sufficient to obtain a reliable measure of repeated sprint ability with trained female team-sport athletes, but recommended two as reproducibility of measures improved with a second practice session. As such, it is possible that completion of three full trials of the current protocol may have improved reliability for trial two versus trial three. In addition, if a complete twenty sprint practice trial of the CISP was completed in a rested state further insight into the efficacy of one practice trial would have been gained. However, extending the habituation process for the current protocol is not recommended due to the prolonged, repetitive nature of the
exercise and the possible negative effect on participant motivation if too many sessions are required (Hopkins, 2000a). In addition, in the current investigation, analysis of PPO and MPO for sprints 1 - 5 in the practice vs. trial 1 compared to practice vs. trial 2 revealed no differences in typical errors of measurement, intra-class correlations and 95% confidence limits. This indicates no improvement in reproducibility of measures when further trials were included. Consequently, for trained games players’ who are accustomed to intermittent-sprint exercise, one practice session comprising five sprints provides reliable measures of PPO and MPO in the CISP.

4.5.1. Conclusion

This study was the first to examine the reliability of the CISP, a test previously used to reproduce activity patterns in match play. The protocol is unique for cycling based tests of intermittent-sprint exercise because of its duration. Consequently, it permits the examination of the effect of differing interventions and training on performance and metabolism in intermittent-sprint exercise over durations typical of one half of a team match while also permitting investigation of physiological responses across similar time frames. Results demonstrate the protocol is a reliable measure of intermittent-sprint exercise, but typical errors of measurement, intra-class correlations and ordinary least products regression indicate PPO is the more reliable measure of performance.
CHAPTER V. THE INFLUENCE OF HOT HUMID AND HOT DRY ENVIRONMENTS ON INTERMITTENT-SPRINT EXERCISE PERFORMANCE

5.1. Abstract

This study examined the effect of a hot humid (HH) compared to a hot dry (HD) environment, matched for heat stress, on intermittent sprint performance. In comparison to HD, HH environments compromise evaporative heat loss and decrease exercise tolerance (Maughan et al., 2012). It was hypothesized HH would produce greater physiological strain and reduce intermittent sprint exercise performance compared to HD. Eleven male team sports players completed the cycling intermittent sprint protocol (CISP) in three conditions, temperate (TEMP; 21.2 ± 1.3°C, 48.6 ± 8.4% relative humidity (rh)), HH (33.7 ± 0.5°C, 78.2 ± 2.3% rh) and HD (40.2 ± 0.2°C, 33.1 ± 4.9% rh), with both heat conditions matched for heat stress. All participants completed the CISP in TEMP but three failed to completed the full protocol of twenty sprints in HH and HD. Peak power output (PPO) declined in all conditions (P < 0.05), but was not different between any condition (sprints 1 – 14 (n = 11); HH, 1073 ± 150 W; HD, 1104 ± 127 W; TEMP, 1074 ± 134 W; sprints 15 – 20 (n = 8); HH, 954 ± 114 W; HD, 997 ± 115 W; TEMP, 993 ± 94 W; P > 0.05). Physiological strain (PSI) was not significantly different in HH compared to HD but HH was higher than TEMP (P < 0.05). Data demonstrates intermittent sprint exercise performance of 40 minutes duration is impaired, but not different in hot humid and hot dry environments matched for heat stress despite evidence of a trend towards greater physiological strain in a hot humid environment.

5.2 Introduction

Globalization has increased the incidence of major team-sport competition in hot humid and hot dry environments. Therefore, international team-sport athletes frequently
compete in environments of high heat stress. Under such conditions, performance in intermittent sprinting, representative of activity patterns in team-sports, is impaired compared to temperate conditions (Drust et al., 2005; Sunderland et al., 2005). This impairment occurs without significant difference in metabolic responses to exercise between conditions and may be explained by peripheral and/or central mechanisms (Nybo and Nielsen, 2001a; Racinais et al., 2007).

While it is known performance in intermittent sprinting is impaired in hot compared to temperate conditions, few studies have examined the effect of differing compositions of heat and humidity, contributing to the same total heat stress, on performance of this type. No difference in performance was observed when two sets of 3 x 30 s Wingates with 60 minutes passive rest were repeated in temperate (22°C, 30% rh), hot humid (30°C, 85% rh) and hot dry (40°C, 40% rh) environments, although this may be explained by the exercise and recovery duration used (Backx et al., 2000). Although related to prolonged exercise, recently a progressive impairment in exercise capacity in the heat has been reported with increased relative humidity (Maughan et al., 2012). When four cycling trials were performed at 70% VO$_{2\text{max}}$ in 30.2 ± 0.2°C and either 24, 40, 60 or 80% rh, exercise time was significantly reduced at 60 and 80% rh compared to 24% (Maughan et al., 2012). However, conditions in this study were not matched for heat stress and so it is unknown if clamping relative humidity while increasing dry bulb temperature to replicate heat stress, would produce similar findings.

Typically, dry and evaporative heat exchange mechanisms combine to maintain thermal equilibrium in the resting human (Cheung, 2010). However, during exercise in the heat, dry heat exchange is limited by high ambient and skin temperatures and a reduced thermal gradient for heat transfer. Consequently, 80% of heat loss is achieved through evaporation with approximately 2.4 MJ of heat energy liberated per litre of sweat vaporized (Cheung, 2010). However, the effectiveness of evaporation is primarily determined by relative humidity. High relative humidity increases sweat drippage, decreases sweat vaporization and reduces heat loss through evaporation. Therefore,
compared to a hot dry environment where the evaporative potential of the environment exceeds the evaporative requirement of the athlete, exercise in a hot humid environment of similar overall heat stress could contribute to greater hyperthermia and physiological strain and concomitantly, greater reductions in performance.

To permit accurate interpretation of the influence of differing combinations of heat and humidity on intermittent-sprint exercise, research should use trials matched for heat stress. This requirement is seldom achieved. However, as intermittent-sprint activity is routinely performed as part of team-sport competitions in equatorial or Middle Eastern regions the influence of differing combinations of environmental factors needs to be ascertained. Research has demonstrated composite environmental indices provide better indicators of environmental influence than individual factors alone (Zhang et al., 1992). Wet bulb globe temperature (WBGT) is one such index that can be used to measure and match heat stress. WBGT is criticised for a lack of ability to predict physiological strain when evaporation is limited (Budd, 2008), however, as an index of heat stress it is the accepted international standard and has been adopted by the US military, World Health Organisation and the American College of Sports medicine (Epstein and Moran, 2006). Therefore, the purpose of this study was to determine the effect of differing heat and humidity, hot humid (HH) and hot dry (HD), of the same total heat stress (determined using WBGT) on intermittent-sprint exercise. It was hypothesized HH would result in greater physiological strain and reduce intermittent-sprint performance compared to HD.

5.3. Methods

5.3.1. Participants

Eleven male games players (mean ± SD: Age 22.4 ± 2.7 years, height 180.4 ± 5.3 cm, body mass 78.5 ± 11.2 kg, percent body fat 15.5 ± 2.8%, VO2peak 48.4 ± 6.8 ml.kg⁻¹.min⁻¹) participated in the study. The study was approved by the University Research Ethics and
Governance Committee and conducted in accordance with the Declaration of Helsinki (2008). Before testing, participants completed a medical questionnaire and followed standard pre-trial preparation guidelines for exercise, hydration and diet (section 3.4.).

5.3.2. Experimental design

![Experimental Design Diagram]

Figure 5.1. Schematic of experimental design with measurements.

Participants completed one preliminary and three randomised main trials over a two week period. Trials were completed between September and May and scheduled for the same time of day (Winget et al., 1985; Hill et al., 1992). During preliminary testing baseline data were obtained (section 3.6.), participants completed a lactate-threshold-to-\( \text{VO}_2\text{peak} \) test and practiced the CISP (Castle et al., 2006) in ambient conditions. For the remaining visits participants completed the CISP in one of three conditions with hot trials matched for heat stress [HH: 33.7 ± 0.5°C, 78.2 ± 2.3% rh; HD: 40.2 ± 0.2°C, 33.1 ± 4.9% rh; temperate (TEMP), 21.2 ±1.3°C, 48.6 ± 8.4% rh]. Artificial wind was not used in trials.
due to the possibility of disproportionately altering evaporative capacity in the hot humid trial and preventing accurate assessment of the strain associated with this environment (Budd, 2008). For hot trials, exercise was terminated if participants felt unable to continue, or they reached the institutional ethics committee approved core temperature for termination of exercise in the heat (39.7°C).

5.3.3. Preliminary visit

Body mass and height were determined using a beam scale (Detecto, USA) and percent body fat was determined from four sites using calipers (Harpenden, UK) (section 3.6.). Hydration was assessed (section 3.8) and participants completed a lactate-threshold-to-\(\text{VO}_{2}\)peak test on a cycle ergometer (Monark 620, Sweden) fitted with power cranks (SRM, Julick, Germany) (section 3.5 & 3.6.). Blood for lactate concentration (YSI 2300, USA) was drawn in the last 30 s of each stage (section 3.6.). When lactate threshold was achieved (Bourdon, 2000), intensity was increased 25 W every minute until exhaustion.

Expired air was collected using open-circuit spirometry for 45 s in the final minute of each stage (section 3.6.). Oxygen uptake was determined using a Servomex Xentra 4100 analyser (Servomex, England) (section 3.6.). Heart rate ((HR) Polar FS1, Finland) and RPE (Borg, 1970) were recorded in the last 10 s of each stage (section 3.6). After 25 ± 5 minutes recovery a CISP practice was completed (section 3.6.).

5.3.4. Main trials

Participant’s hydration status and nude body mass were determined (section 3.8.). After five minutes of rest in ambient conditions, heart rate (Polar FS1, Finland) and lactate concentration (YSI, USA) were determined (section 3.10.1. & 3.11.). Resting core temperature (\(T_{re}\)) was determined by disposable rectal thermometer (Henley, UK) (section 3.10.4.) and skin temperature (\(T_{sk}\)), using thermistors attached with porous zinc oxide tape to four sites on the right side of the body (section 3.10.4.) (Ramanathan, 1964).
connected to a data logger (Grant, UK). Participants then completed the CISP (section 3.7.) in one of three conditions. The CISP included the standard warm-up followed immediately by twenty, 2 minute blocks as described (section 3.7.). Power output was recorded by SRM cranks at a rate of 50 Hz and PPO and work done were determined using SRM software (section 3.7.).

5.3.5. Physiological measures

$T_{re}$, $T_{sk}$, Thermal sensation ($T_{sen}$), RPE and HR were recorded immediately pre and post warm-up with the participant seated in the chamber. During the CISP these variables were measured at 1 minute into each 2 minute period (section 3.10.1., 3.10.4., 3.10.6., Figure. 3.4.). Blood and gas samples were taken 1 minute into each fourth 2 minute period (Figure. 3.4.). No fluid was permitted during trials and non-urine fluid loss was assessed to 0.01 kg from pre and post-test nude body mass (section 3.10.5). Physiological strain index (PSI) was calculated using the equation of Moran et al., (1998) (section 3.10.3.). Total body temperature, body heat content and metabolic energy expenditure were calculated using standard equations (section 3.10.2, & 3.10.4.).

5.3.6. Statistical analysis

Data were checked for sphericity and adjusted using the Huynh-Feldt correction. Significance was determined using two-way repeated measures ANOVAs (condition*sprint) with Bonferroni adjustment. Where participants failed to complete the CISP ANOVAs were completed with n = 11 and n = 8 participants for sprints 1-14 and 15-20, respectively. Differences between conditions for WBGT and pre-exercise measures were assessed using a one-way repeated measures ANOVA. To determine the effect of fitness on performance, data were also assessed with participants grouped as trained ($VO_{2peak} \geq 50 \, ml.kg^{-1}.min^{-1}$) and moderately trained ($VO_{2peak} \leq 43 \, ml.kg^{-1}.min^{-1}$) using a two-way mixed ANOVA. Perceptual measures were assessed using Friedman’s ANOVA with Wilcoxon signed ranks test. Effect sizes were estimated using partial Eta squared.
(\eta_r^2) (section 3.13.). All data were analysed using SPSS (18.0) and reported as means ± S.D. Significance was accepted at \( P < 0.05 \).

5.4. Results

5.4.1. Ambient conditions

Mean WBGT was higher in hot trials (HH, 30.97 ± 0.69°C; HD, 30.37 ± 0.81°C; TEMP, 16.61 ± 1.75°C; main effect, \( P < 0.001 \)), but not different between HH and HD (\( P > 0.05 \)).

5.4.2. Preliminary measures and warm up

There was no difference in pre-exercise body mass (kg), \( U_{sp} \), or resting \( T_{re} \) between conditions (main effect, \( P = 0.425, 0.579 \) and 0.073 respectively). \( T_{re} \) did not change with warm-up (main effect, \( P = 0.270 \), Table 5.1.). \( T_{sk} \) was elevated by warm-up in hot conditions (main effect, \( P < 0.001 \)) and post warm-up was different between conditions (\( P < 0.05 \), Table 5.1.).

Table 5.1. Rectal temperature (\( T_{re} \), °C) and mean skin temperature (\( T_{sk} \), °C) pre and post warm-up.

<table>
<thead>
<tr>
<th>Condition</th>
<th>( T_{re} ) (°C)</th>
<th>Post w/up</th>
<th>Pre w/up</th>
<th>Post w/up</th>
</tr>
</thead>
<tbody>
<tr>
<td>HH</td>
<td>37.5 ± 0.2</td>
<td>37.5 ± 0.3</td>
<td>30.6 ± 0.6</td>
<td>34.8 ± 1.4*‡</td>
</tr>
<tr>
<td>HD</td>
<td>37.5 ± 0.2</td>
<td>37.5 ± 0.2</td>
<td>30.8 ± 0.6</td>
<td>36.6 ± 0.6*‡</td>
</tr>
<tr>
<td>TEMP</td>
<td>37.4 ± 0.1</td>
<td>37.4 ± 0.2</td>
<td>30.4 ± 1.4</td>
<td>30.7 ± 2.3‡</td>
</tr>
</tbody>
</table>

Data are mean ± S.D. HH = hot humid; HD = hot dry; TEMP = temperate. * Significantly higher than pre warm-up within condition (\( P < 0.01 \)). ‡ Significantly different between conditions post warm-up (\( P < 0.01 \)).
5.4.3. Number of sprints completed

All participants completed the CISP in TEMP. In the hot trials, eleven participants completed fourteen sprints, but only eight completed the full protocol of twenty sprints. One participant was stopped in both trials due to $T_{re}$ reaching 39.7°C. The remaining two participants stopped after sprint fourteen due to exhaustion.

5.4.4. Main experimental conditions

5.4.4.1. Performance variables

PPO was not different between conditions in sprints 1-14 ($F_{(2,20)} = 1.132, P = 0.342, \eta_p^2 = 0.102$, Table 5.2, Figure 5.2.) or 15-20 ($F_{(2,14)} = 0.902, P = 0.114, \eta_p^2 = 0.102$, Table 5.2, Figure 5.2.). For training state there was also no effect of group on PPO in sprints 1-14 ($F_{(1,26)} = 1.002, P = 0.326, \eta_p^2 = 0.037$) or 15-20 ($F_{(1,10)} = 4.928, P = 0.051, \eta_p^2 = 0.330$). Over time, PPO in HH declined 8% from sprint 16 compared to sprint 4 (all P values < 0.05). In HD an 8% reduction in PPO occurred from sprint 15 compared to sprint 5 (all P values < 0.05) and in TEMP a 6% decline in PPO was observed from sprint 17 compared to sprint 3 (all P values < 0.05).

**Table 5.2.** Peak power output (W) and work done (J) during the CISP in HH, HD and TEMP.

<table>
<thead>
<tr>
<th></th>
<th>Sprints 1-14 (n=11)</th>
<th>Sprints 15-20 (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peak Power Output (W)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HH</td>
<td>1073±150</td>
<td>954±114</td>
</tr>
<tr>
<td>HD</td>
<td>1104±127</td>
<td>997±115</td>
</tr>
<tr>
<td>TEMP</td>
<td>1074±134</td>
<td>993±94</td>
</tr>
<tr>
<td><strong>Work Done (J)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HH</td>
<td>3073 ± 491</td>
<td>2694 ± 405</td>
</tr>
<tr>
<td>HD</td>
<td>3163 ± 427</td>
<td>2822 ± 376</td>
</tr>
<tr>
<td>TEMP</td>
<td>3102 ± 422</td>
<td>2865 ± 299</td>
</tr>
</tbody>
</table>

Data are for the mean (± S.D.) of all sprints for sprints 1 - 14 and 15 - 20. HH = hot humid; HD = hot dry; TEMP = temperate.
Figure 5.2. Mean (± S.D.) Peak Power Output during the CISP in HH, (hot humid) HD (hot dry) and TEMP (temperate) (sprints 1-14 n = 11; sprints 15-20 n = 8). *Significant difference from sprint 4 in HH (P < 0.05). †Significant difference from sprint 5 in HD (P < 0.05). ‡Significantly different from sprint 3 in TEMP (P < 0.05).

Work done was not different between conditions in sprints 1-14 ($F_{(2,20)} = 1.026, P = 0.377$, $\eta^2_p = 0.093$, Table 5.2.) or 15-20 ($F_{(2,14)} = 1.478, P = 0.262$, $\eta^2_p = 0.174$, Table 5.2.). Over time, HH reduced work done by 10% from sprint 17 compared to sprint 2 (all P values < 0.05). In HD an 8% reduction occurred from sprint 15 compared to sprint 5 and in TEMP, a 6% reduction was evident from sprint 17 compared to sprint 3 (all P values < 0.05).

5.4.4.2. Physiological variables

Mean $T_{re}$ in sprints 1-14 was not different between conditions ($F_{(2,20)} = 2.550, P = 0.103$, $\eta^2_p = 0.203$, Table 5.3.) and there was no effect of training state ($F_{(1,26)} = 0.003, P = 0.957$, $\eta^2_p = 0.000$). Mean $T_{re}$ differed over time ($F_{(13,130)} = 156.569, P < 0.001$, $\eta^2_p = 0.940$) and there was a significant interaction ($F_{(26,260)} = 3.881, P < 0.001$, $\eta^2_p = 0.280$, Figure 5.3.). Post hoc analysis revealed $T_{re}$ was different only between HH and TEMP from sprint 13 (all
P values < 0.05). In sprints 15-20 there was an effect of environment \( (F_{(2,14)} = 11.503, \ P = 0.001, \ \eta^2_p = 0.622) \), time \( (F_{(5,35)} = 75.958, \ P < 0.001, \ \eta^2_p = 0.916) \) and a significant interaction \( (F_{(10,70)} = 6.781, \ P < 0.001, \ \eta^2_p = 0.492) \). Follow up analysis revealed no difference in T\(_{re}\) between hot trials but a higher mean T\(_{re}\) was evident in HH compared to TEMP from sprint 15 (all P values < 0.05, Figure 5.3.) and in HD compared to TEMP from sprint 17 (all P values < 0.05, Figure 5.3.). T\(_{re}\) did not differ in sprints 15-20 based on training state \( (F_{(1,10)} = 2.085, \ P = 0.179, \ \eta^2_p = 0.172) \).
### Table 5.3 Peak and mean physiological and perceptual measures during the CISP in HH, HD and TEMP.

<table>
<thead>
<tr>
<th></th>
<th>Sprints 1 - 14</th>
<th></th>
<th></th>
<th>Sprints 15 - 20</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HH</td>
<td>HD</td>
<td>TEMP</td>
<td>HH</td>
<td>HD</td>
<td>TEMP</td>
</tr>
<tr>
<td>$T_{re}$ Peak ($^\circ$C)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>39.1 ± 0.5†</td>
<td>38.9 ± 0.5†</td>
<td>38.5 ± 0.2</td>
</tr>
<tr>
<td>$T_{re}$ mean ($^\circ$C)</td>
<td>38.1 ± 0.5</td>
<td>37.9 ± 0.5</td>
<td>37.9 ± 0.3</td>
<td>38.9 ± 0.4†</td>
<td>38.7 ± 0.4</td>
<td>38.4 ± 0.2</td>
</tr>
<tr>
<td>$T_{sk}$ mean ($^\circ$C)</td>
<td>36.04 ± 0.7‡</td>
<td>37.1 ± 0.8‡</td>
<td>31.3 ± 1.9‡</td>
<td>36.4 ± 0.6‡</td>
<td>37.3 ± 0.9‡</td>
<td>31.9 ± 1.8‡</td>
</tr>
<tr>
<td>$HR_{mean}$ (b.min$^{-1}$)</td>
<td>162 ± 19†</td>
<td>158 ± 16†</td>
<td>147 ± 19</td>
<td>176 ± 12†</td>
<td>171 ± 11†</td>
<td>154 ± 15</td>
</tr>
<tr>
<td>$[B_{la}]_{mean}$ (mM)</td>
<td>4.0 ± 1.6</td>
<td>4.2 ± 1.8</td>
<td>4.2 ± 1.8</td>
<td>3.8 ± 1.6</td>
<td>4.3 ± 1.8</td>
<td>4.3 ± 1.9</td>
</tr>
<tr>
<td>$VO_{2}$mean (L.min$^{-1}$)</td>
<td>2.3 ± 0.2</td>
<td>2.2 ± 0.3</td>
<td>2.2 ± 0.2</td>
<td>2.2 ± 0.2</td>
<td>2.2 ± 0.3</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>BHC (kJ)</td>
<td>10495 ± 1463†</td>
<td>10536 ± 1474†</td>
<td>10203 ± 1449</td>
<td>10245 ± 945†</td>
<td>10256 ± 944†</td>
<td>9913 ± 979</td>
</tr>
<tr>
<td>PSI Peak</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9 ± 1†</td>
<td>8 ± 2</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>PSI mean</td>
<td>6 ± 2†</td>
<td>5 ± 2</td>
<td>5 ± 1</td>
<td>8 ± 1†</td>
<td>7 ± 2</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>RPE Peak</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>18 ± 2†</td>
<td>17 ± 2</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>RPE mean</td>
<td>14 ± 3†</td>
<td>13 ± 3</td>
<td>12 ± 2</td>
<td>18 ± 2†</td>
<td>16 ± 2</td>
<td>15 ± 1</td>
</tr>
<tr>
<td>$T_{Sen}$ Peak</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7 ± 1†</td>
<td>7 ± 1†</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>$T_{Sen}$ mean</td>
<td>6 ± 1†</td>
<td>6 ± 1†</td>
<td>5 ± 1</td>
<td>7 ± 1†</td>
<td>7 ± 1†</td>
<td>6 ± 1</td>
</tr>
</tbody>
</table>

Data are mean ± S.D. (sprint 1 – 14 N = 11; sprints 15 – 20 N = 8). HH = hot humid; HD = hot dry; TEMP = temperate; RPE = rating of perceived exertion; $T_{Sen}$ = Thermal sensation; PSI = Physiological strain index; $VO_{2}$ = Oxygen consumption. Peak values are those recorded in the final 2 minute block of the CISP. †Significant difference from TEMP (P < 0.05). ‡Significant difference between a condition.
Figure 5.3. Mean (± S.D.) rectal temperature and weighted skin temperature during the CISP in HH (hot humid), HD (hot dry) and TEMP (temperate). *Significant difference between HH and TEMP (P < 0.05). †Significant difference between HD and TEMP (P < 0.05). ‡Significant difference between all conditions (sprints 1-14 and 15-20, P < 0.05).

$T_{sk}$ was different between conditions in sprints 1-14 ($F_{(2,20)} = 133.995$, $P < 0.001$, $\eta^2_p = 0.931$, Table 5.3). There was a main effect of time ($F_{(13,130)} = 20.888$, $P < 0.001$, $\eta^2_p = 0.676$) and a significant interaction ($F_{(26,260)} = 4.455$, $P < 0.001$, $\eta^2_p = 0.308$, Figure 5.3.). Follow up analysis revealed $T_{sk}$ was different between conditions from sprint one (all P values < 0.05, Figure 5.3.). $T_{sk}$ in sprint 15-20 was different between conditions ($F_{(2,14)} = 74.714$, $P < 0.001$, $\eta^2_p = 0.914$, Table 5.3.) and there was a main effect of time ($F_{(5,35)} = 7.187$, $P < 0.001$, $\eta^2_p = 0.507$, Figure 5.3.). Follow up analysis revealed $T_{sk}$ was different between conditions from sprint 15 (all P values < 0.05, Figure 5.3.).

HR in sprints 1-14 was different between conditions ($F_{(2,20)} = 20.395$, $P < 0.001$, $\eta^2_p = 0.671$, Table 5.3). There was a main effect of time ($F_{(13,130)} = 64.250$, $P < 0.001$, $\eta^2_p = 0.865$) and a
significant interaction ($F_{(26,260)} = 2.585, P < 0.001, \eta_p^2 = 0.205$, Figure. 5.4.). Follow up analysis revealed HR was not different between hot trials, but HH was different to TEMP from sprint 4 (all P values < 0.05) and HD from sprint 9 (all P values < 0.05). In sprints 15-20 there was a main effect of environment ($F_{(2,14)} = 25.768, P < 0.001, \eta_p^2 = 0.786$), time ($F_{(5,35)} = 7.149, P < 0.001, \eta_p^2 = 0.505$) and interaction ($F_{(10,70)} = 1.985, P < 0.05, \eta_p^2 = 0.181$). Follow up analysis revealed HR was similar in HH and HD (all P values < 0.05), but higher than TEMP (all P values < 0.01). Over time HR was greater in HH from sprint 4 (all P values < 0.05) and in both HD and TEMP from sprint 5 (all P values < 0.05).

**Figure 5.4.** Mean (± S.D.) heart rate during the CISP in HH (hot humid), HD (hot dry) and TEMP (temperate) (sprints 1-14 n = 11; sprints 15-20 n = 8) *Significant difference between HH and TEMP (P < 0.05). †Significant difference between HD and TEMP (P < 0.05).

PSI in sprints 1-14 displayed a significant main effect of environment ($F_{(2,20)} = 6.594, P = 0.006, \eta_p^2 = 0.397$, Table 5.3.), time ($F_{(13,130)} = 119.771, P < 0.001, \eta_p^2 = 0.923$) and interaction ($F_{(26,260)} = 4.429, P < 0.001, \eta_p^2 = 0.307$). Follow up analysis revealed PSI was higher in HH compared to TEMP from sprint 7 (all P values > 0.05) but was not different
between hot trials or HD and TEMP (all P values < 0.05). PSI in sprints 15-20 was different between conditions ($F_{(2,14)} = 8.997, P = 0.003, \eta^2_p = 0.562$, Table 5.3.), time ($F_{(5,35)} = 45.031, P < 0.001, \eta^2_p = 0.865$) and interaction ($F_{(10,70)} = 3.239, P = 0.002, \eta^2_p = 0.316$). Follow up analysis revealed PSI was different only between HH and TEMP (all P values < 0.05).

BHC in sprints 1-14 was different between conditions ($F_{(2,20)} = 89.276, P < 0.001, \eta^2_p = 0.899$) and there was a significant main effect of time and ($F_{(13,130)} = 136.439, P < 0.001, \eta^2_p = 0.932$) interaction ($F_{(26,260)} = 1.809, P = 0.011, \eta^2_p = 0.153$). Follow up analysis revealed that BHC was different between hot trials in the first three sprints only (all P values < 0.05) but both were greater than TEMP from sprint one (all P values < 0.01). In sprints 15-20 there was a significant main effect of environment ($F_{(2,14)} = 89.390, P < 0.001, \eta^2_p = 0.927$), time ($F_{(5,35)} = 72.108, P < 0.001, \eta^2_p = 0.912$) and interaction ($F_{(10,70)} = 2.590, P < 0.05, \eta^2_p = 0.223$) on BHC. Follow up analysis revealed that BHC was not different between hot trials, but both were different to TEMP (all P values < 0.001).

There was no difference between conditions in blood lactate ($F_{(2,20)} = 0.348, P = 0.710, \eta^2_p = 0.034$; Table 5.3.) or oxygen uptake ($F_{(2,20)} = 0.448 P = 0.645, \eta^2_p = 0.043$, Table 5.3.) during the CISP. A similar decrease in nude body mass was observed in HH and HD pre and post CISP (1.2 ± 0.3 kg and 1.0 ± 0.2 kg, $P > 0.05$) and this was greater than TEMP (0.6 ± 0.1 kg, $P < 0.01$).

5.4.4.3. Perceptual measures

In sprints 1-14 there was a significant main effect of environment ($F_{(2,20)} = 11.075, P = 0.001, \eta^2_p = 0.526$, Table 5.3.) and time ($F_{(13,130)} = 57.832, P < 0.001, \eta^2_p = 0.853$) on RPE and a significant interaction ($F_{(26,260)} = 8.180, P < 0.001, \eta^2_p = 0.450$). Follow up analysis revealed RPE was different only between HH and TEMP from sprints 5-14 (all P values < 0.05). In sprints 15-20 there was a significant main effect of environment ($F_{(2,14)} = 17.250,$
P < 0.001, $\eta^2_p = 0.711$), time ($F_{(5,35)} = 17.347$, $P < 0.001$, $\eta^2_p = 0.712$) and interaction ($F_{(10,70)} = 2.949$, $P < 0.05$, $\eta^2_p = 0.296$) on RPE. Follow up analysis revealed RPE was different between HH and TEMP only (all $P$ values < 0.05). Peak RPE was higher in HH compared to HD and TEMP only ($P < 0.05$). Mean and peak $T_{\text{sen}}$ were not different between hot trials ($P > 0.05$), but both hot conditions were greater than TEMP ($P < 0.05$, Table 5.3.).

5.5. Discussion

The main finding of this study was that intermittent-sprint performance and physiological strain were not different in hot humid and hot dry environments matched for heat stress. While variability in power output could have prevented detection of performance differences between environments, previous research examining the effect of HH and HD environments matched for heat stress on repeated-sprint performance has demonstrated similar findings using similar participants and a smaller sample size (Backx et al., 2000). As such, it may be suggested that when intermittent sprinting using exercise-rest ratios representative of team-sports is completed for 40 minutes in a hot environment, the composition of thermal factors comprising the overall heat stress does not differentially impact performance.

Previous research has identified aerobic fitness as an important determinant of thermoregulatory capacity in the heat (Mora-Rodriguez, 2012). Trained individuals display improved heat dissipation and tolerance due to reduced thresholds for vasodilation and sweating (Mora-Rodriguez, 2012). It is conceivable therefore, that variability in participants’ aerobic fitness may have contributed to the lack of difference between conditions. However, when data were analysed based on aerobic fitness no difference was observed in PPO or PSI between groups.
PPO and work done in the current study was not significantly different between hot and control trials. The extent of hyperthermia induced may explain this. Research has identified reduced voluntary activation and impaired performance in the heat due to increased $T_{re}$ and demonstrated the extent of reduction in voluntary activation is dependent on the increase in $T_{re}$ (Nybo and Nielsen, 2001a; Morrison et al., 2004). Consequently, when exercise in a hot trial has induced a marked hyperthermia (> 39.1°C) compared to control, a significant difference has been observed in performance between conditions (Drust et al., 2005; Sunderland et al., 2005). Conversely, when exercise has been performed in the heat without marked differences in hyperthermia between conditions, performance has not been different between trials (Almudehki et al., 2012). Therefore, in the current investigation lack of difference in PPO between conditions may be due to the extent of hyperthermia induced during hot and temperate trials (HH, 38.3 ± 0.5°C; HD, 38.2 ± 0.5°C, TEMP, 38.1 ± 0.3°C).

Performance between trials in the current study was not different. However, while all eleven participants completed the CISP in TEMP, three failed to complete the full protocol of twenty sprints in hot trials. Two of these participants had the greatest body mass (kg), body surface area (m²) and percent body fat of the sample and the greatest rate of increase in body heat content during all CISPs (14.8 kJ.min⁻¹ vs. 9.1 kJ.min⁻¹). It has been suggested larger athletes select lower exercise intensities in self-paced running in the heat to ensure optimal heat storage and prevent dangerous hyperthermia (Marino et al., 2004). As the CISP is not self-paced it may be the fixed intensity in recovery elicited the greater rates of heat storage in the heavier participants, greater physiological strain and consequently, premature cessation of exercise. The final participant unable to complete exercise in hot trials had the second largest absolute $\dot{VO}_{2peak}$ (4.19 L.min⁻¹) and the highest metabolic heat production of the whole sample (493 W.m² vs. 384 W.m²). Greater heat production exists in endurance trained athletes during fixed intensity exercise due to exercising at a higher absolute $\dot{VO}_2$ compared to untrained (Mora-Rodriguez, 2012). Accordingly, for this participant it may be that exercise at 35% $\dot{VO}_{2peak}$ evoked greater metabolic heat production that, in the hot trials, negated the improved heat dissipation.
mechanisms and produced greater physiological strain, necessitating early cessation of exercise.

Over time PPO declined 6 - 8% in all conditions. The typical error for PPO in the CISP has been reported as 2.9% and as such, the reductions observed in this study represent a meaningful change requiring examination (chapter four). Recently, it has been proposed that impaired performance in the heat may be due to cardiovascular strain rather than neuromuscular factors (Cheuvront et al., 2010). In hot environments $T_{sk}$ is elevated and the gradient for dry heat transfer is reduced (Gonzalez-Alonso, 2012). So, it is argued that when $T_{sk}$ is high the function of a rising $T_{re}$ is to maintain a favourable gradient and reduce skin blood flow requirements for heat loss (Cheuvront et al., 2010). However, during exercise in the heat increases in $T_{re}$ and $T_{sk}$ cause a reflex increase in skin blood flow that is proportional to the reduction in $T_{re}$-$T_{sk}$ gradient and may decrease venous return and cardiac output (Cheuvront et al., 2010; Gonzalez-Alonso, 2012). As vascular conductance is maintained in active tissue in maximal exercise any reduction in cardiac output may generate a perfusion issue and an increase in the relative exercise intensity that may reduce performance in the heat (Cheuvront et al., 2010). In the current investigation, $T_{sk}$ was significantly higher in HD compared to HH and both were significantly higher than TEMP. Additionally, the $T_{re}$-$T_{sk}$ gradient was reduced in hot compared to control (2.2°C in HH, 1°C in HD and 6.5°C in TEMP). It is conceivable this resulted in increased skin blood flow, a reduced cardiac output and increased relative exercise intensity contributing to the reductions in performance. While this is supported by the higher heart rates observed in hot trials compared to TEMP, the lack of difference in VO$_2$ or lactate does not lend support to the suggestion of a cardiovascular strain mediated reduction in performance independent of a high $T_{re}$. Alternately, it has been demonstrated that passive hyperthermia significantly reduces voluntary root mean square electromyography amplitude of active muscle at all increments in $T_{re}$ > 0.5°C above baseline and that force production is impaired after $T_{re}$ is increased > 1°C (Ross et al., 2012). Although not measured in the current investigation, in view of the increases in $T_{re}$ during the CISP (HH + 1.6°C; HD, + 1.4°C; TEMP, + 1.1°C) and a lack of difference in metabolism, it is possible reductions in performance resulted from impaired central and voluntary drive due in part...
to reductions in middle cerebral artery blood flow and impaired cerebral heat dissipation (Nybo and Nielsen, 2001a; Racinais et al., 2007).

5.5.1. Conclusion

The current study is the first to examine the effect of hot humid and hot dry environments, matched for heat stress, on intermittent-sprint exercise similar to that observed in team-sports. Data indicate intermittent-sprint performance is impaired, but not different in hot humid compared to hot dry conditions when heat stress is similar. The current work illustrates the need for heat acclimation for games players when competing in hot environments. However, evidence suggests that while the overall heat load in acclimation should match expected competition conditions the composition of thermal factors constituting the heat stress is not a primary concern for intermittent-sprint athletes. The work is limited to forty minutes, approximating one half of a field-based team-sport. Further research is necessary to determine the effect of hot humid and hot dry environments, matched for heat stress, on the ‘second half’ of a team-game.
CHAPTER VI. PHYSIOLOGICAL AND PERCEPTUAL RESPONSES TO PROGRESSIVE COMPARED TO TRADITIONAL HEAT ACCLIMATION AND THE EFFECT ON INTERMITTENT-SPRINT EXERCISE IN A HOT ENVIRONMENT

6.1. Abstract

The main aim of this study was to compare physiological and perceptual adaptation to heat acclimation from a progressive model of acclimation that targets external heat stress to maintain physiological strain to those achieved using a traditional model. A secondary aim was to investigate the effect of progressive heat acclimation on physiological, perceptual and performance responses during forty minutes of intermittent-sprint exercise in 33°C 50% rh. Twenty four University games players matched for VO2peak, peak power and body surface area were divided into three groups; progressive heat acclimation [PA, 4 d 30°C 50% relative humidity (rh), 4 d 33°C 53% RH and 4 d 35°C 60% rh], traditional heat acclimation (TA, 12 d 33°C 56% rh), and training (TG 12 d 19°C 36% rh). Pre and post acclimation, participants completed a 40 minute cycling intermittent sprint protocol (CISP) in 33°C 50% rh, comprising twenty, 5 s sprints. Resting heart rate over day one to twelve was reduced by both acclimation regimes (P < 0.05) but not TG (P > 0.05) and resting T_re displayed a tendency to decline (P > 0.05). Mean exercise heart rate across day one to twelve tended to decline (P > 0.05) but peak heart rate was significantly reduced by TA and TG (P < 0.05). Further, mean and peak exercise T_re were reduced by TA and TG by day twelve (P < 0.05). Consistent with elevated heat stress and physiological strain during PA, mean and peak exercise heart rate was unchanged across day one to twelve and mean exercise T_re was significantly higher on day twelve compared to six. Sweat rate was increased by day six in TA (P < 0.05), day twelve in PA (P < 0.05) but not TG (P > 0.05). Resting heart rate and T_re were not significantly different prior to CISP 2 but tended to decline in PA and TA. During CISP 2 mean heart rate and T_re were reduced by both acclimation regimes (P < 0.05) and peak T_re was significantly reduced by all regimes (P < 0.05). In addition, during CISP 2 T_sk and T_body were reduced by both acclimation regimes (P < 0.05) but not TG (P > 0.05). Both acclimation regimes also
reduced RPE and $T_{sen}$ during CISP 2 ($P < 0.05$) but TG had no effect ($P > 0.05$). Finally, PPO was reduced by 19, 6 and 8% in TA, TG and PA during CISP 1 and was strongly negatively correlated with exercise $T_{re}$ for both acclimation regimes ($r > 0.90$, $P < 0.001$). TA increased PPO in CISP 2 and ameliorated the strong negative correlation between this measure and $T_{re}$ but no such response was observed for PA and TG. Based on the ability to evoke classic acclimation responses compared to TA, PA was judged an effective method for conferring the heat acclimated phenotype. However, while evidence suggests the current design maintained physiological strain during the acclimation regime, the degree of strain was possibly insufficient to elicit a maximal acclimation response.

6.2. Introduction

Hyperthermia impairs intermittent-sprint performance under conditions of heat stress (Maxwell et al., 1996; Morris et al., 2000; Drust et al., 2005). Heat acclimation is frequently used to combat this decline and regimes can be grouped into three types; constant work rate, self-regulated exercise and isothermal strain (Taylor, 2000). Constant work rate protocols, whereby participants are required to exercise at a fixed rate over a specific number of days, represent the traditional model of heat acclimation and are the most frequently used (Taylor, 2000). However, with constant work rates the heat strain in subsequent exposures progressively declines across the protocol and therefore, the potential of this method to elicit maximal heat adaptation is questionable (Taylor, 2000). Similarly, with constant work rate protocols there is increased potential for participant drop-out as exposure to high levels of heat stress occurs early, promoting rapid elevations in core temperature, premature cessation of acclimation sessions and potential incomplete adaptation to the heat (Garden et al., 1966; Wyndham et al., 1976; Horstman et al., 1982; Febbraio et al., 1994).

Self-regulated heat acclimation protocols counter participant drop-out and permit complete participation in initial sessions through self-selection of work rates, but
application of this type of protocol in a research environment is limited due to the variable exercise intensities used (Taylor, 2000). Similarly, isothermal strain regimes evoke heat acclimation by elevating all participants to a pre-determined ‘critical’ core temperature (Taylor, 2000; Garrett et al., 2012) and maintaining it by modifying work-load throughout the session. Based on the premise that attainment and maintenance of an elevated core temperature above the sweating threshold is central to effective heat acclimation, this model may offer more complete adaptation to the heat (Taylor et al., 2000) compared to other models. Currently, however, there is limited evidence comparing isothermal with traditional methods of heat acclimation and therefore, it is difficult to verify whether isothermal heat acclimation is a superior method or confers additional benefit beyond more traditional methods. In addition, inter-individual differences in the work required to elicit a target core temperature and maintain it due to, for example, heat tolerance or training status, may evoke different physiological responses in, for example, immune function and promote over-reaching. Further, although increasing (Garrett et al., 2012), there is limited empirical evidence directly investigating the performance benefits of isothermal heat acclimation against other methods.

Heat acclimation adaptation exhibits a biphasic response pattern. Two-thirds to 75% of physiological adaptation is achieved within 4 - 6 days, while complete sudomotor function alteration necessitates up to fourteen days (Armstrong and Maresh, 1991; Pandolf, 1998). Consequently, despite possible limitations with constant work rate protocols, the majority of earlier literature on heat acclimation proposed exercise at a fixed intensity for a set duration in a controlled environment (e.g. 60 - 90 mins.d⁻¹ for 10 - 14 days at ≥ 40% \( \dot{V}O_{2\text{max}} \)), as the most effective means of eliciting complete adaptation to the heat (Nadel et al., 1974; Horstman 1982; King 1985; Armstrong and Dziados, 1986).

Evidence suggests the time course of adaptation and decay for some physiological markers of heat acclimation may reflect a protocol-dependent limit as opposed to a true maximal physiological response. With constant work rate protocols where physiological
strain is reduced across time, a decline in plasma volume towards baseline by the end of the protocol is observed. However, maintenance of expansion for up to 22 days has also been reported when an isothermal strain protocol (Patterson et al., 2004) that provides a progressive stimulus has been used, highlighting the need for further research using alternate heat acclimation methods.

There is limited research on the efficacy of alternate heat acclimation protocols on exercise performance in the heat. Earlier work focused primarily on the impact of altered exercise intensity and duration, or the effect of continuous versus intermittent protocols on acclimation responses (Fein, 1975; Houmard et al., 1990; Aoyagi et al., 1995; Gill et al., 2001; O’ Brien et al., 2004). Such investigations produced conflicting results and primarily examined benefits to continuous exercise. More recently, research has identified a beneficial effect from short-term heat acclimation protocols for intermittent exercise (Sunderland 2008; Peterson et al., 2010; Brade et al., 2013), however, variation in the extent of adaptation was observed. Considering greater thermal strain has been identified in intermittent compared to continuous exercise (Nevill et al. 1995), there is a need for further research into optimising heat acclimation for intermittent-sprint exercise using non-traditional protocols designed to maximise adaptation and minimise participant drop-out. It is inviting to speculate that progressive heat acclimation, where thermal load is increased over the duration of the heat acclimation protocol by alteration of environmental conditions, may provide a progressive, more complete thermal stimulus that can optimise exercise performance in the heat.

Progressive heat acclimation, where environmental conditions are altered throughout the acclimation period has received little attention in the literature. Daanen et al., (2011) used a 2-stage heat acclimation protocol of constant work and incremental exercise for 9 days at 35°C 29% rh followed by 3 days at 41°C and 33% rh, but reported no beneficial effect of the increase in thermal load on physiological markers of heat acclimation possibly owing to the protocol design whereby a nine day constant work-rate method preceded a brief three day progression to a higher heat stress. In this context, the two
step progression coupled with the brevity of exposure to higher heat stress may have been insufficient to elicit adaptation. In contrast, Costa et al., (2014) recently demonstrated a beneficial effect of progressive heat acclimation in ultra endurance athletes. When three 2 hour treadmill running sessions at 60% VO\textsubscript{2max} were completed in 30°C followed by three in 35°C, additional significant improvements in T\textsubscript{re}, HR and thermal comfort were reported with exposure at 35°C (Costa et al., 2014). Further, comparative evidence from intermittent hypoxic acclimation also suggests a positive effect of progressive heat acclimation. Hamlin and Hellemans, (2007) exposed twenty-two multi-sport endurance athletes to 90 minutes of intermittent normobaric hypoxic exposure at rest versus 90 minutes of placebo exposure at rest for 15 days over a 3-week period. Fraction of inspired oxygen in the hypoxic gas decreased from 13% in week one to 10% by week three. Results showed 3-km run time decreased by 1.7% two days after, and by 2.3% seventeen days after the last hypoxic episode in the training relative to the placebo group. In addition, relative to the placebo group, the training group increased reticulocyte count two days (23.5%) and twelve days post-exposure. Considering the reported positive effect of progressive hypoxic acclimation coupled with the potential practical applications, a progressive model of heat acclimation could offer an alternative and indeed, more thermally-balanced model to existing models of heat acclimation.

The objectives of this study were to examine physiological and perceptual adaptation to heat acclimation using a progressive protocol compared to a traditional model and to investigate the effect of progressive heat acclimation on physiological, perceptual and performance responses during forty minutes of intermittent-sprint exercise in 33°C 50% rh. This is the first study to use a progressive heat acclimation model whereby physiological and thermal strain was elevated throughout the protocol by stepwise increases in heat stress on two occasions during acclimation. It was hypothesised a progressive heat acclimation model with stepwise increases in heat stress would, compared to a traditional model, evoke similar adaptations to the heat and prove a valid alternative heat acclimation protocol. In addition, it was hypothesised that progressive heat acclimation would improve perceptual and physiological responses during
intermittent sprinting in the heat and ameliorate heat induced decrements in performance.

6.3. Methods

6.3.1. Participants

Twenty four University students (twenty two male, two female) who were games players and participated in sport three to five times per week volunteered for the study. Participants were randomly assigned to one of three groups, matched for $\dot{V}O_2^{\text{peak}}$, body surface area (BSA), and experience of the CISP, [(Traditional Acclimation - TA, n = 6, 20.5 ± 0.3 years, 177.3 ± 2.4 cm, 74.9 ± 4.2 kg, BSA = 1.91 ± 0.05 m$^2$, 42.2 ± 2.4 ml·kg$^{-1}$·min$^{-1}$) (Progressive Acclimation - PA, n = 9, 20.5 ± 1.5 years, 179.1 ± 2.7 cm, 71.1 ± 3.8 kg, BSA = 1.89 ±0.06 m$^2$, 46.9 ± 2.7 ml·kg$^{-1}$·min$^{-1}$) (Training Group - TG, n = 9, 21.6 ± 1.5 years, 175.2 ± 5.6 cm, 73.8 ± 5.9 kg, BSA = 1.89 ± 0.10 m$^2$, 47.7 ± 3.3 ml·kg$^{-1}$·min$^{-1}$)]. All participants were informed as to the demands, risks and benefits and gave written informed consent to participate in the study that was approved by the University Research Ethics and Governance committee and conducted in accordance with the Declaration of Helsinki (2008).

6.3.2. Environments

Three differing environments were compared in this study with the TA and PA groups matched for overall heat stress. For TA the 12 day acclimation programme was completed at 33.0 ± 0.4°C and 56.0 ± % rh. In PA, environmental conditions were elevated on two occasions to increase heat stress. On day 1 - 4 of PA participants exercised at 29.1 ± 3.4°C and 54.3 ± 6.7% rh. On day 5 - 8 environmental conditions were set at 33.1 ± 0.5°C and 52.9 ± 3.8% rh and on day 9 - 12 at 34.6 ± 4.2°C and 60.5 ± 2.8% rh. For TG all sessions were at 19.0 ± 1.6°C and 36.4 ± 1.4% rh.
6.3.3. Experimental design

Each participant was required to visit the laboratory on fifteen separate occasions (Figure. 6.1.). This included one preliminary trial, a pre and post CISP in 33 ± 0.2°C, 50 ± 3% rh and on day 2 - 7 and 9 - 14 inclusive, 12 days of training or acclimation (60 minutes at 50% VO₂peak) depending on the group they had been assigned to. All trials for each participant were completed in ≤ 21 days. All testing was completed between October and December to minimize the potential for natural heat acclimatization and all main trials were completed at the same time of day to minimize the influence of circadian variation (Winget et al., 1985; Hill et al., 1992).
### 6.3.3.1. Preliminary visit

Prior to main trials, each participant completed a preliminary visit including anthropometric assessment, a peak oxygen consumption test and a CISP practice. Height was recorded to within 0.1 cm in the Frankfurt plane using a beam scale, body mass was measured to within 0.1 kg using a standard laboratory scales (SECA, UK) and body surface area was calculated using the method of Dubois and Dubois (1916). Percentage body fat was determined from four sites (Tricep, Bicep, Suprailium, Subscapular) using skinfold calipers (Harpenden Instruments, West Sussex, UK) as described by Durnin and Womersley (1974) and the equations of Siri (1961) (section 3.6.).

Lactate threshold and peak oxygen consumption was determined for each participant using a graded exercise test on one of two modified cycle ergometers (Monark 620 Ergomedic, Sweden, with SRM power cranks (SRM; scientific model, Julick, Germany); Monark Ergomedic 874E with SRM power cranks (SRM; FSA Gossamer standard, Julick, Germany)) (section 3.5.1. & 3.6.). 25 ± 5 minutes after cessation of the graded exercise test all participants completed a CISP practice (section 3.6.).

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<table>
<thead>
<tr>
<th>A measures</th>
<th>B measures</th>
<th>C measures (day 1–12)</th>
<th>D measures (day 1, 6, 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (HT)</td>
<td>Peak power output (PPO)</td>
<td>Hydration (U_{H2O} + U_{Na})</td>
<td>Plasma Volume (PV)</td>
</tr>
<tr>
<td>% Body Fat</td>
<td>Work done</td>
<td>Sweat rate</td>
<td>Haematocrit (Hct)</td>
</tr>
<tr>
<td>Lactate threshold and VO_2peak (LT-VO_2peak)</td>
<td>Hydration (U_{H2O} + U_{Na})</td>
<td>Rectal temperature (T_{re})</td>
<td>Haemoglobin (Hb)</td>
</tr>
<tr>
<td>Resting and maximum heart rate (HR)</td>
<td>Sweat rate</td>
<td>Skin temperature (T_{sk})</td>
<td>Systolic rate</td>
</tr>
<tr>
<td>CISP practice</td>
<td>VO_2</td>
<td>[Na]_serum + [K]_serum + [Cl]_serum</td>
<td>[Na]_serum + [K]_serum + [Cl]_serum</td>
</tr>
<tr>
<td></td>
<td>[Lactate]_serum</td>
<td>Thermal Sensation (T_{th})</td>
<td>[Lactate]_serum</td>
</tr>
<tr>
<td></td>
<td>Rating of perceived exertion (RPE)</td>
<td>Rating of Perceived Exertion (RPE)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Physiological strain index (PSI)</td>
<td></td>
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</tr>
</tbody>
</table>

**Figure 6.1.** Schematic of experimental design with measurements.
6.3.3.1.1. Cycling Intermittent-Sprint Protocol

Pre and post acclimation or training (≥ 1 day) all participants completed the CISP (section 3.7.), on one of the modified Monark cycle ergometers (section 3.5.1.), at the same time of day in an environmental chamber at 33.0 ± 0.2°C, 50.0 ± 3% rh. Peak power output (PPO), mean power output (MPO) and work done were recorded and determined as described in section 3.5.1.

Prior to main trials all participants were requested to follow standard pre-trial preparation guidelines for diet, exercise and hydration (section 3.4.). On arrival at the laboratory nude body mass and hydration status were assessed (section 3.8.). Participants were prepared for measurement of rectal temperature ($T_{re}$) and skin temperature ($T_{sk}$) and resting values recorded (section 3.10.4). A resting blood sample was obtained from a hyperemized finger for lactate concentration (section 3.11.) and resting heart rate was determined (section 3.10.1). On completion of resting measures and immediately prior to entering the environmental chamber, a Tegaderm sweat patch (HFL Sport Science Limited, Fordham, Cambridgeshire, UK) was applied between the shoulder blades of each participant for the analysis of Na, K, and Cl− concentrations in sweat over the duration of the CISP.

On entering the chamber participants completed the CISP warm-up and the CISP (section 3.6 and 3.7). Thermoregulatory, perceptual and heart rate measures were recorded every two minutes (section 3.7., 3.10.) during the CISP. Every fourth two minute block of the CISP, arterialised whole blood samples were collected from a hyperemised finger and expired air sampled (section 3.6., 3.11.). On completion of the CISP, or if participants felt unable to continue, or reached a critical core temperature, they were removed from the chamber and monitored until suitably recovered (section 3.2.). Post CISP, prior to administering fluid in recovery weight loss was determined (3.10.5.) and the Tegaderm sweat patch removed and placed in a 10 ml syringe (Becton, Dickson & company, San Augustin del Guadalix, Spain) The sample was then transferred to a 2 ml micro tube.
(Sarstedt, Germany) and immediately frozen at -18°C for analysis (HFL Sport Science Ltd., Fordham, Cambridgeshire). Defrosted samples were analysed in a Beckman chemistry analyser (AU640, Beckman coulter INC, Japan) via an ion selective electrode. The ion selective electrode measured the potential of a specific ion in solution against a stable reference electrode of constant potential. The potential difference between the electrodes depended upon the activity of the ion in solution and was directly related to concentration of that ion (HFL Sport Science Ltd., Fordham, Cambridgeshire).

6.3.3.2. Acclimation

All participants completed 12 days of HA or training, 60 min.d⁻¹ at 50% VO₂peak, in the appropriate environmental conditions depending on the group they had been assigned to. Prior to main trials all participants were requested to follow standard pre-trial preparation guidelines for diet, exercise and hydration (section 3.4.) and all heat acclimation sessions were conducted at the same time of day for each participant.

On arrival to the laboratory, participants’ baseline measures were obtained as nude body mass, hydration status, resting rectal temperature and heart rate were measured in every session after 15 minutes rest (section 3.8. & 3.10.). During all acclimation and training sessions rectal temperature, heart rate, thermal sensation and RPE were recorded in the final 20 s of every fifth minute. On day 1, 6 and 12 of acclimation and training, a 20 ml venous blood sample was obtained pre and post exercise for analysis of aldosterone, cortisol, haemoglobin and haematocrit (section 3.11., 3.12.1 & 3.12.2).

6.3.4. Statistical analysis

Data were checked for normality and sphericity and adjusted where necessary using the Huynh-Feldt method. In addition, if Levene’s test indicated the assumption of homogeneity of variance was violated data were log transformed. Physiological responses to twelve days PA, TA or TG and intermittent-sprint exercise were assessed using a three way mixed
ANOVA (regime*day*time) with repeated measures on two factors (day*time). Peak physiological responses during acclimation and intermittent-sprinting were assessed using a two-way mixed ANOVA (regime*day) with repeated measures on one factor (day). Non parametric perceptual measures were assessed using a Kruskal Wallis test with Wilcoxon signed rank test. All data were analysed using SPSS (version 20.0) and are reported as mean ± standard deviation. Statistical significance was accepted as $P < 0.05$. Effect sizes were calculated as described in section 3.13.

6.4. Results

6.4.1. Physiological and perceptual responses to twelve days of heat acclimation or training

6.4.1.1. Body mass and hydration

Pre-exercise body mass and hydration status were not different between days in any group ($P > 0.05$), indicating participants were in a similar physiological state prior to exercise on day one, six and twelve. Venous blood for hormone analysis and sweat samples was collected from six participants in each group only due to material constraints.

6.4.1.2 Rectal temperature

Resting $T_{re}$ decreased over time (main effect: $F_{(2,42)} = 10.995, P < 0.001, \eta_p^2 = 0.343$) but, despite reductions of 0.3, 0.2 and 0.3°C in TA, PA and TG, respectively, no significant interaction (main effect regime*day: $F_{(4,42)} = 2.115, P = 0.096, \eta_p^2 = 0.168$, Table 6.1.) resulted. Exercise $T_{re}$ was reduced across days (main effect day: $F_{(2,42)} = 9.127, P = 0.001, \eta_p^2 = 0.303$) and there was a significant interaction (main effect regime*day: $F_{(4,42)} = 3.502, P = 0.018, \eta_p^2 = 0.250$). Exercise $T_{re}$ in TA and TG was reduced 0.45 and 0.33°C on day twelve compared to one (all $P$ values < 0.05), but exercise $T_{re}$ in PA was not different ($P > 0.05$),
consistent with the elevated heat stress of the progressive protocol. During exercise $T_{re}$ increased in all conditions (main effect: $F_{(11,231)}$ 185.957, $P < 0.001$, $\eta_p^2 = 0.899$), was significantly higher in PA and TA compared to TG on day twelve from 40 minutes onwards and significantly higher in TA compared to TG on day one from 50 minutes onwards (main effect day*time*regime: $F_{(44,462)}$ 3.598, $P = 0.003$, $\eta_p^2 = 0.255$). Peak exercise $T_{re}$ was reduced by TA (0.53°C) and TG (0.4°C) on day twelve (Table 6.1.). In PA, consistent with elevated heat stress, peak $T_{re}$ on day twelve was 0.3°C higher compared to day six (main effect regime*day: $F_{(4,42)}$ 5.255 $P = 0.002$, $\eta_p^2 = 0.334$; Post Hoc, all $P$ values < 0.05, Table 6.1.).

6.4.1.3 Heart rate

Resting heart rate on day one, six and twelve was reduced (main effect: $F_{(2,42)}$ 4.994, $P < 0.001$, $\eta_p^2 = 0.428$) and there was a significant interaction (main effect regime*day: $F_{(4,42)}$ 2.681, $P = 0.045$, $\eta_p^2 = 0.211$). Resting heart rate ($b.min^{-1}$) in TA decreased 11 $b.min^{-1}$ by day six ($P < 0.05$) and no further reductions were observed by day twelve (Table 6.1.). Similarly, resting heart rate in PA was reduced 13 $b.min^{-1}$ by day six (76 ± 9 vs. 63 ± 6, $P < 0.05$) and did not decline any further by day twelve (Table 6.1.). In TG resting heart rate was not different over days one to twelve ($P > 0.05$). Exercise heart rate was reduced in TA, PA and TG by 15 $b.min^{-1}$, 6 $b.min^{-1}$ and 14 $b.min^{-1}$ respectively from day one to twelve, but was not different across days within regimes (main effect regime*day: $F_{(4,42)}$ 1.655, $P = 0.180$, $\eta_p^2 = 0.142$). Peak exercise heart rate was reduced across days (main effect day: $F_{(2,42)}$ 17.044, $P < 0.001$, $\eta_p^2 = 0.460$) and there was a significant interaction (main effect regime*day: $F_{(4,42)}$ 3.006 $P = 0.029$, $\eta_p^2 = 0.231$). TA reduced peak heart rate 12 and 18 $b.min^{-1}$ by day six and twelve, respectively (all $P$ values < 0.05). In PA peak heart rate was not different across days, consistent with the elevated heat stress (all $P$ values > 0.05) and in TG a significant reduction of 18 $b.min^{-1}$ in peak heart rate occurred by day twelve (all $P$ values < 0.05). On day one peak heart rate was significantly higher in TA compared to TG and on day twelve, both TA and PA produced higher end exercise heart rates (all $P$ values < 0.05, Table 6.1.).
Table 6.1. Physiological responses to twelve days of TA, PA and TG.

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 6</th>
<th>Day 12</th>
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</thead>
<tbody>
<tr>
<td><strong>Resting HR (b.min⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>76 ± 8</td>
<td>65 ± 7*</td>
<td>65 ± 6*</td>
</tr>
<tr>
<td>PA</td>
<td>76 ± 9</td>
<td>63 ± 6*</td>
<td>64 ± 5*</td>
</tr>
<tr>
<td>TG</td>
<td>71 ± 28</td>
<td>74 ± 25</td>
<td>69 ± 17</td>
</tr>
<tr>
<td><strong>Exercise HR (b.min⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>163 ± 14</td>
<td>155 ± 13</td>
<td>148 ± 12</td>
</tr>
<tr>
<td>PA</td>
<td>162 ± 19</td>
<td>156 ± 15</td>
<td>156 ± 14</td>
</tr>
<tr>
<td>TG</td>
<td>147 ± 19</td>
<td>145 ± 19</td>
<td>133 ± 11</td>
</tr>
<tr>
<td><strong>Peak HR (b.min⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>179 ± 7</td>
<td>167 ± 8*</td>
<td>162 ± 9*#</td>
</tr>
<tr>
<td>PA</td>
<td>173 ± 18</td>
<td>167 ± 12</td>
<td>170 ± 11†</td>
</tr>
<tr>
<td>TG</td>
<td>156 ± 18#</td>
<td>149 ± 16</td>
<td>138 ± 10**†</td>
</tr>
<tr>
<td><strong>Resting T_re (°C)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>37.2 ± 0.4</td>
<td>36.9 ± 0.4</td>
<td>36.9 ± 0.3</td>
</tr>
<tr>
<td>PA</td>
<td>37.2 ± 0.3</td>
<td>37.0 ± 0.6</td>
<td>37.0 ± 0.4</td>
</tr>
<tr>
<td>TG</td>
<td>37.2 ± 0.4</td>
<td>37.1 ± 0.3</td>
<td>36.9 ± 0.4</td>
</tr>
<tr>
<td><strong>Exercise T_re (°C)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>38.2 ± 0.7</td>
<td>37.9 ± 0.7</td>
<td>37.8 ± 0.7*</td>
</tr>
<tr>
<td>PA</td>
<td>37.9 ± 0.5</td>
<td>37.8 ± 0.4</td>
<td>37.9 ± 0.5</td>
</tr>
<tr>
<td>TG</td>
<td>37.9 ± 0.5</td>
<td>37.8 ± 0.5</td>
<td>37.6 ± 0.4**+</td>
</tr>
<tr>
<td><strong>Peak T_re (°C)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>39.0 ± 0.7</td>
<td>38.6 ± 0.7</td>
<td>38.5 ± 0.6*</td>
</tr>
<tr>
<td>PA</td>
<td>38.4 ± 0.6</td>
<td>38.3 ± 0.3</td>
<td>38.7 ± 0.3†</td>
</tr>
<tr>
<td>TG</td>
<td>38.3 ± 0.3</td>
<td>38.2 ± 0.3</td>
<td>37.9 ± 0.2**†</td>
</tr>
<tr>
<td><strong>Sweat rate (L.hr⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>0.91 ± 0.15</td>
<td>1.21 ± 0.29*</td>
<td>1.32 ± 0.28*</td>
</tr>
<tr>
<td>PA</td>
<td>0.95 ± 0.37</td>
<td>1.00 ± 0.35</td>
<td>1.31 ± 0.59**†</td>
</tr>
<tr>
<td>TG</td>
<td>0.62 ± 0.26</td>
<td>0.71 ± 0.63#</td>
<td>0.63 ± 0.21#</td>
</tr>
<tr>
<td><strong>Haemoglobin (g.dl⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>16.6 ± 1.6</td>
<td>14.6 ± 0.9</td>
<td>16.0 ± 1.7</td>
</tr>
<tr>
<td>PA</td>
<td>14.9 ± 0.8</td>
<td>14.3 ± 10</td>
<td>14.7 ± 1.6</td>
</tr>
<tr>
<td>TG</td>
<td>16.1 ± 1.9</td>
<td>15.1 ± 1.1</td>
<td>15.5 ± 1.7</td>
</tr>
<tr>
<td><strong>Haematocrit</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>0.48 ± 0.03</td>
<td>0.43 ± 0.03*</td>
<td>0.43 ± 0.03*</td>
</tr>
<tr>
<td>PA</td>
<td>0.45 ± 0.02†</td>
<td>0.44 ± 0.02</td>
<td>0.44 ± 0.03</td>
</tr>
<tr>
<td>TG</td>
<td>0.47 ± 0.03</td>
<td>0.45 ± 0.02</td>
<td>0.44 ± 0.03*</td>
</tr>
<tr>
<td><strong>PV change (%)</strong></td>
<td>Day 1-6</td>
<td>Day 6-12</td>
<td>Day 1-12</td>
</tr>
<tr>
<td>TA</td>
<td>13.4 ± 10.9</td>
<td>-5.23 ± 8.7</td>
<td>8.9 ± 14.0</td>
</tr>
<tr>
<td>PA</td>
<td>9.2 ± 5.7</td>
<td>1.6 ± 5.9</td>
<td>10.9 ± 8.4</td>
</tr>
<tr>
<td>TG</td>
<td>7.5 ± 14.4</td>
<td>-2.2 ± 16.3</td>
<td>7.6 ± 8.8</td>
</tr>
</tbody>
</table>

Data are mean (± S.D.) *Significant difference from day 1.†Significant difference from day 6. **Significant difference between TG and PA (all P values < 0.05). †Significant difference between TA and TG. ‡Significant difference between PA and TA.
6.4.1.4. Aldosterone and cortisol

Plasma aldosterone changes were not significantly different within or across days and were not different between regimes (main effect day*time*regime: $F_{(4,28)} = 2.279$, $P = 0.086$, $\eta_p^2 = 0.246$, Figure 6.2.). Similarly, cortisol concentration was not different within or across days and was not different between regimes (main effect day*time*regime: $F_{(4,28)} = 2.524$, $P = 0.063$, $\eta_p^2 = 0.265$, Figure 6.3.).

Figure 6.2. Mean (± S.D.) Plasma aldosterone (pg.ml^{-1}) pre and post exercise on day 1, 6 and 12 in TA, PA and TG.
Figure 6.3. Mean (± S.D.) Plasma cortisol (ng.ml\(^{-1}\)) pre and post exercise on day 1, 6 and 12 in TA, PA and TG.

6.4.1.5. Sweat rate and sweat sodium, potassium and chloride

Sweat rate was significantly different across days (main effect day: F\(_{(2,42)}\) 7.962, P = 0.001, \(\eta^2_p = 0.275\)) and a significant interaction occurred (main effect regime*day: F\(_{(4,42)}\) 3.004, P = 0.029, \(\eta^2_p = 0.222\)). Sweat rate was significantly higher during TA compared to TG on day six and twelve and higher in PA compared to TG on day twelve (all P values < 0.05, Table 6.1.). No significant difference was observed in sweat rate between PA and TA (all P values > 0.05, Figure 6.4.). Within groups, sweat rate in TA was significantly higher from day six and in PA from day twelve compared to day one (all P values < 0.05, Table 6.1., Figure 6.4.). No changes were observed in sweat rate in TG across days (all P values > 0.05, Figure 6.4.). There was no effect of day (main effect day: F\(_{(2,30)}\) 2.149, P = 0.134, \(\eta^2_p =\)
0.150, Table 6.2.) on sweat sodium during acclimation. Similarly, potassium concentration did not vary with the day (main effect day: $F_{(2,30)} = 2.362$, $P = 0.112$, $\eta_p^2 = 0.136$, Table 6.2.) and there was no interaction (main effect day*regime: $F_{(4,30)} = 0.604$, $P = 0.663$, $\eta_p^2 = 0.075$, Table 6.2.). Finally, chloride concentration was not different over days (main effect day: $F_{(2,30)} = 0.135$, $P = 0.875$, $\eta_p^2 = 0.009$, Table 6.2) and there was no significant interaction (main effect day*regime: $F_{(4,30)} = 0.448$, $P = 0.773$, $\eta_p^2 = 0.056$, Table 6.2.).

Figure 6.4. Mean (± S.D.) sweat rate (L.h⁻¹) in TA, PA and TG. *Significant difference from day 1. †Significant difference from TA day 6. #Significant difference between TA and TG. * Significant difference between TG and PA (P < 0.05).
Table 6.2. Sweat sodium, potassium and chloride during TA, PA and TG.

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 6</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (mmol.L⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>90.9 ± 43.9</td>
<td>80.1 ± 34</td>
<td>76.9 ± 31</td>
</tr>
<tr>
<td>PA</td>
<td>91.8 ± 36.7</td>
<td>81.3 ± 5.8</td>
<td>76.3 ± 20</td>
</tr>
<tr>
<td>TG</td>
<td>85.4 ± 20.3</td>
<td>93.2 ± 18.6</td>
<td>78.2 ± 17.7</td>
</tr>
<tr>
<td>Potassium (mmol.L⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>7.1 ± 2.4</td>
<td>6.9 ± 1.4</td>
<td>5.78 ± 1.4</td>
</tr>
<tr>
<td>PA</td>
<td>7.3 ± 2.8</td>
<td>5.9 ± 1.4</td>
<td>6.2 ± 1.1</td>
</tr>
<tr>
<td>TG</td>
<td>6.2 ± 1.9</td>
<td>6.3 ± 2</td>
<td>5.4 ± 1.2</td>
</tr>
<tr>
<td>Chloride (mmol.L⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>86.7 ± 50</td>
<td>86.8 ± 30.1</td>
<td>95.2 ± 44</td>
</tr>
<tr>
<td>PA</td>
<td>85.4 ± 35.7</td>
<td>74.1 ± 23.4</td>
<td>70.7 ± 19.2</td>
</tr>
<tr>
<td>TG</td>
<td>78.2 ± 17.6</td>
<td>85.2 ± 17.1</td>
<td>79.6 ± 27.1</td>
</tr>
</tbody>
</table>

Data are mean (± S.D.).

6.4.1.6. Plasma volume haemoglobin and haematocrit

Haemoglobin was not different between or within groups over time (main effect day*regime: $F_{(2,40)} = 0.781, P = 0.544, \eta^2_p = 0.072$, Table 6.1.). Haematocrit varied across days and was significantly different between and within regimes (main effect day*regime: $F_{(4,40)} = 4.902, P = 0.003, \eta^2_p = 0.329$, Table 6.1.). Haematocrit was different between TA and PA on day 1 ($P < 0.05$) and was significantly reduced during TA by day six and during TG by day twelve (All $P$ values < 0.05). Large inter-individual variation was observed in plasma volume change in all groups. Plasma volume tended to be increased 13.5 ± 10.9 and 9.2 ± 5.7% in TA and PA respectively from day one to six. From day six to twelve plasma volume tended to decrease in TA (-5.2 ± 8.7%) and increase in PA (1.6 ± 5.9%). TG tended to increase PV over day one to six (7.5 ± 14.2%), but plasma volume was reduced from day six to twelve (-2.24 ± 16.3). Due to the large inter-individual differences there were no significant changes within or between groups (main effect day*regime: $F_{(2,40)} = 0.515, P = 0.725, \eta^2_p = 0.049$, Table 6.1.).
6.4.1.7. RPE, T\textsubscript{sen} and PSI

There was a main effect of day on RPE (main effect day: F\textsubscript{(2,42)} 17.355, P < 0.001, \eta\textsuperscript{2} = 0.452), but no significant interaction (main effect regime*day: F\textsubscript{(4,42)} 1.387, P = 0.255, \eta\textsuperscript{2} = 0.117). RPE increased during exercise (main effect time: F\textsubscript{(11,231)} 88.728, P < 0.001, \eta\textsuperscript{2} = 0.809) and there was a significant interaction (main effect regime*time: F\textsubscript{(22,231)} 3.875, P = 0.013, \eta\textsuperscript{2} = 0.270). The change in RPE over time on day one, six and twelve was not dependent on the regime (main effect day*time*regime: F\textsubscript{(44,462)} 1.069, P = 0.358, \eta\textsuperscript{2} = 0.092). There was a significant main effect of day (main effect day: F\textsubscript{(2,42)} 18.467, P < 0.001, \eta\textsuperscript{2} = 0.468) on peak RPE, but no significant interaction (main effect regime*day: F\textsubscript{(4,42)} 1.547, P = 0.206, \eta\textsuperscript{2} = 0.128).

Thermal sensation was significantly different over time within and between groups (main effect; Chi squared = 1.082, P < 0.001, ES = 0.477). T\textsubscript{sen} was significantly lower in TG and TA by day six and twelve respectively and was significantly higher in PA by day six compared to day one, but was also significantly elevated on day twelve compared to six (all P values < 0.05, Table 6.3.). Between groups, T\textsubscript{sen} was higher in both acclimation regimes compared to TG on all days, but was only different between acclimation regimes on day twelve when T\textsubscript{sen} in PA was significantly higher (all P values < 0.05, Table 6.3.). Peak T\textsubscript{sen} was different over time and between groups (main effect; Chi squared = 35.169, P < 0.001, ES = 0.495). Peak T\textsubscript{sen} was significantly lower in TA on day twelve and in PA was significantly higher on day six and twelve compared to day one (all P values < 0.05, Table 6.3.). Peak T\textsubscript{sen} was not different in TG across days (all P values > 0.05, Table 6.3.). Between conditions peak T\textsubscript{sen} was significantly higher in PA compared to TG on day six and twelve and significantly higher in PA compared to TA on day twelve only (all P values < 0.05). No differences were observed between TG and TA across days (all P values > 0.05). PSI was different across days (main effect day: F\textsubscript{(2,42)} 5.388, P = 0.008, \eta\textsuperscript{2} = 0.204), but there was no significant interaction (main effect regime*day: F\textsubscript{(4,42)} 1.954 P = 0.119, \eta\textsuperscript{2} = 0.157) and there was no difference within or between groups in peak PSI.
Table 6.3. PSI, RPE and $T_{sen}$ during twelve days of TA, PA and TG.

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Day 6</th>
<th>Day 12</th>
<th>Day 1</th>
<th>Day 6</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSImean</td>
<td>PSI peak</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>6 ± 1</td>
<td>5 ± 1</td>
<td>7 ± 1</td>
<td>7 ± 2</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>PA</td>
<td>6 ± 1</td>
<td>6 ± 1</td>
<td>8 ± 2</td>
<td>8 ± 1</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>TG</td>
<td>5 ± 1</td>
<td>4 ± 1</td>
<td>7 ± 1</td>
<td>6 ± 1</td>
<td>5 ± 1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>RPEmean</th>
<th>RPEpeak</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA</td>
<td>15 ± 3</td>
</tr>
<tr>
<td>PA</td>
<td>14 ± 3</td>
</tr>
<tr>
<td>TG</td>
<td>14 ± 2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>$T_{sen}$mean</th>
<th>$T_{sen}$peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA</td>
<td>6.7 ± 1.0</td>
</tr>
<tr>
<td>PA</td>
<td>5.8 ± 0.6</td>
</tr>
<tr>
<td>TG</td>
<td>5.5 ± 1.14#</td>
</tr>
</tbody>
</table>

Data are mean (± S.D.). * Significant difference from day 1. †Significant difference from day 6. ‡ Significant difference between PA and TG. †Significant difference between TA and TG. Significance difference between PA and TA (all P values < 0.05).

6.4.2. Performance, physiological and perceptual responses to intermittent-sprint exercise in 33°C, 50% rh pre-post twelve days of PA, TA or TG.

6.4.2.1. Participants

Pre-exercise body mass and hydration status were not different between CISP 1 and 2 in any group (all main effect P values > 0.05) indicating participants were in a similar physiological state prior to intermittent-sprint exercise in the heat. One participant failed to complete CISP one due to volitional exhaustion after fifteen sprints (30 minutes) and was removed from the environmental chamber.

6.4.2.2. PPO and work done

There was a main effect of day on PPO ($F_{(1,20)}$ 9.016, $P = 0.007$, $\eta^2_p = 0.311$) and there was a significant interaction (main effect regime*day: $F_{(2,20)}$ 4.052, $P = 0.033$, $\eta^2_p = 0.288$, Table 6.4). PPO was significantly higher in TA in CISP 2 compared to CISP 1 ($P = 0.002$, Table 6.4), but was not different in PA or TG (all P values > 0.05). There was a main effect of time on PPO ($F_{(19,380)}$ 8.418, $P < 0.001$, $\eta^2_p = 0.296$) and there was a significant interaction of day and regime (main effect regime*day: $F_{(38,380)}$ 3.828, $P < 0.001$, $\eta^2_p = 0.277$). PPO was
significantly higher in CISP 2 compared to CISP 1 after TA from sprint eight to twenty (all P values < 0.05, Figure 6.5.). There was no significant difference in PPO in PA and TG (all P values > 0.05, Figure 6.5.).

Table 6.4. PPO (W) and Work Done (J) during CISP 1 and 2 in TA, PA and TG.

<table>
<thead>
<tr>
<th></th>
<th>CISP 1 (Pre intervention)</th>
<th>CISP 2 (Post intervention)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PPO (W)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>931 ± 207</td>
<td>1062 ± 133*</td>
</tr>
<tr>
<td>PA</td>
<td>963 ± 187</td>
<td>990 ± 143</td>
</tr>
<tr>
<td>TG</td>
<td>967 ± 255</td>
<td>971 ± 268</td>
</tr>
<tr>
<td><strong>Work Done (J)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>2567 ± 623</td>
<td>2973 ± 421</td>
</tr>
<tr>
<td>PA</td>
<td>2687 ± 581</td>
<td>2780 ± 431</td>
</tr>
<tr>
<td>TG</td>
<td>2705 ± 709</td>
<td>2725 ± 763</td>
</tr>
</tbody>
</table>

Data are mean (± S.D.). *Significant difference from CISP 1 (P < 0.05)
Figure 6.5. Mean (± S.D.) PPO during CISP 1 and 2 in TA, PA and TG.*Significant difference from CISP 1 (P < 0.05).

Work done was different from CISP 1 to 2 (main effect day: $F_{(1,20)}$ 9.525, $P = 0.006$, $\eta^2_p = 0.323$) and there was a significant interaction (main effect regime*day: $F_{(2,20)}$ 3.148, $P = 0.041$, $\eta^2_p = 0.273$, Table 6.4.). Mean work done was significantly higher in CISP 2 compared to CISP 1 in TA only ($P = 0.002$, Table 6.4.). There was an effect of time on work done during the CISP ($F_{(19,380)}$ 9.506, $P < 0.001$, $\eta^2_p = 0.322$) and there was a significant interaction of day and regime (main effect regime*day*time: $F_{(38,380)}$ 3.810, $P < 0.001$, $\eta^2_p = 0.276$). Work done in TA was significantly higher in CISP 2 compared to CISP 1 from sprint ten onwards (all $P$ values < 0.05, Figure 6.6.). No significant difference was observed in work done during the CISPs in PA or TG (all $P$ values > 0.05, Figure 6.6.).
Figure 6.6. Mean (± S.D.) Work Done during CISP 1 and 2 in TA, PA and TG.*Significant difference from CISP 1 (P < 0.05).
Table 6.5. Physiological responses during CISP 1 and 2 in TA, PA and TG.

<table>
<thead>
<tr>
<th></th>
<th>CISP 1 (Pre intervention)</th>
<th>CISP 2 (Post intervention)</th>
<th>Change</th>
</tr>
</thead>
<tbody>
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<td><strong>Resting HR (b.min⁻¹)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>79 ± 11</td>
<td>70 ± 9</td>
<td>-9</td>
</tr>
<tr>
<td>PA</td>
<td>73 ± 14</td>
<td>62 ± 9</td>
<td>-11</td>
</tr>
<tr>
<td>TG</td>
<td>77 ± 23</td>
<td>72 ± 19</td>
<td>-5</td>
</tr>
<tr>
<td><strong>Exercise HR (b.min⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>174 ± 23</td>
<td>155 ± 20*</td>
<td>-19</td>
</tr>
<tr>
<td>PA</td>
<td>169 ± 18</td>
<td>153 ± 19*</td>
<td>-16</td>
</tr>
<tr>
<td>TG</td>
<td>163 ± 23</td>
<td>158 ± 19</td>
<td>-5</td>
</tr>
<tr>
<td><strong>Peak HR (b.min⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>185 ± 24</td>
<td>162 ± 19</td>
<td>23</td>
</tr>
<tr>
<td>PA</td>
<td>181 ± 15</td>
<td>164 ± 15</td>
<td>17</td>
</tr>
<tr>
<td>TG</td>
<td>172 ± 21</td>
<td>166 ± 14</td>
<td>6</td>
</tr>
<tr>
<td><strong>Resting T_{re} (°C)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>37.7 ± 0.3</td>
<td>37.4 ± 0.3</td>
<td>-0.3</td>
</tr>
<tr>
<td>PA</td>
<td>37.6 ± 0.3</td>
<td>37.2 ± 0.2</td>
<td>-0.4</td>
</tr>
<tr>
<td>TG</td>
<td>37.5 ± 0.4</td>
<td>37.5 ± 0.3</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>Exercise T_{re} (°C)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>38.4 ± 0.6</td>
<td>38.1 ± 0.6*</td>
<td>-0.3</td>
</tr>
<tr>
<td>PA</td>
<td>38.3 ± 0.6</td>
<td>37.8 ± 0.5*</td>
<td>-0.5</td>
</tr>
<tr>
<td>TG</td>
<td>38.1 ± 0.5</td>
<td>37.9 ± 0.4</td>
<td>-0.2</td>
</tr>
<tr>
<td><strong>Peak T_{re} (°C)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>39.2 ± 0.6</td>
<td>38.7 ± 0.6*</td>
<td>0.4</td>
</tr>
<tr>
<td>PA</td>
<td>38.9 ± 0.5</td>
<td>38.2 ± 0.3*</td>
<td>-0.7</td>
</tr>
<tr>
<td>TG</td>
<td>38.6 ± 0.3</td>
<td>37.4 ± 0.3*</td>
<td>-0.2</td>
</tr>
<tr>
<td><strong>T_{aw} (°C)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>35.73 ± 1.19</td>
<td>34.78 ± 0.93*</td>
<td>-0.95</td>
</tr>
<tr>
<td>PA</td>
<td>35.33 ± 0.62</td>
<td>34.63 ± 0.74*</td>
<td>-0.70</td>
</tr>
<tr>
<td>TG</td>
<td>35.61 ± 1.04</td>
<td>35.86 ± 0.75*</td>
<td>+0.25</td>
</tr>
<tr>
<td><strong>BHC (J)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>9855 ± 1232</td>
<td>9829 ± 1274</td>
<td>26</td>
</tr>
<tr>
<td>PA</td>
<td>9699 ± 1202</td>
<td>9607 ± 1102</td>
<td>-92</td>
</tr>
<tr>
<td>TG</td>
<td>9839 ± 1763</td>
<td>9822 ± 1776</td>
<td>-17</td>
</tr>
<tr>
<td><strong>Peak BHC (J)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>9790 ± 1393</td>
<td>9644 ± 1292*</td>
<td>-146</td>
</tr>
<tr>
<td>PA</td>
<td>10458 ± 1088</td>
<td>10376 ± 1038*</td>
<td>-82</td>
</tr>
<tr>
<td>TG</td>
<td>10265 ± 2061</td>
<td>10153 ± 2091</td>
<td>-112</td>
</tr>
<tr>
<td><strong>Sweat rate (l.hr⁻¹)</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>TA</td>
<td>1.38 ± 0.49</td>
<td>1.65 ± 0.46</td>
<td>+0.27</td>
</tr>
<tr>
<td>PA</td>
<td>1.37 ± 0.64</td>
<td>1.49 ± 0.57</td>
<td>+0.12</td>
</tr>
<tr>
<td>TG</td>
<td>1.13 ± 0.41</td>
<td>1.23 ± 0.39</td>
<td>+0.10</td>
</tr>
<tr>
<td><strong>Blood [lactate] (mM)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>6.2 ± 2.5</td>
<td>5.2 ± 1.9</td>
<td>-1.0</td>
</tr>
<tr>
<td>PA</td>
<td>4.9 ± 1.3</td>
<td>3.4 ± 1.0</td>
<td>-1.5</td>
</tr>
<tr>
<td>TG</td>
<td>3.9 ± 1.1</td>
<td>3.4 ± 1.1</td>
<td>-0.5</td>
</tr>
<tr>
<td><strong>VO₂ (l.min⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>2.41 ± 0.54</td>
<td>2.16 ± 0.44</td>
<td>-0.25</td>
</tr>
<tr>
<td>PA</td>
<td>2.30 ± 0.45</td>
<td>2.07 ± 0.45</td>
<td>-0.23</td>
</tr>
<tr>
<td>TG</td>
<td>2.11 ± 0.45</td>
<td>1.90 ± 0.40</td>
<td>-0.21</td>
</tr>
<tr>
<td><strong>Heat production (W.m⁻²)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>440 ± 97</td>
<td>392 ± 85</td>
<td>-48</td>
</tr>
<tr>
<td>PA</td>
<td>398 ± 71</td>
<td>356 ± 74</td>
<td>-42</td>
</tr>
<tr>
<td>TG</td>
<td>383 ± 60</td>
<td>350 ± 55</td>
<td>-33</td>
</tr>
</tbody>
</table>

Data are mean (± S.D.) *Significant difference from day 1. †Significant difference from day 6. ‡Significant difference between TG and PA (all P values < 0.05). §Significant difference between TA and TG. ¶Significant difference between PA and TA.
### 6.4.2.3. Heart rate

Resting heart rate decreased over time (main effect: $F_{(1,21)} = 30.000$, $P < 0.001$, $\eta^2_p = 0.588$) but, despite reductions of 9, 11 and 5 b.min$^{-1}$ in TA, PA and TG respectively no significant interaction (main effect regime*time: $F_{(2,21)} = 1.415$, $P = 0.265$, $\eta^2_p = 0.119$, Table 6.5.) resulted. Exercise heart rate during intermittent sprinting was reduced from CISP 1 to CISP 2 (main effect day: $F_{(1,20)} = 38.045$, $P < 0.001$, $\eta^2_p = 0.664$, Table 6.5.) and there was a significant interaction (main effect regime*day: $F_{(2,20)} = 4.119$, $P = 0.031$, $\eta^2_p = 0.282$). PA and TA reduced mean exercise heart rate (all P values < 0.001, Table 6.5.), but there was no effect of TG (all P values > 0.05). Exercise heart rate increased during intermittent-sprinting (main effect time: $F_{(19,38)} = 70.106$, $P < 0.001$, $\eta^2_p = 0.770$) and there was a significant interaction (main effect regime*day*time: $F_{(38,380)} = 1.572$, $P = 0.019$, $\eta^2_p = 0.130$). TA reduced exercise heart rate from sprint two onwards in CISP 2 compared to CISP 1 (all P values < 0.05, Figure 6.7.). Similarly, PA reduced exercise heart rate across all sprints in CISP 2 compared to CISP 1 (all P values < 0.05, Figure 6.7.). After TG exercise heart rate was reduced only in the first two sprints in CISP 2 compared to CISP 1. Peak exercise heart rate was reduced over time (main effect: $F_{(1,21)} = 34.158$, $P < 0.001$, $\eta^2_p = 0.619$). While peak heart rate was lower in CISP 2 by 23, 17 and 6 b.min$^{-1}$ after TA PA and TG, respectively, there was no significant interaction (main effect regime*time: $F_{(2,21)} = 3.269$, $P = 0.058$, $\eta^2_p = 0.237$).
Figure 6.7. Mean (± S.D.) heart rate (b.min⁻¹) in CISP 1 and 2 following TA, PA and TG.

*Significant difference from CISP 1 (P < 0.05).

6.4.2.4 T<sub>re</sub>, T<sub>sk</sub>, T<sub>body</sub> and body heat content

Resting T<sub>re</sub> was reduced over time from CISP 1 to CISP 2 (main effect: F<sub>(1,21)</sub> 4.466, P = 0.047, η<sub>p</sub>² = 0.175) but, despite decreases of 0.3 and 0.4°C in TA and PA respectively, no significant interaction (main effect regime*time: F<sub>(2,21)</sub> 2.840, P = 0.081, η<sub>p</sub>² = 0.213, Table 6.5.) resulted. Exercise T<sub>re</sub> during CISP 2 was reduced compared to CISP 1 (main effect time: F<sub>(1,20)</sub> 40.691, P < 0.001, η<sub>p</sub>² = 0.670) and there was a significant interaction (main effect regime*day: F<sub>(2,20)</sub> 4.314, P = 0.028, η<sub>p</sub>² = 0.301). Mean exercise T<sub>re</sub> during CISP 2 was reduced 0.3 and 0.5°C by TA and PA, respectively (all P values < 0.05, Table 6.5.), but there was no reduction after TG (P < 0.05). T<sub>re</sub> increased during intermittent sprinting in the heat during CISP 1 and 2 (main effect time: F<sub>(19,380)</sub> 325.286, P < 0.001, η<sub>p</sub>² = 0.942) and there was a significant interaction effect (main effect regime*day*time: F<sub>(38,380)</sub> 1.657, P = 0.010, η<sub>p</sub>² = 0.142, Figure 6.8.). Both TA and PA reduced T<sub>re</sub> from sprint one to twenty
during CISP 2 compared to CISP 1 (all P values < 0.05, Figure 6.8.) but there was no
significant effect of TG (all P values > 0.05). Peak exercise $T_{re}$ was also reduced from CISP
1 to 2 (main effect time: $F_{(1,21)}$ 39.553, $P < 0.001$, $\eta_p^2 = 0.653$) and there was a significant
interaction (main effect regime*time: $F_{(2,21)}$ 3.493, $P = 0.049$, $\eta_p^2 = 0.250$). Peak $T_{re}$ during
intermittent sprinting was significantly reduced by all interventions (all P values < 0.05,
Table 6.5.).

![Figure 6.8. Mean (± S.D.) $T_{re}$ (°C) in CISP 1 and 2 following TA, PA and TG.*Significant
difference from CISP 1 ($P < 0.05$).](image)

$T_{sk}$ was reduced during CISP 2 compared to CISP 1 (main effect day: $F_{(1,20)}$ 9.106, $P = 0.007$,
$\eta_p^2 = 0.313$) and there was a significant interaction (main effect regime*day: $F_{(2,20)}$ 3.913,
$P = 0.037$, $\eta_p^2 = 0.281$, Table 6.5.). Both TA and PA reduced $T_{sk}$ in CISP 2 compared to CISP
1 (all P values < 0.05), but there was no effect of TG ($P > 0.05$). Peak $T_{body}$ was reduced
over time (main effect day: $F_{(1,20)}$ 34.894, $P < 0.001$, $\eta_p^2 = 0.636$) and there was a
significant interaction (main effect regime*day: $F_{(2,20)}$ 4.938, $P = 0.018$, $\eta_p^2 = 0.331$). TA
and PA reduced peak $T_{\text{body}}$ (all P values < 0.05), but there was no effect of training (P > 0.05). Body heat content was reduced across days (main effect day: $F_{(1,20)} 9.106, P = 0.007, \eta_p^2 = 0.313$), but there was no significant interaction (main effect regime*day: $F_{(2,20)} 2.486, P = 0.109, \eta_p^2 = 0.199$, Table 6.5.). While body heat content was increased during exercise (main effect time: $F_{(19,38)} 15.738, P < 0.001, \eta_p^2 = 0.440$), there was no effect of day or regime (main effect regime*day*time: $F_{(38,380)} 0.960, P = 0.540, \eta_p^2 = 0.088$). Peak body heat content was however, reduced by both TA and PA in CISP 2 compared to CISP 1 (main effect regime*day: $F_{(2,20)} 4.657, P = 0.022, \eta_p^2 = 0.318$, post hoc all P values < 0.05, Table 6.5.), but there was no effect of TG (P > 0.05).

### 6.4.2.5. Blood lactate, VO$_2$ and metabolic heat production

Blood lactate was reduced in CISP 2 compared to CISP 1 (main effect day: $F_{(1,20)} 16.974, P = 0.001, \eta_p^2 = 0.485$), but there was no significant interaction (main effect regime*day: $F_{(2,20)} 2.379, P = 0.121, \eta_p^2 = 0.209$, Table 6.5.). Similarly, VO$_2$ was reduced in CISP 2 compared to CISP 1 (main effect day: $F_{(1,20)} 8.115, P = 0.010, \eta_p^2 = 0.289$), but there was no significant interaction (main effect regime*day: $F_{(2,20)} 0.347, P = 0.711, \eta_p^2 = 0.034$, Table 6.5.). Metabolic heat production was reduced by ~10% in CISP 2 compared to CISP 1 (main effect day: $F_{(1,21)} 7.338, P = 0.013, \eta_p^2 = 0.259$), but there was no significant interaction (main effect regime*day: $F_{(2,21)} 0.192, P = 0.827, \eta_p^2 = 0.018$).

### 6.4.2.6. Sweat rate and sweat sodium, potassium and chloride during intermittent-sprint exercise

Sweat rate was increased in CISP 2 compared to CISP 1 (main effect day: $F_{(1,20)} 8.486, P = 0.008, \eta_p^2 = 0.288$), but there was no significant interaction (main effect regime*day: $F_{(2,21)} 0.882, P = 0.429, \eta_p^2 = 0.078$, Table 6.5.). Sweat sodium, potassium and chloride were reduced between CISP 1 and 2 (main effect day: all P values < 0.05), but there was no significant interaction (main effect regime*day: all P values > 0.05, Table 6.6.)
Table 6.6. Sweat sodium, potassium and chloride during CISP 1 and 2 in TA, PA and TG.

<table>
<thead>
<tr>
<th></th>
<th>CISP 1 (Pre intervention)</th>
<th>CISP 2 (Post intervention)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sodium (mmol.L⁻¹)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>90.9 ± 43.9</td>
<td>76.5 ± 28.7</td>
</tr>
<tr>
<td>PA</td>
<td>92.4 ± 31.2</td>
<td>81.8 ± 20.8</td>
</tr>
<tr>
<td>TG</td>
<td>105.7 ± 30.1</td>
<td>96.2 ± 19.2</td>
</tr>
<tr>
<td><strong>Potassium (mmol.L⁻¹)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>7.1 ± 2.4</td>
<td>4.8 ± 0.9</td>
</tr>
<tr>
<td>PA</td>
<td>6.1 ± 1.9</td>
<td>5.3 ± 1.4</td>
</tr>
<tr>
<td>TG</td>
<td>6.0 ± 1.1</td>
<td>5.2 ± 0.72</td>
</tr>
<tr>
<td><strong>Chloride (mmol.L⁻¹)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>86.7 ± 50.1</td>
<td>69.5 ± 26.8</td>
</tr>
<tr>
<td>PA</td>
<td>82.7 ± 28.2</td>
<td>73.3 ± 19.8</td>
</tr>
<tr>
<td>TG</td>
<td>96.1 ± 27.3</td>
<td>87.9 ± 19.3</td>
</tr>
</tbody>
</table>

Data are mean (± S.D.)

6.4.2.7. RPE, T sen and PSI

RPE was reduced in CISP 2 compared to CISP 1 (main effect day: F(1,20) 26.324, P < 0.001, \( \eta_p^2 = 0.568 \)) and there was a significant interaction (main effect regime*day: F(2,20) 4.046, P = 0.033, \( \eta_p^2 = 0.288 \)). Both TA and PA reduced mean RPE during exercise in CISP 2 compared to CISP 1 (all P values < 0.05, Table 6.7.), but there was no effect of TG (P > 0.05). RPE increased during exercise (main effect time: F(2,20) 90.698, P < 0.001, \( \eta_p^2 = 0.819 \)), but there was no significant interaction of day or regime (main effect regime*day*time: F(2,20) 4.046, P = 0.033, \( \eta_p^2 = 0.288 \)). Peak RPE was not different between groups (main effect regime*day: F(8,80) 0.852, P = 0.560, \( \eta_p^2 = 0.079 \)).
Table 6.7. RPE, PSI and $T_{sen}$ during CISP 1 and 2 in TA, PA and TG.

<table>
<thead>
<tr>
<th></th>
<th>CISP 1 (Pre intervention)</th>
<th>CISP 2 (Post intervention)</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exercise RPE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>16 ± 3</td>
<td>14 ± 3*</td>
<td>-2</td>
</tr>
<tr>
<td>PA</td>
<td>15 ± 3</td>
<td>11 ± 2*</td>
<td>-4</td>
</tr>
<tr>
<td>TG</td>
<td>14 ± 3</td>
<td>13 ± 2</td>
<td>-1</td>
</tr>
<tr>
<td><strong>Peak RPE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>18 ± 3</td>
<td>15 ± 3</td>
<td>-3</td>
</tr>
<tr>
<td>PA</td>
<td>17 ± 2</td>
<td>13 ± 2</td>
<td>-4</td>
</tr>
<tr>
<td>TG</td>
<td>16 ± 3</td>
<td>15 ± 2</td>
<td>-1</td>
</tr>
<tr>
<td><strong>Exercise PSI</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>TA</td>
<td>5.9 ± 2.1</td>
<td>5.3 ± 1.4</td>
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</tr>
<tr>
<td>PA</td>
<td>6.4 ± 2.0</td>
<td>5.1 ± 1.6</td>
<td>-1.3</td>
</tr>
<tr>
<td>TG</td>
<td>5.9 ± 2.1</td>
<td>5.3 ± 1.4</td>
<td>-0.6</td>
</tr>
<tr>
<td><strong>Peak PSI</strong></td>
<td></td>
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</tr>
<tr>
<td>TA</td>
<td>7.8 ± 1.7</td>
<td>6.7 ± 0.9</td>
<td>-1.1</td>
</tr>
<tr>
<td>PA</td>
<td>8.7 ± 1.8</td>
<td>6.7 ± 1.3</td>
<td>-2</td>
</tr>
<tr>
<td>TG</td>
<td>7.8 ± 1.7</td>
<td>6.7 ± 0.9</td>
<td>-1.1</td>
</tr>
<tr>
<td><strong>Exercise $T_{sen}$</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>6.3 ± 1.4</td>
<td>5.5 ± 0.9*</td>
<td>-0.8</td>
</tr>
<tr>
<td>PA</td>
<td>6.2 ± 0.6</td>
<td>5.5 ± 0.6*</td>
<td>-0.7</td>
</tr>
<tr>
<td>TG</td>
<td>6.0 ± 0.7</td>
<td>6.1 ± 0.5*†</td>
<td>+0.1</td>
</tr>
<tr>
<td><strong>Peak $T_{sen}$</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>7.2 ± 0.9</td>
<td>6.1 ± 1.1</td>
<td>-1.1</td>
</tr>
<tr>
<td>PA</td>
<td>6.7 ± 0.4</td>
<td>5.9 ± 0.6*</td>
<td>-0.6</td>
</tr>
<tr>
<td>TG</td>
<td>6.4 ± 0.7</td>
<td>6.1 ± 0.5</td>
<td>-0.3</td>
</tr>
</tbody>
</table>

Data are mean (± S.D.). *Significant difference from day 1. †Significant difference from day 6. ‡Significant difference between TG and PA (all P values < 0.05). ††Significant difference between TA and TG. ‡‡Significant difference between PA and TA.

$T_{sen}$ was reduced during CISP 2 compared to CISP 1 (main effect: $\chi^2$ 39.075, $P < 0.001$, $ES = 0.186$). Between groups, $T_{sen}$ during CISP 2 was lower after TA and PA compared to TG (all $P$ values < 0.0167, Table 6.7.). Within groups, TA produced lower $T_{sen}$ in sprint four, twelve and twenty in CISP 2 (main effect; $P < 0.001$; Post Hoc, all $P$ values < 0.0167) and PA resulted in a lower $T_{sen}$ in CISP 2 (main effect: $P < 0.001$) from sprint twelve to twenty (all $P$ values < 0.0167). TG did not lower $T_{sen}$ during intermittent sprinting in the heat (all $P$ values > 0.05). Peak $T_{sen}$ was reduced after PA only ($P = 0.010$).

There was a main effect of day on PSI ($F_{(1,20)}$ 22.760, $P < 0.001$, $\eta^2 = 0.532$), but no significant interaction (main effect regime*day: $F_{(2,20)}$ 1.408, $P = 0.268$, $\eta^2 = 0.123$, Table 6.7.). PSI increased during CISP 1 and CISP 2 (main effect time: $F_{(19,38)}$ 309.496, $P < 0.001$, $\eta^2 = 0.939$) and there was a significant interaction of day and regime (main effect...
regime*day*time: $F_{(38,380)} = 1.632$, $P = 0.012$, $\eta_p^2 = 0.140$). In TA PSI was reduced in CISP 2 compared to CISP 1 from sprint two onwards (all $P$ values < 0.05) and in PA PSI was reduced from sprint four to twenty. After TG, PSI was only reduced in sprints nineteen and twenty during CISP 2. Peak PSI was not different between or within groups (main effect: $P > 0.05$).

6.4.2.8. Correlation analysis

During CISP 1 a strong negative correlation was observed for PPO and $T_{re}$ ($r = -0.938$, $P < 0.001$) in TA, but this correlation was reduced in CISP 2 ($r = -0.314$, $P = 0.178$). In PA, a strong negative correlation was also observed in CISP 1 ($r = -0.900$, $P < 0.001$), but this was not altered by progressive acclimation (CISP 2; $r = -0.928$, $P < 0.001$). In TG a moderate negative correlation was observed between PPO and $T_{re}$ during CISP 1 ($r = -0.598$, $P = 0.005$) and this relationship remained after twelve days of training ($r = -0.928$, $P < 0.001$).

6.5. Discussion

The objectives of this study were to examine physiological and perceptual adaptation to heat acclimation using a progressive compared to a traditional heat acclimation protocol and to investigate the subsequent effect of progressive heat acclimation on physiological, perceptual and performance responses during forty minutes of intermittent-sprint exercise in the heat. This is the first study to use a progressive heat acclimation model whereby physiological and thermal strain was elevated throughout the protocol by stepwise increases in heat stress on two occasions during acclimation. It was hypothesised a progressive heat acclimation model with stepwise increases in heat stress would, compared to a traditional model, evoke similar adaptations to the heat and prove a valid alternative heat acclimation protocol. In addition, it was hypothesised that progressive heat acclimation would improve perceptual and physiological responses
during intermittent sprinting in the heat and ameliorate heat induced decrements in performance.

The efficacy of progressive heat acclimation can be judged against its ability to evoke classic heat-induced, adaptive responses consistent with the heat acclimated phenotype, including decreased heart rate and core temperature during rest and exercise, increased sweat rate and increased plasma volume. Similar to isothermal models, the principle of progressive acclimation is to maintain physiological strain for the duration of acclimation to maximise adaptive responses. With the progressive model, such effect is, however, achieved by manipulation of heat stress with planned increases at specific points during acclimation to elevate physiological strain.

6.5.1. Physiological and perceptual responses during acclimation and training

Peak heart rate during exercise was reduced in TA and TG and, although not significant, mean heart rate tended to decline (-15 and -14 b.min\(^{-1}\) in TA and TG, respectively). In contrast, as expected with elevations in heat stress and consequently, physiological strain, peak heart during PA was unchanged and any tendency for decline in mean heart rate was small (-6 b.min\(^{-1}\)). At rest, heart rate was unchanged in TG, but significant bradycardia was evident by day six and persisted to day twelve in both acclimation regimes. Consistent with previous research, (Convertino et al., 1980; Patterson et al., 2004; Magalhaes et al., 2010; Garrett et al., 2011) these data suggest enhanced cardiovascular stability in response to exercise / an exercise-heat acclimation regime that may be explained by altered autonomic activity, hypervolaemia, or a centrally mediated effect.

Expansion of plasma volume is a frequently reported, rapidly occurring response to heat acclimation (Nielsen et al., 1993; Lorenzo et al., 2010; Garrett et al., 2012) of which 40% can be attributed to the thermal stimulus and 60% to exercise (Convertino et al., 1980). In
the current study, accepting that large inter-individual variation and variability in plasma volume change exists, (Weinstein et al, 1998; Racinais et al., 2012), and may have contributed to a non-significant expansion, a trend toward increased plasma volume was observed in TA, PA and TG (530 ± 444, 330 ± 229 and 324 ± 528 ml respectively). This was accompanied by a significant haemodilution (~ 12%, 4% and 6% in TA, PA and TG, respectively, Table 6.1.) and overall may have contributed to the aforementioned heart rate response. Elevated colloid-osmotic pressure in response to protein influx to the vascular compartment may explain plasma volume expansion, but lack of change in plasma protein content has been observed with acclimation (Nielsen et al., 1993, Garrett et al., 2009). Further, enhanced plasma volume may be mediated by increased retention of Na\(^+\) and water in the distal tubules in response to increased expression of aldosterone in acclimation (Nielsen et al., 1993; Garrett et al., 2012, 2014), but this is not supported by previous (Garrett et al., 2009), or the current work as, despite a trend toward increased aldosterone and sweat Na\(^+\), there was no significant change within or across days.

Similar to other work (Senay et al., 1976; Kirby et al., 1986; Armstrong et al., 1989; Nielsen et al., 1993; Aoyagi et al., 1995; Nielsen, 1998; Garrett et al., 2012), the trend towards increased plasma volume in the current study was essentially complete in all groups by day six, yet reductions in heart rate persisted until day twelve. This illustrates two important points; firstly, consistent with previous research, this suggests hypervolemia may not independently mediate decreased heart rate and may indicate a possible central effect whereby increased ventricular contractility contributes to improved cardiac efficiency during the acclimation process (Horowitz, 2002; Garrett et al., 2009). Secondly, Nielsen et al., (1993) and Patterson et al., (2004) demonstrated sustained expansion of plasma volume for twelve to twenty two days using an isocardiac / isothermal model of acclimation to maintain strain for the duration of the intervention. Therefore, given a similar aim to the progressive model used in this study of maximising the hypervolemic response the trend toward no change in plasma volume from day six to twelve, while consistent with the biphasic nature of heat acclimation, may highlight a
deficiency in the progressive heat acclimation model design. Specifically, it may signify that the initial starting WBGT (24.4°C) was too low and therefore two increases in heat stress (WBGT 27.7 and 32.0°C) were insufficient to provoke sustained expansion.

The final increase in heat stress in PA significantly elevated peak exercise heart rate on day twelve compared to day six (Table 6.1.). Earlier work in this thesis (chapter 5) demonstrated that the combination of heat and humidity contributing to the overall heat stress (when matched between conditions) does not differentially impact exercise performance over 40 minutes, but trends toward increased physiological strain with greater humidity, as identified by Smolander et al., (1987). The significant elevation in heart rate on day twelve compared to day six may therefore, reflect the increased humidity in the final phase of PA. That stress was enhanced in the current study by the progressive nature of the acclimation protocol is partly supported by perceptual responses during acclimation, as $T_{\text{sen}}$ was significantly higher on day six and twelve compared to day one in PA and was significantly higher on day twelve in PA compared to TA and TG (Table 6.3.). By comparison $T_{\text{sen}}$ was reduced by day 12 in TA and TG.

Twelve days of TA and TG reduced mean and peak $T_{\text{re}}$ during exercise, but in PA, consistent with the maintenance of physiological strain, peak $T_{\text{re}}$ was significantly higher on day twelve compared to six and mean $T_{\text{re}}$ during exercise was unchanged. Lower mean and peak $T_{\text{re}}$ during exercise may indicate decreased production or dissipation of heat, but approximately one third of the average lowering of final $T_{\text{re}}$ may be attributed to lowering of resting $T_{\text{re}}$ (Kampmann et al., 2008). Previous research has identified the magnitude of decrease in resting $T_{\text{re}}$ to be 0.3 – 0.5°C with heat acclimation (Wyndham et al., 1954; Patterson et al., 2004; Castle et al., 2011). In the current study, although $T_{\text{re}}$ declined, the change (0.3, 0.2 and 0.3°C in TA, PA and TG respectively) was not significant. While consistent with earlier research (Garrett et al., 2009), this was unexpected as acclimation-induced reductions in thermoregulatory and vasodilatory setpoints, reduced heat storage and improved cardiovascular adjustments (Garden et al., 1966; Buono et al., 1998, Armstrong, 1998) coupled with attenuation of hyperpyrexic mediators (Shin et al.,
2013) should have contributed to a decreased resting $T_{re}$. However, the extent of decrease in resting $T_{re}$ is primarily due to the physical work performed (Kampmann et al., 2008), therefore, lack of change in the current study may reflect the intensity of exercise during acclimation and training. In contrast, previous work using identical exercise intensities and environmental conditions for acclimation (Castle et al., 2011) has reported significant change in resting $T_{re}$ and therefore, it may be that inter-individual differences and large standard deviations prevented detection of a difference. Alternately, it has been demonstrated that heat acclimation at a fixed time of day evokes significant changes in acclimatory markers only during the time period in which the heat acclimation was completed (Shido et al., 1999). As such, given that measurements on day one, six and twelve were completed between 7.30 and 9.30 am to ensure consistent hormonal responses, but some acclimation sessions were completed at a different time due to logistical constraints, a time of day effect may have contributed to findings.

Sweat rate during exercise was significantly elevated during TA and PA, but was unchanged during TG in a temperate environment (Table 6.1., Figure 6.4.). Consistent with previous research (Patterson et al., 2004; Machado-Moreira et al., 2005; Buono et al., 2009), improved sweat rate with acclimation may be due to lowering of the zero point of the central nervous system for sweating, i.e. a central adaptation (Nadel et al., 1974). Peripheral adaptation may, however, also augment sweat rate through eccrine gland hypertrophy, increased cholinergic sensitivity of the gland, increased peri-glandular concentrations of acetylcholine and improved capacity (Patterson et al., 2004; Buono et al., 2009). Alternatively, rather than an adaptive response in PA, increased sweat rate on day twelve may simply reflect the increased heat stress and strain inherent in a protocol of this type contributing to an increased volume of sweat on day six and twelve.
6.5.2. Physiological and perceptual responses during Intermittent-sprint exercise in 33°C 50% rh

Mean exercise heart rate was significantly reduced in CISP 2 compared to CISP 1 in response to both acclimation regimes and, peak heart rate, although not significantly different, showed a trend toward a decline (-17 and -23 b.min\(^{-1}\) in PA and TA respectively). Further, mean \(T_{re}\) during CISP 2 was significantly reduced by TA and PA (-0.3 and -0.5°C respectively). Collectively, these data indicate that both PA and TA conferred improved cardiovascular stability and thermoregulatory function during intermittent-sprint exercise in contrast to TG. Although reductions in exercise heart rate during CISP 2 may be explained by mechanisms previously described, elucidating mechanisms responsible for the reduction in \(T_{re}\) during exercise is less clear. Previously, it has been suggested a decrease in hypothalamic thermoregulatory set point contributes to a decreased resting \(T_{re}\) that subsequently contributes to a decreased \(T_{re}\) in exercise (Garden et al., 1966; Buono et al., 1998, Armstrong, 1998). Prior to CISP 2 however, resting \(T_{re}\) was not different within any condition, but tended toward a decline in PA and TA and demonstrated a potentially meaningful effect (-0.4 and -0.3°C respectively, \(\eta_p^2 = 0.213\)). In addition, decreased heat production may contribute to enhanced thermoregulatory function during exercise. However, despite significant reductions in \(T_{body}\) and peak BHC in CISP 2 compared to CISP 1, mean BHC during exercise was not significantly different compared to CISP 1, but demonstrated a trend toward a meaningful decline (\(\eta_p^2 = 0.199\)). Further, as oxygen uptake tended to be lower during CISP 2 compared to CISP 1, metabolic heat production was decreased by 10% in CISP 2, but again was not significantly different from CISP 1. Finally, improved heat dissipation with acclimation may contribute to enhanced thermoregulatory function and improve physiological responses during exercise in the heat. In the current study, \(T_{sk}\) was significantly reduced by PA and TA. Consequently, given the reductions in exercise \(T_{re}\) during CISP 2 the core: skin temperature gradient for both TA and PA was increased from CISP 1 to 2 (2.7 vs. 3.3°C and 2.9 vs. 3.2°C in TA and PA, respectively) and may have improved heat dissipation while optimising skin blood flow allowing improved cardiovascular function (Cheuvront et al., 2010). Further heat dissipation can be achieved by an increase in sweat volume produced. However, sweat rate during intermittent-sprint
exercise in the current study was not different in any regime. When sweat rate from intermittent-sprinting is compared to day one, six and twelve of acclimation it is interesting to note that volumes achieved during the CISPs were only matched on day twelve of acclimation. Greater thermal strain has been reported in intermittent compared to continuous exercise (Nevill et al., 1995) and therefore, this lack of change may represent an insufficient stimulus during acclimation to promote enhanced sudomotor function during the CISPs. It has been suggested that humid heat acclimation elicits greater increases in sweat rate (Shvartz et al., 1973: Henane, 1980). As such, use of greater humidity during acclimation or earlier in PA when elevating heat stress may have evoked a greater sweat response. Griefahn (1997), however refutes this contention, demonstrating no difference in sweat rate between hot humid and dry heat acclimation. As stated, sudomotor motor adaptation may evolve from central and peripheral mechanisms. Use of whole body sweat rate in the current work prevents insight to the extent of contribution from these mechanisms, therefore it is unknown whether set-point and / or sweat gland adaptation occurred.

Consistent with reduced physiological strain, RPE during exercise was significantly reduced after PA and TA, but not TG and could be indicative of altered group III and IV afferent feedback in response to acclimation (Amann, 2011), or an effect of specific brain regions and endogenous opioids (Marcora, 2009). Further, \( T_{\text{sen}} \) was decreased by both acclimation regimes, but not TG. Peak \( T_{\text{sen}} \) was decreased in PA only indicating a possible beneficial effect of the greater heat stress used in the final phase of progressive compared to traditional acclimation.

6.5.3. Performance responses during Intermittent-sprint exercise in 33°C 50% rh

The drop in PPO over time during CISP 1 in PA, TG and TA was 8.2, 6.0 (P > 0.05) and 19.1% (P < 0.05) respectively. Given the TEM of the CISP for PPO identified in chapter four, these decrements suggest a meaningful drop in performance over the duration of exercise. Further to the drop in PPO, a strong negative correlation existed between PPO
and exercise T\text{re} in TA and PA (r = -0.938 and -0.900 respectively, all P values < 0.001). On completion of acclimation, PPO was significantly increased in TA during CISP 2 from sprint ten, the decrease over time was < 1% and no significant correlation was observed for this variable and exercise T\text{re} (r = -0.314, P = 0.178). In contrast, no change in PPO was observed for PA and TG between CISP 1 and 2 with similar reductions over time and the persistence of a strong negative relationship between PPO and T\text{re} in PA (r = -0.928, P < 0.001). Further, mean work done was significantly higher in CISP 2 compared to CISP 1 only in TA. These data indicate a beneficial effect of TA on performance in intermittent-sprinting in the heat, as reported in previous work using an identical protocol (Castle et al., 2011) and may be due to acclimation-induced reductions in muscle temperature, epinephrine concentration and muscle glycogen use (Castle et al., 2011). However, while the marked difference in percent decrement between TA and the other conditions could not be explained by relative power output (W.kg\textsuperscript{-1}), body surface area or initial sprint performance (Bishop, 2012), it is also important to note in the TA group that two participants displayed marked negative physiological responses to exercise in the heat in both intermittent sprinting and acclimation. For these two participants, the increase in T\text{re} during intermittent-sprinting in the heat was the highest of all participants in the study at 0.04°C.min\textsuperscript{-1}. Further, these individuals had the greatest absolute and relative rate of increase in body heat content during intermittent exercise (9.7, 8.4 J.min\textsuperscript{-1} and 0.15, 0.10 J.min\textsuperscript{-1}.kg\textsuperscript{-1}), yet two of the lowest sweat rates. Both individuals also possessed the lowest relative aerobic fitness of all participants and the highest percentage body fat and from a performance perspective, displayed the greatest decrease in PPO over time during CISP 1 of all participants in this thesis (43% and 25%, respectively). During acclimation these participants displayed the greatest physiological strain, required the greatest amount of time to acquire the heat acclimated phenotype and demonstrated some of the largest plasma volume expansions of the whole cohort (25% and 28% respectively). Lack of tolerance to the heat has previously been reported in young fit individuals (Moran et al., 2007) and may be inherent or acquired, temporary or permanent and due, for example, to lack of fitness, body composition, previous heat injury or infectious disease and subsequent inability to regulate heat storage. As both of the individuals previously mentioned were free of any infection for the study duration it may be their intolerance to heat based on aforementioned criteria was markedly lower than the remainder of the TA
group but also the study cohort and therefore, when combined with the small group size, may have contributed to the marked decrement in performance during the first CISP.

Similar to TA, PPO after PA tended to be higher (2.7%), but was not significantly different. Considering Castle et al., (2011) demonstrated significance with a 2% improvement in PPO after heat acclimation the result in the current study, although consistent with earlier work in this thesis (chapter 5), is most likely explained by the greater variance in the peak power data recorded from participants in the current work compared to Castle et al., (2011). Alternately, findings may reflect a Type II error as post hoc power analysis demonstrated a sample size of twelve would be required for significance in PPO. In contrast to TA, PA in the current study did not ameliorate the decrement in PPO or the negative correlation with $T_{re}$ during the CISP (Decrement CISP 1 = 6.0%, CISP 2 = 4.4%). Although similar to decrements reported in study two (chapter 5), the small decrease in PPO in CISP 1 would have contributed to the persistence of a negative correlation between PPO and $T_{re}$ after PA. It is however, also important to note that during CISP 1 mean and peak heart rate, $T_{re}$, $T_{sk}$ and blood lactate, although not significantly different, tended to be lower in PA compared to TA. Further, although not significantly different, aerobic fitness tended to be higher in PA compared to TA (46.9 ± 2.7 ml.kg$^{-1}$.min$^{-1}$ vs. 42.2 ± 2.4 ml.kg$^{-1}$.min$^{-1}$, respectively) and heat production was lower during CISP 1. Rate of heat production has been shown to mediate an anticipatory reduction in power output (Tucker et al., 2006). Further, inhibitory feedback from group III and IV afferents sensitive to the mechanical, biochemical and thermal state of the tissue is known to modulate CNS drive to locomotor muscle (Amann et al., 2008, 2013). As such, it is plausible the tendency for lower physiological responses in the PA group combined to produce better maintenance of central drive to locomotor muscle compared to the TA group resulting in less reduction in PPO. That PA then did not alter the decrement in drive may also be due to the protocol whereby the extent of physiological strain induced by this protocol in its current form may not have evoked sufficient strain for a sufficient period in the more trained PA group. Little is known of the effect of intermittent-sprint exercise in the heat on central and peripheral fatigue and no studies have examined how exercise-acclimation
may modulate fatigue of this type. As such, further work is required to confirm or refute the aforementioned explanations for the lack of effect of PA.

6.5.4. Limitations

Research provides compelling evidence of a common mechanism for thermotolerance and heat acclimation and consequently, evidence of heat shock protein involvement in acquisition of the heat acclimated phenotype (Kuennen et al., 2011). Further, more recent work proposes that extra-cellular HSP72 concentration is significantly increased only in very hot ambient conditions where the exogenous heat stress evokes a minimum endogenous criteria of rapid and sustained increases in core temperature to > 38.5°C and enhanced sympathetic activity (Gibson et al., 2013). While this provides explanation for the previously held notion that effective heat acclimation is only achieved through attainment of a critical core temperature of ~ 38.5°C, it also provides a criterion reference by which responses to PA may be examined and the protocol itself judged. During PA, the maximum increase in exercise $T_{re}$ on day one (1.1°C), six (1.3°C) and twelve (1.5°C) evoked $T_{re}$ responses beyond 38.5°C only in the final stage of the protocol (day 9 - 12) and then only for ~ 15 minutes in each session in the final phase. Similarly, in the final phase of PA, mean exercise $T_{re}$ reached only 37.9 ± 0.5°C. Consequently, while sympathetic activity (measured as increased heart rate) surpassed the minimum endogenous requirement as identified in previous work (Gibson et al., 2013), it may be the progressive protocol used, while superior to TG in maintenance of physiological strain and beneficial as it allows all participants to complete sessions in the initial stages of the protocol thereby maximising exposure to heat, did not evoke sufficient strain to optimise acclimation responses. This may explain non-significant findings in some key markers of heat acclimation e.g. plasma volume and resting $T_{re}$ during acclimation sessions but also during the CISPs and is reflected in the non-significant findings for plasma cortisol within or between regimes. That said, accepting that improved performance during intermittent-sprinting after TA may have been distorted by markedly lower heat tolerance of some participants in that group, key markers of physiological and perceptual strain during intermittent-sprinting
were reduced by both PA and TA, indicating progressive acclimation is as effective as traditional acclimation in this respect. Although TA and TG both evoked improvements in markers of heat acclimation from day one to twelve it is important to remember that such observations may merely represent decreased physiological strain as previously reported with constant work-rate protocols (Taylor, 2000) and therefore, may reflect an inability to optimise physiological responses to acclimation within a specific time frame. Data presented for PA indicate that, despite a sufficient lack of strain to achieve a minimum endogenous $T_{re}$ criteria of 38.5°C, the increases in heat stress on day five and nine were sufficient to maintain physiological strain across the protocol thereby indicating the efficacy of this method in inducing the heat acclimated phenotype. Further work is required to elucidate the optimal heat stress and increments required to achieve maximal adaptation within a standard heat acclimation time frame.

6.5.5. Conclusion

In conclusion, this was the first study to examine progressive heat acclimation as a method to maximise heat adaptation by elevating physiological strain throughout an acclimation regime with stepwise increase in heat stress on two occasions. Further, this was the first study to examine the effect of progressive heat acclimation on responses during intermittent-sprint exercise in the heat. Overall, when compared against traditional acclimation, data demonstrate progressive acclimation is a valid method for conferring the heat acclimated phenotype. Due to the reduced heat stress in the initial stages of progressive acclimation this protocol may permit better tolerance of initial heat exposure and maximise exposure time in the early stages of acclimation by reducing incidence of heat illness and limiting participant drop out. Therefore, progressive heat acclimation may permit greater individualisation of heat acclimation and be of benefit to certain athletes, especially those less tolerant to the heat. With the current protocol design, increased physiological strain was evident during the acclimation regime, however, findings indicate the degree of strain was possibly insufficient to evoke the minimum endogenous criteria needed to elicit a maximal acclimation response for a sufficient period of time and a subsequent performance-enhancing effect.
CHAPTER VII. THE EFFECT OF PROGRESSIVE HEAT ACCLIMATION ON FATIGUE FOLLOWING INTERMITTENT-SPRINT EXERCISE IN THE HEAT

7.1. Abstract

The aims of this study were to examine central and peripheral contributions to fatigue during intermittent-sprint exercise in 33°C, 50% relative humidity (rh) using transcranial magnetic stimulation (TMS) and electrical femoral nerve stimulation (FNS) and to investigate whether progressive heat acclimation could ameliorate fatigue following exercise of this type. Seventeen male games players matched for peak oxygen uptake (\(\text{VO}_{2\text{peak}}\)), peak power and body surface area were divided into two groups; progressive heat acclimation (PA, \(n = 9\); 4 d, 50% \(\text{VO}_{2\text{peak}}\), 30.8 ± 0.7°C, 49 ± 5% rh, 4 d 33.2 ± 0.6°C, 50 ± 6% rh and 4 d 35.4 ± 0.6°C, 62 ± 6% rh) and training (TG, \(n = 8\); 12 d, 50% \(\text{VO}_{2\text{peak}}\), 21.2 ± 0.9°C, 31 ± 6% rh). Pre and post acclimation or training, participants completed a 40 minute cycling intermittent-sprint protocol (CISP) in 33.7 ± 0.6°C, 50 ± 3.5% rh, with neuromuscular fatigue assessment immediately before and after exercise. Maximal voluntary contraction (MVC) and potentiated twitch force in both PA and TG were reduced after CISP 1 (all \(P < 0.05\)), whereas cortical voluntary activation was not significantly different but tended to decline. PA reduced resting \(T_{re}\) and heart rate prior to CISP 2 (\(-0.3°C; -10 \text{ b.min}^{-1}\) respectively, \(P < 0.05\)). Further, exercise heart rate was reduced by PA (\(-17 \text{ b.min}^{-1}, P < 0.05\)) and \(T_{re}\) tended to decline (\(-0.3°C, P = 0.10\)). Despite the improved overall physiological strain, peak power output and work done were not different between CISP 1 and 2. Further, the reduction in MVC and potentiated twitch force during intermittent sprinting remained after twelve days of progressive heat acclimation. These data indicate that during forty minutes of intermittent-sprint exercise in the heat, neuromuscular fatigue may be primarily peripheral in origin and PA or TG does not reduce the extent of fatigue despite a reduced physiological strain.
7.2. Introduction

Fatigue may be described as an exercise-induced reduction in maximal voluntary force in a muscle or group of muscles, whether or not the task can be sustained (Gandevia, 2001; Sidhu et al., 2009a; Millet et al., 2011). The genesis of fatigue is task specific, but may comprise both central and peripheral components. Central fatigue is a progressive exercise-induced reduction in voluntary activation or neural drive to a muscle and may comprise both spinal and supraspinal components. In contrast, peripheral fatigue is produced by changes at or distal to the neuromuscular junction, including altered sarcolemmal excitability and excitation-contraction coupling (Gandevia, 2001; Taylor et al., 2006, 2007; Allen et al., 2008; Boyas and Guevel, 2011). Assessments of central and peripheral contributions to fatigue in intermittent-sprint exercise have generally been determined using the interpolated twitch technique by direct supramaximal electrical stimulation of the motor nerve during and immediately following a maximal voluntary contraction (Merton, 1954). Supramaximal stimulation of the motor nerve during a maximal voluntary contraction (MVC) induces a superimposed twitch, an indication that voluntary drive to the muscle is not maximal (Gandevia, 2001; Taylor et al., 2006). Any increase in the size of the superimposed twitch indicates a reduction in voluntary drive and therefore, provides evidence of central fatigue. Supramaximal stimulation post MVC evokes a potentiated twitch with a decrease post exercise indicative of peripheral fatigue. Although useful for indicating central fatigue, the interpolated twitch technique does not allow the site of central fatigue to be localised beyond proximal to the neuromuscular junction (Goodall et al., 2012a; Ross et al., 2012). Recently, transcranial magnetic stimulation (TMS), applied to the motor cortex, has been used to examine central fatigue and has the advantage that it permits the site of failure of voluntary drive to be localized (Sidhu et al., 2009b; Goodall et al., 2012a; Ross et al.; 2012). Further, TMS is reliable for the assessment of supraspinal fatigue in the knee extensors (Goodall et al., 2009; Sidhu et al., 2009b).
In intermittent-sprint exercise, when 40 m running sprints or 6 s cycling sprints, both interspersed with 30 s recovery, are completed in a temperate environment, fatigue manifests as a significantly reduced maximal voluntary torque in both plantar flexors and knee extensors, increased sprint time and reduced power output (Racinais et al., 2007; Perrey et al., 2010b). In such circumstances, decrements in voluntary activation and reduced RMS/M wave ratio measured by electrical stimulation of the motor nerve indicate a reduction in central drive and the presence of central fatigue. In addition, reduced maximal M wave amplitude is reported suggesting reduced sarcolemmal excitability and peripheral fatigue. Taken together, these indicate both central and peripheral contributions to fatigue following exercise of this type. Recently, however, when TMS was used to assess fatigue following intermittent-sprint exercise, cortical voluntary activation and corticospinal excitability were not altered, but potentiated twitch was significantly depressed (Girard et al., 2013a). This suggests a predominance of peripheral factors in fatigue during exercise of this type (Girard et al., 2013a).

Few studies have examined peripheral and central fatigue following intermittent-sprint exercise in the heat. Using an identical protocol as previously described (Racinais et al., 2007; Perrey et al., 2010) Girard et al., (2013b) demonstrated that fatigue was predominantly peripherally-mediated owing to a reduced potentiated twitch and twitch characteristics, but heat had no effect on the pattern and extent of fatigue (Girard et al., 2013b). In contrast, Drust et al., (2005) demonstrated a greater decline in mean power output in hot compared to control conditions when 5 x 15 s sprints were preceded by 40 minutes of intermittent exercise. Although no direct measures to explore the mechanisms responsible for fatigue were performed, given that no differences were observed between conditions in recognised metabolic fatigue agents, it was suggested the greater reduction in power output in the hot trial may have been mediated by the more pronounced hyperthermia induced in the hot trial augmenting central fatigue (Drust et al., 2005). The discrepancy in findings in the previous studies may be explained by the duration of the protocol and the extent of hyperthermia induced, as in the work of Girard et al., (2013b) nine minutes of exercise was performed and average end core temperature was < 38.5 °C, whereas in the study of Drust et al., (2005) forty minutes of
exercise was performed and core temperature was elevated to > 39°C. Other studies that replicate the durations and work-rest ratios of field-based team-sports during exercise in the heat also demonstrate reduced intermittent-sprint performance (Morris et al., 1998, 2000, 2005). However, despite suggesting that hyperthermia and central fatigue may explain the decrement in performance, none have used methods to assess peripheral fatigue or central fatigue where, for example, TMS would provide greater insight. As such, there is a need for research to examine the central and peripheral contributions to fatigue during intermittent-sprint exercise in the heat using durations and work-rest ratios that replicate field-based team-sports.

Heat acclimation (HA) is a strategy commonly used to improve heat tolerance and reduce performance impairment in the heat and can be achieved using constant work rate, isothermal strain and self-regulated exercise protocols (Taylor, 2000; Hargreaves et al., 2008). Successive heat acclimation exposures elicit beneficial physiological adaptations, that include the lowering of resting and exercise core temperature and heart rate, expanded plasma volume, improved sudomotor function and reduced perception of effort and thermal sensation (Armstrong and Maresh, 1991; Sawka et al., 1996). Typically, adaptations exhibit a biphasic response pattern and it is suggested that complete adaptation requires 7 - 14 days, however, up to 75% of adaptations are complete within 4 - 6 days (Pandolf, 1988; Armstrong and Maresh, 1991). Despite the benefits of heat acclimation to exercise performance little is known of the impact of heat acclimation on central and peripheral contributions to fatigue and no studies have examined the effect of exercise-heat acclimation on neuromuscular fatigue following intermittent-sprint exercise in the heat.

That heat acclimation may ameliorate central and peripheral fatigue following intermittent-sprint exercise is conceivable for a number of reasons. Firstly, hyperthermia that induces core temperatures of between 38.5 - 39.5°C evokes decrements in voluntary force production and voluntary activation (Morrison et al., 2004; Thomas et al., 2006) that are correlated with cerebral blood flow at rest and after exercise in the heat (Nybo and
Nielsen, 2001b; Ross et al., 2012). In hypoxia, decreased cerebral blood flow exacerbates declining cerebral oxygenation and contributes to a two-fold greater decline in voluntary activation (Goodall et al., 2012a). As intermittent-sprint exercise induces greater thermal strain and pronounced hyperthermia when performed in the heat (Nevill et al., 1995, Morris et al., 1998, 2000, 2005; Sunderland et al., 2008) this type of exercise may also exacerbate a decline in cerebral blood flow and oxygenation contributing to a greater decrement in voluntary activation. Heat acclimation, that has been demonstrated in this thesis (chapter six) and other research (Patterson et al., 2004; Castle et al., 2011; Burk et al., 2012) to reduce the extent of hyperthermia and cardiovascular strain during exercise, may ameliorate any decline in cerebral blood flow and cerebral oxygenation by reducing competition for cardiac output and may therefore, ameliorate decrements in voluntary activation and central fatigue.

Secondly, it has been suggested central drive and regulation of motor unit activity may be mediated by afferent feedback from a number of sources including, for example, exercising muscle (Noakes, 2012; Amann et al., 2013). In muscle, activity of group III and IV afferents that are sensitive to the mechanical, biochemical and thermal state of the tissue, may be upregulated during exercise in the heat and provide inhibitory input to the CNS limiting central motor drive and performance (Gandevia, 2001; Amann et al., 2013). Heat acclimation may contribute to a decrease in activity of these afferents subsequently decreasing the extent of inhibitory feedback to the CNS, thereby maintaining central drive due to the improvements in heat dissipation, reduction in extent of hyperthermia and decreased muscle glycogenolysis and lactate formation (Kirwan et al., 1987) that are consistent with the heat acclimation phenotype.

Thirdly, RPE and thermal sensation are improved in response to heat acclimation (Pandolf, 1977; Regan et al., 1996; Molloy et al., 2004; Sunderland et al., 2008; Castle et al., 2011; Burk et al., 2012). Although the mechanisms are not well understood thermal sensation has been correlated with skin temperature (Kamon et al., 1974) and RPE has been correlated to physiological variables such as heart rate, ventilation and blood lactate
(Edwards et al., 1972). As such, as increased perception of effort contributes to decreased power output and fatigue, possibly via group III and IV afferents that project through lamina I neurons to the sensory cortex (Craig, 2002; Enoka and Duchateau, 2008), acclimation-induced decrements in, for example, heart rate and skin temperature, as observed in study three, may reduce thermal sensation and RPE thereby reducing the extent of fatigue during exercise in the heat. Recent work, however, indicates afferent feedback from the heart and lungs does not contribute significantly to the perception of effort during exercise (Marcora, 2009). As such, alterations in central factors with heat acclimation, for example, neural pathways and dopamine may be involved (Marcora et al., 2009). Finally, it has been suggested that, with hyperthermia, relaxation rate of muscle is significantly increased requiring faster motor unit firing rates to produce fusion of force. Therefore, an inability of descending voluntary drive to compensate for altered augmented muscle relaxation rate may contribute to central fatigue and if heat acclimation can reduce the extent of hyperthermia it may reduce the extent of fatigue (Todd et al., 2005).

Theoretically at least, based on the mechanisms discussed, the potential for heat acclimation to reduce central and peripheral fatigue following intermittent-sprint exercise in the heat exists. Recently, however, Brazaitis and Skurvydas (2010) demonstrated no change in central fatigue during a two minute MVC exercise following seven sessions of passive hyperthermia. However, the passive intermittent nature of the protocol used in this study has limited application and may have limited the potential for heat acclimation-induced change. As such, more work needs to examine the effect of heat acclimation on central and peripheral fatigue during intermittent-sprint exercise in the heat using TMS when work-rest ratios and durations replicate those observed in field-based team-sports. Therefore, the purpose of this study was to examine central and peripheral contributions to fatigue following intermittent-sprint exercise in the heat using TMS and femoral nerve stimulation (FNS) and to investigate whether progressive heat acclimation could ameliorate fatigue during exercise of this type. It was hypothesised intermittent-sprint exercise completed for forty minutes in the heat would exhibit central and peripheral fatigue and progressive heat acclimation would reduce both these components of fatigue.
during exercise in the heat compared to control as a result of reduced physiological strain.

7.3. Methods

7.3.1. Participants

Seventeen male University students volunteered for the study. Participants were randomly assigned to one of two groups; Progressive Acclimation (PA, n = 9) and Training (TG, n = 8) matched for $\overline{V}O_{2\text{peak}}$ and body surface area. Participant characteristics were as follows (mean ± S.D. for PA and TG, respectively): age 21.1 ± 1.5 and 21.4 ± 1.6 years; height 180.5 ± 5.2 and 177.3 ± 3.6 cm; mass 81.9 ± 9.6 and 78.5 ± 12.1 kg; BSA 2.01 ± 0.13 and 1.95 ± 0.13 m$^2$; Body fat 16.1 ± 3.7 and 18.6 ± 6.1 %; $\overline{V}O_{2\text{peak}}$ 46.3 ± 5.1 and 44.6 ± 7.3 ml.kg$^{-1}$.min$^{-1}$; Peak power 3.9 ± 0.3 and 3.6 ± 0.5 W.kg$^{-1}$. All participants were moderately-trained games players and participated in sport three to five times per week. The study was approved and conducted as described in section 3.2.

7.3.2. Experimental design

Each participant was required to visit the laboratory on sixteen separate occasions. This included one familiarisation visit to acquaint the participant with neuromuscular function testing (NMF), one preliminary visit and two intermittent-sprint tests in 33°C 50% rh pre and post twelve days of PA or TG (Figure 7.1.).
7.3.2.1. Visit 1: familiarisation

The purpose of the familiarisation visit was to provide participants with a thorough familiarisation to the methods for electromyography, femoral nerve stimulation and transcranial magnetic stimulation (TMS). Prior to familiarisation, all participants...
completed a standard medical form and were screened to determine suitability to TMS using a specific medical questionnaire (Appendix 2). Any participants for whom TMS was contraindicated were excluded from the study.

**Force and Electromyography:** For measurement of voluntary and evoked contractions, participants were seated in a custom-modified isometric chair. The hip and knee were positioned at 90° and a load cell (Model 615, Tedea-Huntleigh, PA, USA), interfaced with a data acquisition system, (PowerLab 15T and Lab Chart 7.2, AD Instruments, Oxford, UK) attached to a non-compliant strap positioned just superior to the malleoli of the right ankle. EMG activity of the right vastus lateralis and bicep femoris was recorded. Participants’ skin was shaved, swabbed and cleaned with isopropyl alcohol and self-adhesive electrodes (H59P, Kendall, Mansfield, MA, USA) were placed 2 cm apart over the muscle bellies and the patella. The position of electrodes was marked with indelible ink, measured and recorded using digital images to permit accurate replication on subsequent visits. All EMG signals were amplified, band pass filtered between 20 Hz and 2 kHz, sampled at a rate of 4 kHz and later analysed using commercially available software (Lab Chart 7.2, AD Instruments, UK).

**Femoral nerve stimulation:** With participants seated as described above, single electrical stimuli of 200 μs were delivered via 31.7 mm stimulating electrodes (Model 3100C, Uni-Patch™, MN, USA) to the right femoral nerve using a constant current stimulator (Figure 7.2., Model DS7A, Digitmer Ltd., Hertfordshire, UK). Electrodes were positioned high in the femoral triangle (cathode) and midway between the iliac crest and greater trochanter (Goodall et al., 2010). Plateaus in twitch amplitude and M wave were determined using an incremental protocol starting at 100 mA and increasing by 20 mA until plateaus were achieved (Goodall et al., 2012a). To ensure stimulation intensity was supra-maximal, stimulations were then increased by 30% and kept constant throughout the trials. Mean stimulation intensity was 1478 ± 226 mA. Participants then performed three control MVCs to determine peak torque and peak surface EMG and a further three contractions
(5 s) with femoral nerve stimulation during each MVC and an additional stimulus at rest (~2 s). Each MVC was separated by ~ 30 s.

**Figure 7.2.** Digitimer constant current stimulator.

**Transcranial magnetic stimulation:** With participants seated as described, the vertex of the cranium, (intersection of the mid-sagittal and inter-aural lines), was identified and marked. Optimal placement for producing a large motor evoked potential (MEP) in the vastus lateralis and a small MEP in the bicep femoris was determined in relation to the vertex by using a magnetic stimulator and 110 mm double-cone coil positioned over the motor cortex (Figure 7.3., Magstim 200, Magstim LTD, UK). For participants, this location was 1.5 ± 0.78 cm contralateral to the vertex. Single pulse TMS at 30 - 40% of stimulator output was initially used to habituate the participant to the sensation of TMS and once achieved, single pulse TMS at 60% of stimulator output was used to determine optimal coil placement. Once determined, the optimal placement was recorded and marked with indelible ink for replication in future visits. Resting motor threshold (rMT) for the knee extensors was then established using a magnetic stimulator (Magstim 200, Magstim LTD, UK) by starting at an output of 30% and increasing in 5% increments until the motor evoked potential (MEP) was less than 0.05 mV in more than 50% of eight single pulse stimuli (Groppa et al., 2012). Resting motor threshold occurred at 61.2 ± 13.4% of maximum stimulator output. Subsequently, TMS was delivered at 1.2 times resting motor threshold during MVCs at 50, 75 and 100% to determine cortical voluntary activation (Figure 7.4.). This stimulation intensity (73.5 ± 16%) elicited a large MEP in the vastus lateralis (area > 50% of maximum M wave) during knee extensor contraction at ≥ 50%
MVC (Figure 7.5.). Applicability and reliability of TMS for measuring voluntary activation of knee extensors has been previously determined with typical error or measurement ranging from 3.1 - 3.7% and intra-class correlations greater than 0.85 (Goodall et al., 2009; Sidhu et al., 2009b).

**Figure 7.3.** Magstim 200 and 110 mm double cone coil.

**Figure 7.4.** Set up for transcranial magnetic stimulation over the motor cortex and femoral nerve stimulation.
Figure 7.5. Relationship between MEP area from the vastus lateralis and contraction strength during TMS over the motor cortex. Largest MEP area was recorded during a 50% contraction and at this contraction strength mean MEP area was 60% of maximum M wave.

Once familiarisation to the techniques of electromyography and FNS and TMS were achieved, participants practiced the neuromuscular fatigue assessment protocol that would be used pre and post intermittent-sprint exercise in the heat (Figure 7.6.). This included a warm-up of two 50 and 75% MVCs followed by three 5 s MVCs separated by 10 s with FNS during and post MVC. TMS was then superimposed over the motor cortex during brief (5 s) 100, 75 and 50% MVCs, separated by 5 s. This constituted one set of TMS and this process was repeated twice more interspersed with 15 s rest to give a total of three sets. Completion of the full NMF protocol required 2.58 minutes.
7.3.2.2. Visit 2: preliminary testing and CISP practice

Prior to the preliminary visit, participants were instructed on exercise and dietary guidelines as outlined in section 3.4. Participants arrived at the laboratory three hours postprandial and provided a urine sample for assessment of hydration status (section 3.8.). Height, nude body mass and skinfolds were measured and percentage body fat and body surface area calculated (section 3.6.). Participants then completed a graded exercise test to determine lactate threshold and \( \text{VO}_2\text{peak} \) (section 3.6.).

25 ± 5 minutes post graded exercise testing participants completed a CISP practice that required completion of one quarter of the CISP (section 3.6.) to ensure thorough familiarisation with the intermittent-sprint protocol to be used during the study.

7.3.2.3. Visit 3 and 16: CISP and neuromuscular function (NMF) testing

Prior to intermittent-sprint exercise in the heat, all participants were advised on exercise and dietary practice (section 3.4.) and required to record their diet and fluid intake for 24 hours prior to their pre acclimation or training CISP (≥ 1 day before PA or TG) so that nutrition and exercise pattern could be replicated prior to the post acclimation CISP. All
CISPs were completed in an environmental chamber (Section 3.7. & 3.9.) at 33.7 ± 0.6°C, 50.2 ± 3.5% rh pre and post PA or TG and NMF testing in the environmental laboratory at 21.3 ± 0.4°C, 51.7 ± 4.6% rh.

On arrival to the laboratory, all participants provided a urine sample for hydration assessment and measured nude body mass (section 3.8.). Vertex and optimal coil position were re-established and EMG and stimulating electrodes were re-applied. Participants inserted a rectal probe and skin thermistors were attached (section 3.10.4.) and after 15 minutes of rest in a seated position, resting core and skin temperature were measured and a fingertip blood sample was obtained for determination of resting blood glucose and lactate (section 3.11.).

NMF assessment

For neuromuscular function assessment, participants were seated in a custom modified isometric chair as previously described (Figure 7.4.). Thresholds for TMS and FNS were re-established and participants completed a warm-up comprising two 50 and two 75% MVCs. Participants then completed the full neuromuscular function assessment protocol as previously described.

CISP

On completion of neuromuscular function assessment all EMG electrode positions were marked with indelible ink and then removed. Participants entered the environmental chamber (33.7 ± 0.6°C, 50.2 ± 3.5 % rh) and began the standard CISP warm-up (section 3.6.) followed by 40 minutes of intermittent-sprint cycling (section 3.7.) on a Monark ergometer fitted with an SRM powermeter that continuously recorded and stored power output at a rate of 50 Hz for the duration of exercise to permit calculation of peak power output (PPO) and work done (WD) (section 3.5.1.). During intermittent-sprint cycling $T_{re}$, $T_{sk}$, HR, RPE and $T_{sen}$ were recorded at 1 minute into each two minute block of the CISP
(section 3.10.1, 3.10.4, 3.10.6). In addition, arterialised whole blood samples were collected from a hyperemised finger and gas sampled for ~45 s at 1 minute into each fourth 2 min block (sprints 4, 8, 12, 16 and 20) during the active recovery. No fluid was permitted during the CISP and all CISPs were repeated at the same time of day to limit circadian effect (Winget, et al., 1985; Hill et al., 1992). Physiological strain index, $T_{sk}$, $T_{body}$, body heat content and heat production were calculated using methods described previously (section 3.10.2., 3.10.3., 3.10.4.). Criteria for exercise termination were as described (section 3.2.). On completion of the CISP, participants immediately exited the chamber, towel dried, re-applied all electrodes and repeated NMF testing as previously described. Mean time from completion of the CISP to completion of NMF assessment was 4.6 ± 0.3 minutes.

7.3.2.4. Visit 4 – 15: progressive acclimation or training

PA comprised twelve days of exercise on a cycle ergometer, 60 min.d$^{-1}$ at 50% $VO_2$peak with heat stress increased in a stepwise manner on two occasions. On days 1 - 4 participants exercised in the environmental chamber at 30.8 ± 0.7°C, 49.0 ± 5.4% rh. On day 5 - 8 heat stress was increased to 33.2 ± 0.6°C, 50.1 ± 6.2% rh and finally on days 9 - 12 the heat stress was again increased to 35.4 ± 0.6°C, 62.1 ± 6.4% rh. In TG, participants completed twelve days of exercise in the environmental laboratory at 21.2 ± 0.9°C, 31.5 ± 6.7% rh. All sessions were completed at the same time of day and at the same time as intermittent-sprints were performed (5 – 8 pm) to maximise any physiological responses (Shido et al., 1999). Prior to all PA and TG sessions, participants were instructed on diet and exercise as previously described (section 3.4.) and requested to report to the laboratory in a euhydrated state. Prior to every PA or TG session participants’ hydration state was assessed and no further fluid was permitted during sessions. In addition, during each session HR, $T_{re}$, $T_{sen}$ and RPE were recorded in the final 20 s of every fifth minute and sweat rate was determined from nude body mass measured pre and post exercise (section 3.10.5.).
On day one, six and twelve of PA and TG resting HR and \(T_{re}\) were recorded after 15 minutes in a seated position (section 3.10.1, 3.10.4). In addition, pre and post exercise an arterialised capillary blood sample was obtained from a hyperemised finger for determination of haemoglobin, haematocrit and plasma volume (section 3.11.). Also on day one, six and twelve, HR, \(T_{re}\), RPE and \(T_{sen}\) were assessed every fifth minute in exercise.

7.3.3. Data analyses

All data were analysed offline using standard data acquisition software (Labchart 7, ADI Instruments, UK). Peripheral voluntary activation (PVA) was determined using the interpolated twitch technique (Merton, 1954). Specifically, the mean torque response to femoral nerve stimulation from a superimposed twitch (SIT) during and potentiated twitch (POT) post (~ 2 s, Figure 7.7.) from three initial MVCs in the NMF assessment (Figure 7.6.) was used to determine peripheral voluntary activation using the equation:

Equation 7.1. Calculation of voluntary activation

\[
VA (\%) = \left[ 1 - \frac{SIT}{POT} \right] \times 100
\]

(Ross et al., 2007)

Figure 7.7. Data from a single participant demonstrating a potentiated twitch (\(Q_{tw,pot}\))
Cortical voluntary activation (CVA) was determined from mean torques produced during sets of TMS at 100, 75 and 50% of MVC during the NMF assessment. As corticospinal excitability increases during voluntary contraction it was necessary to estimate the resting twitch amplitude for each participant (Goodall et al., 2009, Ross et al., 2012). Linear regression was derived from the torque responses to TMS of the motor cortex during contractions (SIT) at 100, 75 and 50% MVC and the y intercept of the regression line was taken as the estimated resting twitch (ERT, Figure 7.9.). Cortical voluntary activation was then calculated as;

**Equation 7.2. Calculation of cortical voluntary activation**

\[ CVA \, (\%) = [1 - (\text{SIT}/\text{ERT}) \times 100] \]

(Goodall et al., 2009)
After eliciting an MEP in a target muscle there is a period of silence in EMG signal, referred to as the cortical silent period (Figure 7.10.). During analysis, cortical silent period (ms) was measured from the point of stimulation to the resumption of EMG activity. Resumption of EMG was accepted as ± 2 S.D. of pre stimulus EMG for > 100 ms (Goodall et al., 2010, 2012b). Accuracy of assessment was also confirmed by visual inspection (Girard et al., 2013a).
TMS-induced motor evoked potential (MEP) amplitude for the determination of corticospinal excitability was measured offline as the absolute difference between the maximum and minimum points (mV) (Sidhu et al., 2009b). Peak-to-peak amplitude of the M wave (mV) evoked by femoral nerve stimulation was also determined offline using the same method as for MEPs. MEP area and maximum M wave area were calculated as the integral of the absolute value of the entire MEP or M wave (Sandiford et al., 2005, Sidhu et al., 2009a). MEP area was then normalised to maximum M wave area from a nearby MVC to ensure TMS was activating a high proportion of the knee extensor motor units (Todd et al., 2005; Goodall et al., 2012a; Ross et al., 2012).

Contractility of muscle was assessed by measuring the amplitude of the potentiated twitch (N) in addition to the maximal rate of force development (MRFD), maximal rate of relaxation (MRR) and time to half relaxation ($RT_{0.5}$) (ms). Maximal rate of force development was determined as the maximal slope of the curve between 10 and 90% amplitude, maximal rate of relaxation as the steepest decline in the twitch curve and $RT_{0.5}$ as the time for the force to decay to half peak twitch amplitude (Todd et al., 2005; Goodall et al., 2010).

7.3.4. Statistical analysis

Data were checked for normality, sphericity and variance and adjusted where necessary using the Huynh-Feldt method or log transformation. To examine the effect of intermittent-sprint exercise in the heat on peak power output, work done and neuromuscular fatigue prior to PA or TG, one way repeated measures ANOVA (PPO, WD) and dependent samples t-tests (NMF) were performed on pooled data for all seventeen participants (Part A). Pearson’s Correlation coefficient was used to assess relationships between PPO, WD and $T_{re}$. Physiological responses to twelve days PA or TG was assessed using a three way mixed ANOVA (regime*day*time) (Part B). Subsequently, PPO, WD and physiological responses (Part C) and neuromuscular responses (Part D) during intermittent-sprint cycling in the heat pre-post PA or TG were assessed using a three way
mixed ANOVA (regime*trial*time) with repeated measures on two factors (trial*time).
Non parametric perceptual measures were assessed using a Kruskal Wallis test with Wilcoxon signed rank test. All data were analysed using SPSS (version 20.0) and are reported as mean ± standard deviation. Statistical significance was accepted as $P < 0.05$. Effect sizes were calculated as described (section 3.13.)

7.4. Results

7.4.1. Part A: PPO, WD and neuromuscular fatigue responses to intermittent-sprint exercise in 33°C, 50% rh (pooled data for all seventeen participants).

7.4.1.1. PPO and work done

PPO (W) was reduced during intermittent-sprint exercise in 33°C, 50% rh (main effect; $F_{(19,247)} = 3.402$, $P < 0.001$, $\eta^2_p = 0.207$) from sprint ten onwards (Figure 7.11., all $P$ values < 0.05). Compared to sprint one the mean reduction in PPO in the final ten sprints was 4.0%. Work done was also reduced in the heat (main effect; $F_{(19,247)} = 3.961$, $P < 0.001$, $\eta^2_p = 0.221$) from sprint thirteen onwards (all $P$ values < 0.05). Compared to the first sprint, mean work done in the final eight sprints was reduced by 4.5%. Both PPO and WD were strongly negatively correlated with $T_{re}$ during intermittent-sprint exercise ($r = -0.926$, $P < 0.001$ and $r = -0.931$, $P < 0.001$, PPO and WD respectively, Figure 7.12).
Figure 7.11. Mean (± S.D.) PPO (W) and work done (J) during CISP 1 in 33°C, 50% rh.

*Significant difference from sprint one (P < 0.05).
Figure 7.12. PPO (W) and work done (J) correlated with $T_{re}$ (°C).

7.4.1.2. Neuromuscular fatigue

Maximal voluntary contraction force was significantly reduced after Intermittent-sprint exercise in 33°C and 50% rh (Figure 7.13., 503 ± 90 vs 403 ± 79 N, -19%, $P < 0.001$).
Figure 7.13. Maximal voluntary contraction force (N) pre-post CISP 1. *Significant difference between pre and post CISP 1.

Cortical voluntary activation was reduced after intermittent-sprint exercise in 33°C, 50% rh (Figure 7.14.; 90.9 ± 5.5 vs 84.6 ± 8.4%, P < 0.001). Similarly, peripheral voluntary activation was reduced (Figure 7.14.; 89.7 ± 4.4 vs 86.4 ± 5.2%, P = 0.005).

Figure 7.14. Cortical (CVA) and peripheral voluntary activation (PVA) (%) pre-post CISP 1. *Significant difference between pre and post CISP 1 (P < 0.05).
Potentiated twitch force (-21.5%), maximal rate of force development (-18.9%) and time to half relaxation (-37.9%) were significantly reduced after intermittent-sprint exercise in 33°C, 50% rh. Maximal relaxation rate and measures of corticospinal excitability and conduction time were not different after intermittent-sprinting in the heat. Similarly, Mmax amplitude, MEP area to Mmax area and RMS:M wave ratio were not significantly different after exercise in the heat.

Table 7.1. Neuromuscular variables pre-post CISP 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q_{tw,pot} (N)</td>
<td>181 ± 33</td>
<td>142 ± 35*</td>
</tr>
<tr>
<td>MRFD (N.ms(^{-1}))</td>
<td>5.915 ± 4.329</td>
<td>4.794 ± 3.346*</td>
</tr>
<tr>
<td>MRR (N.ms(^{-1}))</td>
<td>0.826 ± 0.391</td>
<td>0.945 ± 0.584</td>
</tr>
<tr>
<td>RT(_{0.5}) (ms)</td>
<td>89.4 ± 26.2</td>
<td>55.5 ± 21.3*</td>
</tr>
<tr>
<td>Mmax amplitude (mV)</td>
<td>7.6 ± 4.6</td>
<td>7.8 ± 4.4</td>
</tr>
<tr>
<td>MEP_{area}:Mmax_{area}</td>
<td>52.7 ± 15.7</td>
<td>51.7 ± 17.6</td>
</tr>
<tr>
<td>CSP (ms)</td>
<td>142 ± 10</td>
<td>140 ± 50</td>
</tr>
<tr>
<td>Latency (ms)</td>
<td>20 ± 9</td>
<td>22 ± 1</td>
</tr>
<tr>
<td>RMS:M-wave (au)</td>
<td>0.048 ± 0.011</td>
<td>0.052 ± 0.020</td>
</tr>
<tr>
<td>ERT (N)</td>
<td>133 ± 41</td>
<td>95 ± 29*</td>
</tr>
</tbody>
</table>

Data are mean (± S.D.). Q_{tw,pot} (Potentiated twitch), MRFD (maximal rate of force development), MRR (maximal rate of relaxation), RT\(_{0.5}\) (half relaxation time), CSP (cortical silent period), au (arbitrary units), MEP (motor evoked potential).* Significant difference between pre and post CISP 1 (all P values < 0.05)

7.4.2. Part B: Physiological and perceptual responses to twelve days of PA and TG

7.4.2.1. Body mass and hydration

Pre-exercise body mass and hydration status were not different between days in any group (all main effect time*regime P values > 0.05, Table 7.2.) indicating participants were in a similar physiological state prior to acclimation or training on day one, six and twelve.
Table 7.2. Body mass and hydration during PA and TG on day 1, 6 and 12.

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 6</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body Mass (kg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>81.76 ± 8.58</td>
<td>81.62 ± 8.49</td>
<td>81.00 ± 9.12</td>
</tr>
<tr>
<td>TG</td>
<td>77.70 ± 11.73</td>
<td>77.91 ± 10.86</td>
<td>77.74 ± 11.63</td>
</tr>
<tr>
<td><strong>Usg</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>1.011 ± 0.01</td>
<td>1.010 ± 0.01</td>
<td>1.004 ± 0.01</td>
</tr>
<tr>
<td>TG</td>
<td>1.011 ± 0.01</td>
<td>1.012 ± 0.01</td>
<td>1.010 ± 0.01</td>
</tr>
<tr>
<td><strong>Uosm (mOsm.kg⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>384 ± 190</td>
<td>360 ± 231</td>
<td>154 ± 120</td>
</tr>
<tr>
<td>TG</td>
<td>358 ± 287</td>
<td>400 ± 245</td>
<td>354 ± 269</td>
</tr>
</tbody>
</table>

Data are mean (± S.D.).

7.4.2.2. Heart rate

There was a main effect of time on resting heart rate (b.min⁻¹) across days one to twelve, (F(2,30) 5.171, P = 0.012, \( \eta_p^2 = 0.256 \), Table 7.3.). Resting heart rate in PA decreased from 65 ± 10 b.min⁻¹ on day one to 56 ± 8 b.min⁻¹ on day twelve (P = 0.027), but no difference was observed in TG (P = 1.000). Heart rate increased during exercise in PA and TG each day (main effect time: F(11,165) 94.253, P < 0.001, \( \eta_p^2 = 0.863 \)) and differed between groups in the final five minutes of exercise on day one and from fifteen minutes onwards on day six and twelve (main effect day*time*regime: F(22,330) 3.233, P < 0.001, \( \eta_p^2 = 0.177 \): Post hoc; all P values < 0.05). Mean exercise heart rate was significantly higher in PA on day six (150 ± 16 vs. 134 ± 13 b.min⁻¹ PA and TG respectively) and twelve (153 ± 19 vs. 128 ± 10 b.min⁻¹ PA and TG, respectively) and in TG, exercise heart rate was significantly lower on day twelve compared to day one (137 ± 12 vs. 128 ± 10 b.min⁻¹) with no difference in acclimation (main effect day*regime: F(2,30) 3.907, P = 0.031, \( \eta_p^2 = 0.207 \): Post hoc; all P values < 0.05, Table 7.3.). Peak heart rate was significantly higher in PA compared to TG on day one, six and twelve and was significantly reduced in TG on day twelve compared to day six and was significantly elevated on day twelve compared to day six during PA (main effect day*regime: F(2,30) 7.932, P = 0.002, \( \eta_p^2 = 0.346 \): Post hoc; all P values < 0.05, Table 7.3.).
7.4.2.3. Rectal temperature

Resting $T_{re}$ was reduced over time (main effect: $F_{(2,30)} 7.067$, $P = 0.003$, $\eta^2_p = 0.320$). With PA resting $T_{re}$ was reduced by 0.3°C on day six ($P < 0.05$, Table 7.3.). Resting rectal temperature was not different with TG over twelve days ($P > 0.05$, Table 7.3.). $T_{re}$ increased during exercise in PA and TG each day (main effect time: $F_{(11,165)} 4.063$, $P < 0.001$, $\eta^2_p = 0.938$) and was higher in PA in the final five minutes of exercise on day six and from twenty minutes onwards on day twelve (main effect day*time*regime: $F_{(22,330)} 9.802$, $P < 0.001$, $\eta^2_p = 0.395$: Post hoc; all $P$ values < 0.05). Mean exercise $T_{re}$ was not different between PA and TG over day one to twelve and was not different within groups over the duration of PA or TG (main effect day*regime: $F_{(2,30)} 2.221$, $P = 0.126$, $\eta^2_p = 0.129$). Peak $T_{re}$ was higher in PA compared to TG on day six and twelve and was significantly higher in PA on day six and twelve (main effect day*regime: $F_{(2,30)} 8.238$, $P = 0.001$, $\eta^2_p = 0.355$: Post hoc; all $P$ values < 0.05, Table 7.3.).

7.4.2.4. RPE and $T_{sen}$

Mean RPE was not different between groups on day one and six, but was significantly higher during PA on day twelve ($13.5 \pm 2.2$ vs $10.8 \pm 1.7$, PA and TG respectively) and in TG, RPE was significantly reduced on day six and twelve compared to day one (Table 7.3., main effect day*regime: $F_{(2,30)} 7.562$, $P = 0.002$, $\eta^2_p = 0.335$: Post hoc; all $P$ values < 0.05). Peak RPE was significantly higher in PA compared to TG on day twelve, was significantly reduced in TG on day twelve compared to day one and was significantly higher in PA on day twelve compared to day six (main effect day*regime: $F_{(2,30)} 7.216$, $P = 0.003$, $\eta^2_p = 0.325$: Post hoc; all $P$ values < 0.05, Table 7.3.). RPE increased significantly during exercise (main effect time: $F_{(11,165)} 81.803$, $P < 0.001$, $\eta^2_p = 0.845$) and was significantly higher at all time points in exercise in PA compared to TG on day twelve (main effect day*time*regime: $F_{(22,330)} 3.167$, $P < 0.001$, $\eta^2_p = 0.174$: Post hoc; all $P$ values < 0.05). $T_{sen}$ was significantly different between and within groups during PA and TG (Chi Squared = 56.716, $P < 0.001$, ES = 0.7988). $T_{sen}$ in PA was significantly elevated on day six and twelve compared to day one (all $P$ values < 0.05, Table 7.3.) and in TG, $T_{sen}$ was reduced on day six and twelve compared to day one (all $P$ values < 0.05, Table 7.3.). Peak $T_{sen}$ was
significantly different between and within groups during PA and TG (Chi Squared = 40.304, P < 0.001, ES = 0.8061). Peak T_{sen} was higher in PA compared to TG on day one, six and twelve. TG reduced Peak T_{sen} by day six and in PA, Peak T_{sen} was significantly elevated on day twelve.

Table 7.3. Physiological and perceptual responses at rest and exercise in PA and TG on day 1, 6 and 12.

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 6</th>
<th>Day 12</th>
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<tbody>
<tr>
<td><strong>Resting HR (b.min(^{-1}))</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>65 ± 10</td>
<td>61 ± 6</td>
<td>56 ± 8*</td>
</tr>
<tr>
<td>TG</td>
<td>70 ± 14</td>
<td>66 ± 8</td>
<td>67 ± 12</td>
</tr>
<tr>
<td><strong>Exercise HR_{mean} (b.min(^{-1}))</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>150 ± 18</td>
<td>150 ± 16</td>
<td>153 ± 19</td>
</tr>
<tr>
<td>TG</td>
<td>137 ± 12</td>
<td>134 ± 13†</td>
<td>128 ± 10*†</td>
</tr>
<tr>
<td><strong>Exercise HR_{peak} (b.min(^{-1}))</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>158 ± 18</td>
<td>161 ± 14</td>
<td>167 ± 8†</td>
</tr>
<tr>
<td>TG</td>
<td>141 ± 12†</td>
<td>143 ± 8†</td>
<td>135 ± 10†</td>
</tr>
<tr>
<td><strong>Resting T_{re} (°C)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>37.4 ± 0.2</td>
<td>37.1 ± 0.2*</td>
<td>37.1 ± 0.2*</td>
</tr>
<tr>
<td>TG</td>
<td>37.5 ± 0.2</td>
<td>37.3 ± 0.2</td>
<td>37.3 ± 0.2</td>
</tr>
<tr>
<td><strong>Exercise T_{re mean} (°C)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>37.8 ± 0.5</td>
<td>37.8 ± 0.4</td>
<td>37.9 ± 0.5</td>
</tr>
<tr>
<td>TG</td>
<td>37.9 ± 0.4</td>
<td>37.7 ± 0.3</td>
<td>37.7 ± 0.3</td>
</tr>
<tr>
<td><strong>Exercise T_{re peak} (°C)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>38.2 ± 0.4</td>
<td>38.2 ± 0.2</td>
<td>38.6 ± 0.2**</td>
</tr>
<tr>
<td>TG</td>
<td>38.1 ± 0.3</td>
<td>37.9 ± 0.1†</td>
<td>37.9 ± 0.3†</td>
</tr>
<tr>
<td><strong>Sweat rate (l.hr(^{-1}))</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>0.96 ± 0.28</td>
<td>1.15 ± 0.32*</td>
<td>1.19 ± 0.51*†</td>
</tr>
<tr>
<td>TG</td>
<td>0.68 ± 0.35</td>
<td>0.65 ± 0.21†</td>
<td>0.69 ± 0.22†</td>
</tr>
<tr>
<td><strong>Exercise RPE_{mean}</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>14 ± 2</td>
<td>13 ± 2</td>
<td>14 ± 2</td>
</tr>
<tr>
<td>TG</td>
<td>13 ± 2</td>
<td>12 ± 2*</td>
<td>11 ± 2*†</td>
</tr>
<tr>
<td><strong>Exercise RPE_{peak}</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>15.3 ± 2.2</td>
<td>14.2 ± 2.2</td>
<td>16.0 ± 2.0†</td>
</tr>
<tr>
<td>TG</td>
<td>15.0 ± 2.5</td>
<td>13.8 ± 1.6</td>
<td>11.8 ± 1.9†*</td>
</tr>
<tr>
<td><strong>Exercise T_{sen mean}</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>5.9 ± 0.6</td>
<td>6.3 ± 0.5*</td>
<td>6.7 ± 0.5*</td>
</tr>
<tr>
<td>TG</td>
<td>5.1 ± 0.6</td>
<td>4.8 ± 0.5*</td>
<td>4.9 ± 0.4†*</td>
</tr>
<tr>
<td><strong>Exercise T_{sen peak}</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>6.4 ± 0.5†</td>
<td>6.6 ± 0.4†</td>
<td>7.2 ± 0.3**†</td>
</tr>
<tr>
<td>TG</td>
<td>5.6 ± 0.5</td>
<td>5.0 ± 0.6*</td>
<td>5.1 ± 0.2*</td>
</tr>
<tr>
<td><strong>Haemoglobin (g.dl(^{-1}))</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>14.4 ± 1.5</td>
<td>13.9 ± 1.4</td>
<td>14.3 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 6</td>
<td>Day 12</td>
</tr>
<tr>
<td>------------------</td>
<td>---------------</td>
<td>---------------</td>
<td>---------------</td>
</tr>
<tr>
<td><strong>TG</strong></td>
<td>15.1 ± 1.6</td>
<td>14.6 ± 8.9</td>
<td>14.5 ± 1.1</td>
</tr>
<tr>
<td><strong>Haematocrit</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>0.44 ± 0.02</td>
<td>0.44 ± 0.03</td>
<td>0.44 ± 0.02</td>
</tr>
<tr>
<td>TG</td>
<td>0.45 ± 0.04</td>
<td>0.45 ± 0.03</td>
<td>0.44 ± 0.04</td>
</tr>
<tr>
<td><strong>PV change (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>3.5 ± 7.6</td>
<td>0.03 ± 4.3</td>
<td>3.3 ± 13.4</td>
</tr>
<tr>
<td>TG</td>
<td>4.8 ± 12.1</td>
<td>-0.92 ± 8.77</td>
<td>3.6 ± 13.4</td>
</tr>
</tbody>
</table>

Data are mean (± S.D.). *Significant difference from day 1 (P < 0.05). †Significant difference between groups (P < 0.05). ‡Significant difference from day 6 (P < 0.05).

7.4.2.5. Sweat rate

Sweat rate was significantly higher in PA compared to TG on days six (1.15 ± 0.32 vs 0.65 ± 0.21 \text{l.min}^{-1}) and twelve (1.19 ± 0.51 vs 0.69 ± 0.22 \text{l.min}^{-1}) respectively and was significantly higher during PA on day six and day twelve compared to day one (main effect day*regime: \(F_{(2,30)} 7.562, P = 0.002, \eta_p^2 = 0.335\): Post hoc; all P values < 0.05). Sweat rate did not change over time with TG (all P values > 0.05).

7.4.2.6. Haemoglobin, haematocrit and plasma volume

Haemoglobin was not different between or within groups over time (main effect day*regime: \(F_{(2,30)} 0.383, P = 0.685, \eta_p^2 = 0.025\), Table 7.3.). Similarly, haematocrit was not different between or within groups over time (main effect day*regime: \(F_{(2,30)} 0.337, P = 0.717, \eta_p^2 = 0.022\), Table 7.3.). Plasma volume was increased 3.4 ± 7.6 and 4.8 ± 12.1% in PA and TG, respectively, from day one to six. From day six to twelve plasma volume increased 0.03 ± 4.3% and decreased by -0.9 ± 8.7% in PA and TG, respectively. The change in plasma volume was not significant within or between groups (main effect day*regime: \(F_{(2,30)} 1.622, P = 0.215, \eta_p^2 = 0.104\), Table 7.3.).
7.4.3. Part C: Performance, physiological and perceptual responses to intermittent-sprint exercise in 33°C, 50% rh pre-post twelve days of PA or TG

7.4.3.1. Participants

Two participants (one from PA and TG respectively) were unable to complete the final CISP due to injury. Consequently, data analyses for performance, physiological, perceptual and neuromuscular responses to intermittent-sprint exercise were analysed on fifteen participants (PA = 8, TG = 7). Pre-exercise body mass and hydration status were not different between CISP 1 and 2 in any group (all main effect P values > 0.05) indicating participants were in a similar hydration state prior to intermittent-sprint exercise in the heat.

7.4.3.2. PPO and Work Done

PPO during CISP 1 and 2 was not significantly different between or within groups after PA or TG (CISP 1, 1058 ± 148 and 935 ± 182 W in PA and TG, respectively; CISP 2, 1060 ± 120 and 995 ± 157 W in PA and TG, respectively) (main effect day*regime: $F_{(1,13)} = 1.232$, $P = 0.289$, $\eta^2_p = 0.093$, Figure 7.15.). During exercise, PPO was reduced in CISP 1 by 6.7% in PA and by 5.3% in TG. Post PA or TG, PPO was reduced in CISP 2 by 6.8% in PA and 3.2% in TG. The reductions in performance over time were not significant between or within conditions (main effect regime*day*time: $F_{(19,228)} = 0.304$, $P = 0.998$, $\eta^2_p = 0.025$, Figure 7.15.). There was a significant effect of regime on work done in CISP 1 and 2 (CISP 1, 3047 ± 409 and 2751 ± 495 W in PA and TG respectively; CISP 2, 2995 ± 349 and 2836 ± 157 W PA and TG respectively) (main effect day*regime: $F_{(1,13)} = 5.507$, $P = 0.037$, $\eta^2_p = 0.315$). Work done was reduced during CISP 1 by 4.8% in PA and 6.2% in TG. After PA and TG, work done during CISP 2 was reduced 1 and 3% in PA and TG, respectively (main effect regime*day*time: $F_{(19,228)} = 0.340$, $P = 0.996$, $\eta^2_p = 0.028$, Figure 7.15.).
Figure 7.15. Mean (± S.D.) PPO (W) and Work Done (J) in CISP 1 and CISP 2.

7.4.3.3. Heart rate

Resting heart rate prior to intermittent sprinting was not significantly different between groups pre CISP 1 or CISP 2, but was reduced by PA (main effect day*regime: $F_{(1,13)} \ 4.814$, $P = 0.047$, $\eta^2_p = 0.104$: Post hoc; $P < 0.05$, Table 7.4.). Mean exercise heart rate was reduced in CISP 2 compared to CISP 1 (main effect day: $F_{(1,12)} \ 18.544$, $P = 0.001$, $\eta^2_p = 0.607$). Heart rate increased during intermittent-sprint exercise (main effect time: $F_{(19,228)} \ 56.816$, $P < 0.001$, $\eta^2_p = 0.826$) and mean exercise heart rate was significantly lower in CISP 2 after PA (main effect day*regime: $F_{(1,12)} \ 7.777$, $P = 0.016$, $\eta^2_p = 0.393$: Post hoc; $P < 0.05$, Table 7.4) from sprint one to twenty (all $P$ values $< 0.05$). TG did not reduce mean exercise heart rate during the CISP ($P = 0.335$). Although peak heart rate tended to decline after both PA and TG (-13 and -7 beats.min$^{-1}$ in PA and TA respectively, Table 7.4).
the change was not significant from CISP 1 to 2 (main effect day*regime: $F_{(1,12)} = 2.324$, $P = 0.151$, $\eta_p^2 = 0.152$).

Table 7.4. Physiological and perceptual measures during intermittent-sprint exercise before and after PA or TG.

<table>
<thead>
<tr>
<th></th>
<th>CISP 1 (pre intervention)</th>
<th>CISP 2 (post intervention)</th>
<th>Absolute</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Resting HR (b.min$^{-1}$)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>65 ± 9</td>
<td>55 ± 4.2*</td>
<td>-10</td>
</tr>
<tr>
<td>TG</td>
<td>67 ± 13</td>
<td>64 ± 10</td>
<td>-3</td>
</tr>
<tr>
<td><strong>Exercise HR$_{\text{mean}}$ (b.min$^{-1}$)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>159 ± 17</td>
<td>142 ± 18*</td>
<td>-17</td>
</tr>
<tr>
<td>TG</td>
<td>160 ± 20</td>
<td>155 ± 18</td>
<td>-5</td>
</tr>
<tr>
<td><strong>Exercise HR$_{\text{peak}}$ (b.min$^{-1}$)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>168 ± 19</td>
<td>155 ± 19</td>
<td>-13</td>
</tr>
<tr>
<td>TG</td>
<td>173 ± 14</td>
<td>166 ± 18</td>
<td>-7</td>
</tr>
<tr>
<td><strong>Resting $T_{\text{re}}$ (°C)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>37.3 ± 0.2</td>
<td>37.0 ± 0.2*</td>
<td>-0.3</td>
</tr>
<tr>
<td>TG</td>
<td>37.5 ± 0.4</td>
<td>37.5 ± 0.3</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>Exercise $T_{\text{re mean}}$ (°C)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>37.9 ± 0.4</td>
<td>37.6 ± 0.4</td>
<td>-0.3</td>
</tr>
<tr>
<td>TG</td>
<td>38.0 ± 0.5</td>
<td>37.8 ± 0.4</td>
<td>-0.2</td>
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<tr>
<td><strong>Exercise $T_{\text{re peak}}$ (°C)</strong></td>
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<td></td>
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<tr>
<td>PA</td>
<td>38.5 ± 0.36</td>
<td>38.1 ± 0.3</td>
<td>-0.4</td>
</tr>
<tr>
<td>TG</td>
<td>38.5 ± 0.44</td>
<td>38.2 ± 0.3</td>
<td>-0.3</td>
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<tr>
<td><strong>Exercise PSI$_{\text{mean}}$</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>5.5 ± 1.8</td>
<td>4.7 ± 1.4</td>
<td>-0.8</td>
</tr>
<tr>
<td>TG</td>
<td>5.5 ± 1.7</td>
<td>4.6 ± 1.5</td>
<td>-0.9</td>
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<tr>
<td><strong>Exercise PSI$_{\text{peak}}$</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>7.2 ± 1.8</td>
<td>6.1 ± 1.4</td>
<td>-1.1</td>
</tr>
<tr>
<td>TG</td>
<td>7.3 ± 1.3</td>
<td>6.3 ± 1.1</td>
<td>-1.0</td>
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<tr>
<td><strong>$T_{sk}$ (°C)</strong></td>
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<tr>
<td>PA</td>
<td>35.15 ± 0.8</td>
<td>34.82 ± 0.65*</td>
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</tr>
<tr>
<td>TG</td>
<td>35.38 ± 0.7</td>
<td>35.08 ± 0.67</td>
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<tr>
<td><strong>BHC (J)</strong></td>
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<tr>
<td>PA</td>
<td>10615 ± 1143</td>
<td>10471 ± 1075</td>
<td>-144</td>
</tr>
<tr>
<td>TG</td>
<td>10224 ± 1490</td>
<td>10181 ± 1425</td>
<td>-43</td>
</tr>
<tr>
<td><strong>Sweat rate (l.hr$^{-1}$)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>1.31 ± 0.19</td>
<td>1.48 ± 0.21</td>
<td>+0.17</td>
</tr>
<tr>
<td>TG</td>
<td>1.32 ± 0.52</td>
<td>1.39 ± 0.35</td>
<td>+0.07</td>
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<tr>
<td><strong>Blood [lactate] (mM)</strong></td>
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</tr>
<tr>
<td>PA</td>
<td>3.9 ± 1.1</td>
<td>3.2 ± 0.7</td>
<td>-0.7</td>
</tr>
<tr>
<td>TG</td>
<td>4.1 ± 1.2</td>
<td>3.9 ± 1.2</td>
<td>-0.2</td>
</tr>
<tr>
<td><strong>VO$_2$ (l.min$^{-1}$)</strong></td>
<td></td>
<td></td>
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<tr>
<td>PA</td>
<td>2.3 ± 0.3</td>
<td>2.3 ± 0.2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>CISP 1 (pre intervention)</td>
<td>CISP 2 (post intervention)</td>
<td>Absolute</td>
</tr>
<tr>
<td>-------------------</td>
<td>--------------------------</td>
<td>-----------------------------</td>
<td>----------</td>
</tr>
<tr>
<td><strong>Exercise RPE&lt;sub&gt;mean&lt;/sub&gt;</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>14 ± 3</td>
<td>12 ± 2</td>
<td>-2</td>
</tr>
<tr>
<td>TG</td>
<td>14 ± 3</td>
<td>12 ± 2</td>
<td>-2</td>
</tr>
<tr>
<td><strong>Exercise RPE&lt;sub&gt;peak&lt;/sub&gt;</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>16 ± 2</td>
<td>13 ± 2</td>
<td>-3</td>
</tr>
<tr>
<td>TG</td>
<td>16 ± 3</td>
<td>12 ± 2</td>
<td>-4</td>
</tr>
<tr>
<td><strong>Exercise T&lt;sub&gt;sen mean&lt;/sub&gt;</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>6.2 ± 0.7</td>
<td>5.7 ± 0.5*</td>
<td>-0.5</td>
</tr>
<tr>
<td>TG</td>
<td>6.2 ± 0.8</td>
<td>5.7 ± 0.5*</td>
<td>-0.5</td>
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<tr>
<td><strong>Exercise T&lt;sub&gt;sen peak&lt;/sub&gt;</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>6.9 ± 0.6</td>
<td>6.3 ± 0.7</td>
<td>-0.6</td>
</tr>
<tr>
<td>TG</td>
<td>6.8 ± 0.8</td>
<td>6.0 ± 0.5</td>
<td>-0.8</td>
</tr>
</tbody>
</table>

Data are mean (± S.D.). *Significant difference from CISP 1.

7.4.3.4. T<sub>re</sub> and T<sub>sk</sub>

Resting T<sub>re</sub> prior to intermittent sprinting was not significantly different between groups pre CISP 1 or CISP 2 but was reduced by PA (main effect regime*day: F<sub>(1,13)</sub> 5.188, P = 0.046, η<sup>p</sup><sup>2</sup> = 0.342: Post hoc; P < 0.05, Table 7.4.). Mean exercise T<sub>re</sub> was reduced in CISP 2 compared to CISP 1 (main effect day: F<sub>(1,12)</sub> 19.893, P = 0.001, η<sup>p</sup><sup>2</sup> = 0.624). The reduction in T<sub>re</sub> from CISP 1 to CISP 2 was 0.3°C in PA and 0.2°C in TG, but was not different between or within groups (main effect day*regime: F<sub>(1,12)</sub> 3.037, P = 0.107, η<sup>p</sup><sup>2</sup> = 0.202, Table 7.4.). Similarly, T<sub>re</sub> increased during intermittent-sprint exercise (main effect time: F<sub>(19,228)</sub> 1.523, P < 0.001, η<sup>p</sup><sup>2</sup> = 0.938) but the increase was not different between or within PA nor TG. Although peak exercise T<sub>re</sub> tended to decline after both PA and TG (0.4°C, 0.3°C respectively) the change was not significant from CISP 1 to CISP 2 (main effect regime*day: F<sub>(1,13)</sub> 2.334, P = 0.151, η<sup>p</sup><sup>2</sup> = 0.152, Table 7.4.). T<sub>sk</sub> was lower in CISP 2 compared to CISP 1 from sprint twelve onwards following PA (main effect regime*day*time: F<sub>(19,228)</sub> 3.166, P < 0.001, η<sup>p</sup><sup>2</sup> = 0.209: Post hoc; all P values < 0.05, Table 7.4.). TG did not reduce T<sub>sk</sub> during intermittent-sprint exercise in the heat (all P values > 0.05).
7.4.3.5. Sweat rate, PSI, BHC and heat production

Sweat rate was not different within or between groups in CISP 1 and 2 after PA or TG (main effect regime*day: $F_{(1,13)}$ 0.597, $P = 0.453$, $\eta^2_p = 0.044$). PSI was reduced in CISP 2 (main effect day: $F_{(1,12)}$ 13.222, $P = 0.003$, $\eta^2_p = 0.524$), but was not significantly different between or within groups after PA or TG (main effect regime*day: $F_{(1,12)}$ 0.388, $P = 0.545$, $\eta^2_p = 0.031$). Peak PSI was reduced from CISP 1 to CISP 2 (main effect day: $F_{(1,13)}$ 0.012, $P < 0.001$, $\eta^2_p = 0.650$), but was not significantly different between or within groups after PA or TG (main effect regime*day: $F_{(1,13)}$ 0.012, $P = 0.914$, $\eta^2_p = 0.001$). BHC (J) was lower in CISP 2 compared to CISP 1 in all twenty sprints following PA (main effect regime*day*time: $F_{(19,228)}$ 3.475, $P < 0.001$, $\eta^2_p = 0.225$: Post hoc; all $P$ values < 0.05, Table 7.4.). TG did not reduce BHC during intermittent-sprint exercise in the heat (all $P$ values > 0.05). Heat production (W and W.m$^{-2}$-1) in CISP 1 and CISP 2 in PA (755 ± 103 vs. 766 ± 85 W and 378 ± 51 vs. 383 ± 42 W.m$^{-2}$-1) and TG (697 ± 94 vs. 743 ± 126 W and 358 ± 44 vs. 381 ± 59 W.m$^{-2}$-1) was not significantly different within or between groups (all main effect $P$ values > 0.05).

7.4.3.6. Blood lactate and $\dot{V}O_2$

Mean blood lactate concentration was not significantly different between or within PA or TG (main effect regime*trial*time: $F_{(4,52)}$ 1.273, $P = 0.292$, $\eta^2_p = 0.089$, Table 7.4.). Similarly, mean $\dot{V}O_2$ did not differ between or within groups over time (main effect regime*trial*time: $F_{(4,52)}$ 1.189, $P = 0.328$, $\eta^2_p = 0.090$, Table 7.4.).

7.4.3.7. RPE and $T_{sen}$

RPE was not significantly different between or within groups after PA or TG (main effect regime*day: $F_{(1,13)}$ 0.528, $P = 0.480$, $\eta^2_p = 0.039$) but was reduced from CISP 1 to CISP 2 (main effect day: $F_{(1,13)}$ 8.830, $P = 0.011$, $\eta^2_p = 0.404$). RPE increased during intermittent-sprint exercise (main effect time: $F_{(19,247)}$ 55.233, $P < 0.001$, $\eta^2_p = 0.809$), but was not different within or between groups from CISP 1 to 2 (main effect regime*time; $F_{(19,247)}$...
Peak RPE was reduced from CISP 1 to CISP 2 (main effect day: $F_{(1,13)} = 16.911$, $P = 0.001$, $\eta_p^2 = 0.565$), but was not significantly different between or within groups after PA or TG (main effect regime*day: $F_{(1,13)} = 0.194$, $P = 0.667$, $\eta_p^2 = 0.015$). $T_{\text{sen}}$ was not significantly different between PA and TG in CISP 1 or CISP 2 (Chi Squared = 0.388, $P = 0.533$, ES = 0.009 and 2.927, 0.087, 0.075 respectively). $T_{\text{sen}}$ was, however, reduced by both PA (Chi Squared = 5.615, $P = 0.018$, ES = 0.1439) and TG (Chi Squared = 13.248, $P < 0.001$, ES = 0.2561). After PA, $T_{\text{sen}}$ was lower in CISP 2 compared to CISP 1 from sprint seven (all P values < 0.05). After TG, $T_{\text{sen}}$ was lower in CISP 2 compared to CISP 1 from sprint 16 – 20 (all P values < 0.05). Peak $T_{\text{sen}}$ was not different between or within groups (Chi Squared = 1.825, $P = 0.177$, ES = 0.1216).

### 7.4.4. Part D: Neuromuscular responses to intermittent-sprint exercise in the heat

#### 7.4.4.1. Muscular function

MVC force (N) was reduced by intermittent-sprint exercise in the heat (main effect time; $F_{(1,13)} = 152.7$, $P < 0.001$, $\eta_p^2 = 0.922$) and there was a significant interaction (main effect trial*time*regime; $F_{(1,13)} = 5.013$, $P = 0.043$, $\eta_p^2 = 0.278$). Pre post CISP 1 MVC decreased in PA (498 ± 109 vs 397 ± 98 N respectively, Table 7.5.) and was also decreased pre post CISP 2 after twelve days of PA (507 ± 103 vs 423 ± 96, all P values < 0.05, Table 7.5.). In TG, MVC was reduced by intermittent-sprint exercise in the heat in CISP 1 (510 ± 72 vs 411 ± 58 N) and CISP 2 (503 ± 66 vs 382 ± 38 N, all P values < 0.05, Table 7.5.).

A trend toward a reduction in peripheral voluntary activation (%) was observed in both PA and TG in CISP 1 and CISP 2 (Table 7.5.), however, while there was an effect of time (main effect; $F_{(1,13)} = 17.165$, $P = 0.001$, $\eta_p^2 = 0.569$) there was no significant difference within or between groups over time (main effect trial*time*regime; $F_{(1,13)} = 0.270$, $P = 0.612$, $\eta_p^2 = 0.20$). ERT during PA was reduced in CISP 1 and 2 by 30.8 and 21.5% respectively (Table 7.5.). In TG ERT was also reduced in both CISPs (24.9 and 11.3%, CISP 1 and 2 respectively, Table 7.5.). The observed differences were not significantly different within or between groups (main effect trial*time*regime; $F_{(1,13)} = 0.018$, $P = 0.895$, $\eta_p^2 = 0.001$, Table 7.5.).
Q_{tw, pot} (N) was also reduced after intermittent-sprint exercise in the heat and there was a significant interaction (main effect trial*time*regime; F\(_{(1,13)}\) \(5.743, P = 0.032, \eta_p^2 = 0.306\)). Pre-post CISP 1 Q_{tw, pot} decreased in PA (- 24.7%) and was also reduced pre post CISP 2 (-17.6%) (Table 7.5., all P values < 0.05). Similarly, with TG Q_{tw, pot} was reduced pre-post both CISPs (- 20.5 and - 19.1% respectively, all P values < 0.05, Table 7.5.). There was no effect of time and no interaction effect of intermittent-sprint exercise on MRFD, MRR and RT\(_{0.5}\) pre-post CISP 1 or CISP 2 in PA or TG (all main effect P values > 0.05, Table 7.5.).

### 7.4.4.2. EMG activity

Maximum M wave amplitude (mV) and area (\(\mu\text{V.s}^{-1}\)) was not different pre-post CISP 1 or 2 in PA or TG (all main effect P values > 0.05, Table 7.5.). Similarly, raw RMS EMG activity of the vastus lateralis (main effect trial*time*regime; F\(_{(1,13)}\) \(0.385, P = 0.546, \eta_p^2 = 0.031\), Table 7.5.) and normalized activity (RMS/Mmax) (main effect trial*time*regime; F\(_{(1,13)}\) \(1.390, P = 0.261, \eta_p^2 = 0.104\), Table 7.5) were not altered by intermittent sprinting in the heat before or after twelve days of PA or TG.

### 7.4.4.3. Cortical drive

There was no significant difference in cortical voluntary activation between PA and TG in CISP 1 or CISP 2 (main effect regime*trial; F\(_{(1,13)}\) \(0.377, P = 0.550, \eta_p^2 = 0.028\), but it differed over time (main effect time; F\(_{(1,13)}\) \(20.188, P = 0.001, \eta_p^2 = 0.608\)). During CISP 1 cortical voluntary activation decreased in PA (-6.1%) and TG (-9.8%) (all P values > 0.05). Similarly, After twelve days of PA or TG, cortical voluntary activation was reduced in both PA and TG (5.6% and 7.5% respectively, all P values > 0.05), however there was no difference in cortical voluntary activation within or between groups pre and post intervention (main effect regime*trial*time; F\(_{(1,13)}\) \(0.131, P = 0.723, \eta_p^2 = 0.010\)). In PA the mean SIT pre-post CISP 1 and 2 was 4.8 \pm 1.9 vs 4.6 \pm 1.9% and 3.7 \pm 1.6 vs 4.3 \pm 2.3% of MVC respectively. After TG, SIT as a percentage of MVC in CISP 1 and 2 was 2.0 \pm 1.6 vs 3.1 \pm 1.7% and 2.6 \pm 2.9 and 4.3 \pm 3.5% respectively. These differences were not
significant within or between groups (main effect regime*trial*time; $F_{(1,13)}$ 0.052, $P = 0.823$, $\eta^2_p = 0.004$).

MEP amplitude (mV) and MEP area ($\mu V.s^{-1}$) measured during brief voluntary contractions was not affected by intermittent-sprint exercise in the heat pre-post CISP 1 or CISP 2 in PA or TG (all main effect $P$ values > 0.05, Table 7.5.). When MEP area was normalized to Mmax a significant interaction was observed (main effect regime*trial*time; $F_{(1,13)}$ 5.743, $P = 0.032$, $\eta^2_p = 0.306$). MEP$_{area}$/Mmax$_{area}$ was significantly increased after TG in CISP 1 compared to CISP 2 ($P = 0.036$), was significantly higher post CISP before compared to after TG ($P = 0.008$) and was significantly different compared to PA at this timepoint in CISP 1 ($P = 0.032$). PA did not alter MEP$_{area}$/Mmax$_{area}$ (all $P$ values > 0.05). Cortical silent period and latency were not significantly altered pre-post CISP one or two in PA or TG (all main effect $P$ values > 0.05, Table 7.5).
Table 7.5. Mean (± S.D.) neuromuscular function pre-post CISP 1 and 2 in PA and TG.

<table>
<thead>
<tr>
<th></th>
<th>PA</th>
<th>TG</th>
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<tbody>
<tr>
<td></td>
<td>CISP 1 Pre</td>
<td>CISP 1 Post</td>
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<tr>
<td></td>
<td>CISP 1 PRE</td>
<td>CISP 1 Post</td>
</tr>
<tr>
<td><strong>Muscular function</strong></td>
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<tr>
<td>MVC (N)</td>
<td>498 ± 109</td>
<td>397 ± 98*</td>
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<tr>
<td>Qtw,pot (N)</td>
<td>182 ± 31</td>
<td>137 ± 26*</td>
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<tr>
<td>MRR (N.ms⁻¹)</td>
<td>-0.730 ± 0.433</td>
<td>-0.978 ± 0.655</td>
</tr>
<tr>
<td>RT₀.₅ (ms)</td>
<td>91 ± 16</td>
<td>64 ± 24</td>
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<tr>
<td>ERT (N)</td>
<td>144 ± 51</td>
<td>99.6 ± 35</td>
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<tr>
<td>Peripheral VA (%)</td>
<td>88.4 ± 4.8</td>
<td>86.2 ± 6.2</td>
</tr>
<tr>
<td><strong>EMG activity</strong></td>
<td></td>
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<tr>
<td>Mmax amplitude (mV)</td>
<td>7.1 ± 2.5</td>
<td>6.6 ± 2.5</td>
</tr>
<tr>
<td>Mmax area (μV.s⁻¹)</td>
<td>39.7 ± 13.9</td>
<td>35.1 ± 15.6</td>
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<tr>
<td>Raw RMS (μV)</td>
<td>328 ± 117</td>
<td>309 ± 76</td>
</tr>
<tr>
<td>RMS/Mmax</td>
<td>0.047 ± 0.012</td>
<td>0.051 ± 0.015</td>
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<tr>
<td><strong>Cortical drive</strong></td>
<td></td>
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<tr>
<td>MEP amplitude (mV)</td>
<td>2.3 ± 1.1</td>
<td>2.1 ± 0.7</td>
</tr>
<tr>
<td>MEP area (μV.s⁻¹)</td>
<td>21.1 ± 8.5</td>
<td>16.3 ± 4.4</td>
</tr>
<tr>
<td>MEP_area/Mmax_area (%)</td>
<td>53.1 ± 11.1</td>
<td>48.7 ± 15.6</td>
</tr>
<tr>
<td>CSP (ms)</td>
<td>159 ± 67</td>
<td>157 ± 64</td>
</tr>
<tr>
<td>Latency (ms)</td>
<td>18 ± 9</td>
<td>18 ± 7</td>
</tr>
<tr>
<td>SIT (N)</td>
<td>19 ± 13</td>
<td>18 ± 13</td>
</tr>
<tr>
<td>Cortical VA (%)</td>
<td>89.2 ± 6.1</td>
<td>85.5 ± 6.7</td>
</tr>
</tbody>
</table>

Data are mean (± S.D.). *Significant difference from pre to Post in either CISP 1 or CISP 2 (P < 0.05). †Significant difference between groups at designated time point (P < 0.05). +Significant difference within group at designated timepoint (P < 0.05).
7.5. Discussion

The objectives of this study were to assess the neuromuscular responses to intermittent-sprint exercise in the heat and to examine the effect of progressive heat acclimation on central and peripheral fatigue during intermittent-sprint exercise in the heat using TMS and FNS. This study is the first to use TMS to assess fatigue in intermittent-sprint exercise in the heat and a novel finding was the reduction in cortical voluntary activation when intermittent-sprint exercise was completed over forty minutes duration in 33°C, 50% rh. Further, supporting the evidence in chapter six, it was observed that a progressive model of heat acclimation improves physiological strain during intermittent-sprint exercise in the heat. However, contrary to the proposed hypothesis, progressive heat acclimation does not alter the central or peripheral contributions to fatigue during intermittent-sprint exercise of forty minutes duration.

7.5.1. Performance and neuromuscular function during intermittent-sprint exercise in the heat

Pooled data for all seventeen participants completing the study was used to examine the effect of intermittent-sprinting in 33°C and 50% rh on performance and neuromuscular function. In contrast to more recent work (Almudehki et al., 2012; Girard et al., 2013a), completion of the CISP reduced PPO (W) (~4%) and work done (J) (~4.5%). In addition, both performance variables were significantly negatively correlated with $T_{re}$ (°C) ($r = -0.926$, $P < 0.001$ and $r = -0.931$, $P > 0.001$, PPO and WD respectively). The discrepancy between our findings and those of recent studies cited may be due to differences in protocols used and the extent of hyperthermia induced. Specifically, when total exercise duration is brief, the number of sprints completed is low and the recoveries prolonged, the extent of hyperthermia induced is small and performance is not negatively affected (Almudehki et al., 2012; Girard et al., 2013a). In contrast, when protocols that replicate durations and work:rest patterns of field-based, team-sports are used, similar to this study, greater hyperthermia is induced and performance is impaired (Morris et al., 1998,
2000; Castle et al., 2006; 2011). For example, similar to this work, Castle et al., (2006, 2011) reported a 2 - 4% decrement in PPO using the CISP in 33°C, 50% rh. Although heat may alter muscle metabolism, previous work in this thesis (chapter five and six) and other research (Morris et al., 1998, 2000; Drust et al., 2005; Sunderland et al., 2005; Castle et al., 2006) has demonstrated no difference in metabolic factors between hot and control conditions during intermittent-sprint exercise, suggesting that such exercise maximally taxes glycolysis and the addition of a heat stress does not contribute to elevated strain. As such, as in previous work, the performance decrement may be attributed to a CNS mediated effect which was further examined.

The reduction in performance in the current investigation was accompanied by a significant reduction in maximal voluntary contraction force (−19%) pre to post intermittent-sprint exercise (Figure 7.13.), similar to that observed in other studies (Racinais et al., 2007; Perrey et al., 2010b). Previously, decreases in MVC force have been reported to occur in conjunction with reductions in voluntary drive due to both modest and marked elevations in core temperature by passive hyperthermia (Morrison et al., 2004; Thomas et al., 2006; Ross et al., 2012). As such, in the current study the reduced MVC force may, in part, be attributed to the decrement in cortical and peripheral voluntary activation (−4.4 and −3.3% respectively, Figure 7.14.) induced by hyperthermia during intermittent-sprinting in the heat (peak body temperature ≈ 38.5°C). MEP amplitude was not different following intermittent-sprint exercise, indicating that motor cortex excitability was well preserved and did not contribute to the decreased voluntary descending drive. Similarly, cortical silent period was unaltered suggesting that intermittent-sprinting did not alter intracortical inhibitory neuron activity and therefore, did not inhibit descending drive. Taken together with the unchanged RMS:M wave ratio indicating no change in the number of motoneurons recruited, absence of change in MEPs and CSP suggest that the integrity and responsiveness of the motor neurons from motor cortex to muscle was maintained during intermittent-sprint exercise in the heat (Sidhu et al., 2009a). However, recovery of MEP and CSP characteristics post exercise have been reported despite the persistence of impaired voluntary descending drive indicating a faster recovery rate for
these variables (Sidhu et al., 2009a). Therefore, the influence of the time from exercise cessation to completion of the NMF protocol in the current study (~4 minutes) cannot be discounted. Given that impaired voluntary descending drive persisted despite maintenance of motor cortex integrity, it may be that the demand of intermittent-sprint exercise prolonged group III and IV afferent feedback providing increased feedback to the CNS at a level upstream of the motor cortical outputs to impair voluntary descending drive (Taylor et al., 2006, Sidhu et al., 2012a).

Previous research reports conflicting findings with respect to voluntary activation in sprint-type exercise (Racinais et al., 2007; Perrey et al., 2010b; Girard et al., 2013a). Reductions in voluntary activation, similar in magnitude to those reported in this study, were reported in repeated-sprint cycling and running in temperate conditions using the twitch interpolation technique suggesting a central contribution to fatigue in exercise of this type (Racinais et al., 2007; Perrey et al., 2010b). Recently however, TMS was used for the first time to examine repeated-sprint cycling in temperate conditions and, in contrast to previous work, no reduction in voluntary activation was observed in brief MVCs (Girard et al., 2013a). Methodological differences in the assessment of voluntary activation may explain differences between the recent work of Girard et al., (2013a) and the earlier studies of Racinais et al., (2007) and Perrey et al., (2010b). Essentially, the interpolated twitch technique can provide an estimate of drive to muscles, but is proposed not to provide an indication of descending drive to motor neurons or take into account the non-linear input-output relationship of the motor neuron pool (Herbert and Gandevia, 1999). In addition, increased isometric force, indicative of central fatigue, has been observed in intact single fibres when such an occurrence should not be possible (Place et al., 2008). Therefore, it is proposed that an alternate mechanism, increased Ca$^{2+}$ concentration, may contribute to the relative increase in the extra force produced by an interpolated twitch as opposed to increased central fatigue. Therefore, it is suggested the interpolated twitch technique over-estimates voluntary activation and central fatigue (Place et al., 2010).
While methodological limitations may explain the differences in voluntary activation recorded in previous studies, why the current work differs from that of Girard et al., (2013a) when both have used TMS to assess cortical voluntary activation, remains unclear. Greater sprint number and protocol duration in the current study may provide an explanation. In addition, completion of the exercise in heat stress and the subsequent hyperthermia may have contributed to findings. Hyperthermia is known to evoke decrements in central drive and voluntary force at core temperatures similar to those observed during intermittent-sprint exercise in the current study (Morrison et al., 2004; Thomas et al., 2006). Further, hyperthermia has been shown to reduce cerebral blood flow (Nybo and Nielsen, 2001b; Ross et al., 2012). In hypoxia, decreased cerebral blood flow contributes to decreased cerebral oxygenation and a two-fold greater decrease in cortical voluntary activation compared to control conditions (Goodall et al., 2012a). As such, it is feasible that the duration of exercise and extent of hyperthermia induced in the current work contributed to decreased cerebral blood flow and oxygenation that together contributed to a decline in cortical voluntary activation. However, TMS used to assess sustained and incremental exercise in temperate and hot environments suggests that decrements in voluntary activation are dependent on the exercise, but independent of the environment (Racinais and Girard, 2012). Whether this is the same when intermittent-sprint exercise that reflects work:rest patterns and durations of field-based team-sports is completed in a hot environment remains unclear. More recent work in a field-based setting (Nybo et al., 2013), demonstrated using the interpolated twitch technique that voluntary activation although reduced post exercise was not different when a simulated football match was completed in ~ 43°C and ~ 21°C. While this again suggests fatigue is task dependent, but environment independent it is important to note that, due to the self-paced nature of the football match (Nybo et al., 2013), total game distance and high intensity running declined by 7% and 26% compared to control during the match in hot conditions which may have contributed to extent of fatigue observed post exercise.

Suppression of MVC force after forty minutes of intermittent-sprint exercise in 33°C, 50% rh in the current study was accompanied by a significant reduction in $Q_{tawpot}$ (-21.4%)
within the values reported previously for repeated-sprinting exercise (Racinais et al., 2007, Perrey et al., 2010b, Girard et al., 2013a). As an indicator of peripheral fatigue, reductions in $Q_{tw,tot}$ may be mediated by altered sarcolemmal excitability whereby failure of the $Na^+/K^+$ pump to redress the efflux of $K^+$ to the extracellular environment may cause membrane depolarization and impair action potential transmission and propagation (Martin et al., 2010; Perrey et al., 2010b). Altered sarcolemmal excitability manifests in reduced M wave amplitude, however, in repeated sprinting M wave amplitude has been reported to increase (Racinais et al., 2007) not change (Girard et al., 2013a) or decrease (Perrey et al., 2010b) providing conflicting evidence as to the role of sarcolemmal excitability in repeated-sprint exercise. In the current study, M wave amplitude was also unchanged pre-post intermittent-sprint exercise in the heat indicating that sarcolemmal excitability was not impaired and that peripheral fatigue in exercise of this type was not mediated by impaired action potential transmission or propagation. Discrepancies in findings pertaining to M wave kinetics during repeated or intermittent-sprint exercise may be explained by the mode of exercise and type of muscle contraction involved, whereby exercise with primarily concentric work (for example, cycling) may not impair membrane excitability, but exercise with a distinct stretch-shortening cycle (for example, running) might (Perrey et al., 2010b).

Alterations in contractile properties of muscle have also been implicated in peripheral fatigue (Perrey et al., 2010b; Ross et al., 2012). In the current study both MRFD and $RT_{0.5}$ were reduced after intermittent-sprint exercise in the heat and MRR, although not significant, was 12.5% faster. A reduced MRFD may contribute to peripheral fatigue through impaired functioning of the sarcoplasmic reticulum pump altering $Ca^{2+}$ release and excitation-contraction coupling leading to reduced sprinting efficiency (Perrey et al., 2010b). In addition, the faster relaxation rate of muscle due to exercise in the heat may have contributed to decreased MVC force due to the higher motor unit firing rates required to fuse force and maintain contraction (Ross et al., 2012). It has, however, been suggested that the latter factor has limited effect in brief MVCs as used in the current study as motor unit discharge may be sufficiently augmented during brief efforts to maintain force (Ross et al., 2012) and overall, further work needs to be completed.
examining the genesis of peripheral fatigue during intermittent-sprint cycle exercise in the heat.

7.5.2. Responses to progressive heat acclimation and training

Similar to the work in chapter six, a progressive heat acclimation protocol was used in this study to evoke adaptation and confer the heat acclimated phenotype. This protocol employed the same stepwise increases in heat stress on day five and nine to maintain a constant heat strain for the duration of acclimation. Similar to chapter six, the effectiveness of the protocol was judged by its ability to evoke classic heat acclimation responses.

In this study, twelve days of PA significantly reduced $T_{re} \sim 0.3°C$ and heart rate $\sim 9$ b.min$^{-1}$ at rest and this response persisted to CISP 2 $\sim 0.3°C$ and $\sim 10$ b.min$^{-1}$. A significant reduction in resting $T_{re}$ during acclimation is in contrast with the findings of chapter six and may be explained by the fewer groups and comparisons in the current study. Consistent with chapter six, the reduction in resting $T_{re}$ with PA was still below that expected for heat acclimation (Wyndham et al., 1954; Patterson et al., 2004; Castle et al., 2011). Again, as reported in chapter six, while this may support the notion that time of day modulated the $T_{re}$ response, all acclimation sessions (and CISPs) were completed at the same time suggesting other factors may have contributed including possibly, differences in environmental conditions, protocols and participants. (Wyndham et al., 1954; Shido et al., 1999; Patterson et al., 2004; Castle et al., 2011).

In the current work, TG reduced exercise heart rate by day twelve (Table 7.3.). While this may indicate improved cardiovascular stability, supported by, although not significant, a 4.8% increase in plasma volume (Table 7.3.), considering a constant work rate protocol was used it may be argued such an observation merely reflects decreased physiological strain (Taylor, 2000). In PA physiological and thermal strain were maintained across the
protocol by stepwise increase in external heat stress on day five and nine, evidenced by no significant change in mean exercise heart rate and $T_{re}$ on day one, six and twelve (Table 7.3.). With respect to peak values, the final increase in heat stress significantly elevated exercise heart rate and $T_{re}$ compared to day six (Table 7.3.) highlighting the efficacy of progressive acclimation in maintaining physiological strain. That strain was enhanced in the current study by the progressive nature of the acclimation protocol is supported by perceptual responses as RPE and $T_{sen}$ were significantly elevated on day twelve compared to six and were significantly higher than TG in the same period (Table 7.3.). By comparison, RPE and $T_{sen}$ were reduced during TG in the same period.

Over twelve days, PA elevated sweat rate but TG did not have any effect (Table 7.3.). As suggested in chapter six, increased strain in PA may simply be an artefact of the increased heat stress, a fact supported by the lack of significant change in sweat rate during the CISP. However, while not significant, the trend toward an increased sweat rate, (11.5 and 5% respectively), indicates that there may have been some adaptation of sudomotor function at either a central (reduced threshold for sweating) or peripheral (improved sweat gland function) level (Nadel et al., 1974; Buono et al., 2009).

Similar to the findings reported in chapter six, plasma volume in the current work showed a trend toward expansion in both PA and TG that was essentially complete by day six (3.5 ± 7.6% and 4.8 ± 12.1%), but was not significantly different across days. Accepting large interindividual response may have contributed to this result, these data support the suggestion from study three that further work is required to optimise progressive heat acclimation to maximise this adaptation.

Twelve days of PA reduced resting heart rate and core temperature prior to CISP 2 (-10 b.min$^{-1}$ and -0.3°C, respectively). During exercise, this manifested as a decreased mean heart rate and a trend toward a reduced $T_{re}$ (-17 b.min$^{-1}$ and -0.3°C, respectively). RPE tended to be lower post PA and TG, but was not significantly different. Mean thermal
sensation was, however, reduced by both regimes. Taken together, this may suggest the progressive protocol used was not optimal for maximising adaptation to the heat. Given, however, that studies have reported significance with similar change in HR and T\text{re} (e.g. Fox et al., 1963; Patterson et al., 2004) it is possible the variability in measures contributed to the non-significant findings. Further, post hoc statistical power analysis suggests low sample size may have contributed to these findings and indicates that twelve participants per group may have provided significant effects.

7.5.3. Neuromuscular responses to intermittent-sprint exercise in the heat (analysis by group)

When data were analysed by group no difference in PPO and WD were observed during CISP 1 or 2 in PA or TG. That no difference in PPO was reported post acclimation, may reflect the maximal nature of the sprinting involved in the CISP protocol and the duration over which the analysis was conducted (i.e. 40 minutes). As 5 s sprints were ‘all-out’ the same level of fatigue may have been induced in CISP 2 (supported by similar decline in PPO over time in both CISPs) post acclimation that, due to the fixed duration of the protocol, prevented detection of any difference. Further, although WD was not different it is important to note the decline over time tended to be reduced post PA (CISP 1 vs. 2; 4.8% vs 1%), but also TG (6.2% vs. 3%). Given the reliability of the CISP as identified in chapter four, this may suggest that more work could be performed in the latter stages of the CISP post acclimation, that, due to the duration of the protocol and design of the study were not detected.

Although PPO and WD were not different post acclimation, maximal voluntary contraction force was, however, reduced in both PA and TG after CISP 1 and was also reduced in both groups after CISP 2 suggesting no effect of PA or TG. When TMS is applied over the motor cortex during a maximal voluntary contraction evidence of a SIT suggests cortical drive to the locomotor muscle is not maximal. Increases in post exercise SIT indicate a decrease in cortical voluntary drive and possibly centrally (supraspinal)
mediated fatigue. In the current study decrements in cortical drive to exercising muscle could not explain the reduced MVC force as, contrary to pooled data showing a significant decrease, cortical voluntary activation was not significantly different pre-post CISP 1 and was not changed by PA or TG when data was analysed by group (Table 7.5.). Further, despite voluntary activation showing a trend towards a decline post intermittent-sprint exercise before and after PA and TG (CISP 1, ~ -4 and -9% in PA and TG respectively; CISP 2, ~ -5 and -7% in PA and TG respectively) the SIT during MVCs was not different between or within conditions. Such findings may however, be a consequence of the sample size in this part of the study (PA = 8, TG = 7) as post hoc analysis (α = 0.05, 1 – β = 0.8, r = 0.5) suggests a sample size of twelve per group to achieve significance for this variable. As a measure of corticospinal excitability, MEP amplitude was not altered pre post CISP 1 or following PA or TG. Further, although MEP area normalised to Mmax area was significantly greater in CISP 1 in TG, this measure was not affected by PA or TG. Similarly, CSP and latency were unaffected by either intervention.

Only one previous investigation has examined neuromuscular fatigue in sprint exercise using TMS (Girard et al., 2013a). In contrast to the current work, this study used a brief repeated-sprint protocol in a temperate environment, but reported no change or trend toward a decline in cortical drive or excitability during brief maximal voluntary contractions (Girard et al., 2013a). This may be explained by the brevity of the protocol employed and the lack of exogenous heat insult. Previously, passive hyperthermia has resulted in decreased voluntary activation and voluntary force production (Morrison et al., 2004, Thomas et al., 2006) and as such, the extended nature of the protocol in the current study coupled with the inclusion of heat stress may have contributed to the trend in cortical voluntary activation scores that, based on reliability data (Goodall et al., 2009; Sidhu et al., 2009b) may indicate a potential meaningful change. Further work is needed to elucidate this point. Alternately, taken together, data from this study and Girard et al., (2013a) may indicate that cortical drive and cortiospinal excitability are not impaired and thus do not contribute to fatigue in repeated or intermittent-sprint exercise when cycle sprints of < 6 s are repeated 15 - 20 times with short (30 s) or long recoveries (30 s - 2 minutes) for a duration of between nine and forty minutes in temperate or hot
environments. This notion contrasts with previous findings (Racinais et al., 2007; Perrey et al., 2010b) that have demonstrated significant decrements (~3%) in voluntary activation during repeated cycling and running sprints. While the inter-individual differences in TMS response in the current study may explain the non-significance of the up to 9% decrements in voluntary activation, the discrepancy may also be explained by the use of the interpolated twitch technique to estimate voluntary activation (Racinais et al., 2007; Perrey et al., 2010b) as debate exists over the ability of this technique to measure voluntary descending drive, as previously described. In addition, as use of the interpolated twitch technique does not permit the site of impaired voluntary drive to be localised, spinal contributions to fatigue cannot be discounted. Further, the intraday and interday typical error of voluntary activation using the interpolated twitch technique is reported as ~2% - 8% (Place et al., 2007) and therefore, the data presented in the work of Racinais et al., (2007) and Perrey et al., (2010b) may reflect variation inherent in the measurement technique as opposed to a meaningful reduction in central drive. In addition, although normalised EMG RMS activity was reduced pre-post intermittent-sprinting (Racinais et al., 2007) and may be interpreted as decreased voluntary drive, EMG activity should be interpreted with caution as amplitude cancellation and reduced sensitivity to small modification in muscle activation compared to interpolated twitch may compromise findings (Kalmar and Cafarelli, 1999; Farina et al., 2004). Finally, differences in descending drive in current and previous work may relate to the predominant type of contraction during exercise whereby the eccentric-concentric pattern of muscle contraction in repeated running sprints may provide greater activation of and feedback from group III and IV afferents compared to the predominantly concentric contractions observed in cycling.

Interestingly, in the work of Girard et al., (2013a) failure of the motor cortex to maximally activate motorneurons was observed during prolonged (30 s) MVC’s. Consequently, it has been suggested that sustained MVC’s should be used to investigate corticospinal function during intermittent-sprint exercise. Considering however, that mean sprint durations in
intermittent field-based team-sports rarely exceed six seconds, the use of sustained MVC’s to assess central fatigue during exercise would have limited ecological validity.

The reduction in maximal voluntary contraction force observed in the current study was accompanied by a significant decrease in $Q_{tw\text{-}pot}$ that also persisted post CISP 2, again indicating no effect of PA or TG on this variable. Previously, changes in muscle contractile properties have been implicated in neuromuscular fatigue whereby repeated running sprints in temperate environments impaired MRFD, MRR and $RT_{0.5}$, but also temperature-induced changes with passive heating have been suggested to alter contractile properties and contribute to fatigue (Todd et al., 2005; Perrey et al., 2010b; Ross et al., 2012). In the current study, however, when data were analysed by group no significant change in MRFD, MRR or $RT_{0.5}$ were observed and there was no effect of PA or TG. However, MRFD was reduced by 24% during CISP 1 but only 2% during CISP 2 in PA whereas in TG this parameter was reduced 5.5% in CISP 1 and 7.3% in CISP 2. Similarly, MRR was faster by 24% in CISP 1 and only 15% in CISP 2 in PA, but was faster by 3% after CISP 1 and 7% after CISP 2 in TG. Combined with the effect sizes ($\geq 0.2$) it may suggest a meaningful effect and therefore, possibly altered excitation-contraction coupling contributed to the observed reduction in $Q_{tw\text{-}pot}$ and MVC. Previously, low frequency fatigue, indicative of impaired excitation-contraction coupling due to reduced efficiency of the calcium cycle has been reported during repeated running sprints (Perrey et al., 2010b). While low frequency fatigue was not assessed during the current study and may be specific to the type of exercise protocol used (running vs. cycling), it may have contributed to the findings of a reduced $Q_{tw\text{-}pot}$. Mmax amplitude was also not altered within or between groups after PA or TG indicating that membrane excitability was not changed by PA or TG and that alteration of action potential transmission and propagation did not contribute to fatigue during intermittent-sprint exercise in 33°C and 50% rh over forty minutes duration.

Only one other study has examined the effect of heat acclimation on central and peripheral fatigue (Brazaitis et al., 2010). This study used passive acclimation in a water bath at waist level, repeated every other day for two weeks with a 2 minute MVC pre the
first and last acclimation session. The interpolated twitch technique was used to assess central and peripheral contributions to fatigue. Heat acclimation was confirmed based on attainment of classic heat acclimation criteria yet, despite heat adaptation, voluntary activation and half relaxation time of muscle was not significantly different post acclimation (Brazaitis et al., 2010). While the authors acknowledged the limitations associated with seven non-consecutive days of passive heat acclimation, the current work adds to this finding as similar results were reported for MRFD and MRR in this chapter. However, given that muscle temperature is reduced during exercise after heat acclimation (Febbraio et al., 1994) it is interesting to note that the percent reduction in MRFD and MRR in the current chapter was lower after PA, but did not change with TG. As such it is tempting to speculate whether a lower muscle temperature post PA may have contributed to the reduction in percent decrement observed in PA compared to TG. If so, it may indicate heat acclimation could, if optimized, contribute to changes in mechanical properties of muscle that may improve peripheral fatigue. Further work with a larger sample size (post hoc analysis n = 12 per group) is required to elucidate this possible effect.

7.5.4. Limitations

The current investigation used a progressive acclimation protocol to induce heat adaptations. While the protocol, judged against classic heat acclimation criteria, appears effective, the extent of physiological strain induced was potentially below that required to maximise physiological adaptation. Consequently, accepting that sample size may have contributed to findings, many of the within exercise measures were not altered compared to training, making examination of the effect of acclimation on these measures difficult. As such, greater exogenous heat stress during each phase may have exacerbated hyperthermia, increased duration at a minimum endogenous core temperature to maximise responses compared to training and allowed further insight into central and peripheral fatigue during intermittent-sprint exercise and the effect of progressive acclimation.
With respect to the NMF assessment protocol used, existing studies have demonstrated with TMS, EMG responses are recovered rapidly (Kalmar and Cafarelli, 2006). In addition, skeletal muscle function and NMF measures have been shown to recover within 1 - 2 minutes of exercise (Froyd et al., 2013). In the current study, the time from completion of sprinting to completion of NMF assessment was 4.6 ± 0.3 minutes. As such the potential for recovery of central and peripheral factors during this period that may have contributed to lack of difference pre-to-post intermittent-sprinting cannot be discounted. Further, recent research with repeated sprint exercise has shown impaired corticospinal function during sustained MVC’s (Girard et al., 2013a). As such, sustained MVCs may have provided better insight to corticospinal function during intermittent-sprint exercise as opposed to the brief efforts utilised in this study (Girard et al., 2013a). Finally, single pulse TMS permits identification of supraspinal contributions to fatigue during intermittent sprint exercise. Lack of change in cortical voluntary activation and corticospinal excitability suggests fatigue during intermittent-sprint exercise may be peripherally mediated. However, both supraspinal and spinal mechanisms may contribute to fatigue and spinal mechanisms need further examination to understand fully central and peripheral aspects of fatigue during intermittent-sprint exercise using for example, cervicomedullary stimulations.

The decline in maximal force capacity has been shown to depend on the characteristics of the task being performed with the type of muscle contraction, activation pattern, intensity and duration of activity seen as critical (Martin et al., 2010). In the current study a cycling model requiring predominantly concentric muscle activity was used to examine fatigue in repeated sprint exercise. As such, in the context of the current work, application of findings to field-based team-sports that are running based may be limited. Further, it must be acknowledged that due to logistical constraints no temperate CISP trial was performed. Therefore is not possible to elucidate whether the neuromuscular fatigue observed is a consequence of the exercise pattern or the heat stress. In addition, a non-exercise control group was not used in the current study and as such the effect of training or heat acclimation compared to a non-exercise control on neuromuscular fatigue cannot be established. Finally, use of forty minutes exercise duration replicates
only the first half of a team game and further work is required to elucidate peripheral and central contributions to fatigue in the second half.

7.5.5. Conclusion

In conclusion, this was the first study to utilize TMS and FNS to examine central and peripheral fatigue following intermittent-sprint exercise in the heat where work:rest ratios replicated those of field-based team-sports. Further, it was the first study to use TMS and FNS to explore modification of fatigue in intermittent-sprint exercise by progressive heat acclimation and training. An initial novel finding from pooled data was the existence of reduced cortical voluntary activation post intermittent-sprint exercise in 33°C, 50% rh. When data were analysed by group however, evidence indicated fatigue was primarily peripheral in origin. A second novel finding was that progressive heat acclimation did not alter fatigue during intermittent-sprint exercise despite evidence of classic physiological adaptations and of a trend toward improved muscle contractile properties. Whether use of an alternate heat acclimation regime would produce different findings is unknown. Similarly, whether a greater exogenous heat stress or a greater duration of intermittent sprinting eliciting a more pronounced hyperthermia would alter fatigue also requires further investigation. Overall, data indicate that during forty minutes of intermittent-sprint exercise in 33°C, 50% rh neuromuscular fatigue may be primarily peripheral in origin.
CHAPTER VIII. GENERAL DISCUSSION

This chapter will be presented in three sections. Firstly, the principle findings from each of the studies in chapters four to seven will be presented. Secondly, the important physiological issues arising from the studies will be considered and future research requirements discussed. Finally, the practical applications of this PhD will be considered.

8.1. Principle Findings

Study 1

The purpose of this study was to determine the reliability of an intermittent-sprint cycling protocol (CISP) to be used to assess intermittent-sprint performance throughout the rest of the thesis. In addition, the efficacy of one practice session as a means to minimise a learning effect during main trials was examined. The study demonstrated that the CISP is a reliable tool for assessing intermittent-sprint performance in a test-retest research paradigm with typical errors of measurement for peak and mean power output of 2.9 and 4.2%, respectively. In addition, findings indicate that one practice trial comprising one quarter of the CISP was sufficient to ameliorate a learning effect during main trials.

Study 2

In this study participants completed the CISP in a hot humid (HH) and a hot dry (HD) environment, matched for heat stress. Previous research has demonstrated that in comparison to HD, HH environments compromise evaporative heat loss and decrease exercise tolerance (Maughan et al., 2012). Therefore, the purpose was to ascertain whether the composition of thermal factors contributing to the environment would have a similar effect on intermittent-sprint performance. Additionally, examining the interplay of differing thermal factors on physiological strain allowed better understanding for
developing a progressive acclimation protocol. Physiological strain index (PSI) was not significantly different in HH compared to HD environments and intermittent-sprint exercise performance of 40 minutes duration was impaired, but not different between these environments when matched for heat stress. As such, while findings reinforce the importance of heat acclimation for team-sport athletes it may be suggested that, when performance is of forty minutes duration, the composition of thermal factors contributing to the heat stress is not a primary factor when designing a heat acclimation programme.

Study 3

The third study compared physiological and perceptual adaptation to a progressive model of heat acclimation, that targets external heat stress to maintain physiological strain, to those achieved using a traditional model. The study also investigated the effect of progressive acclimation on physiological, perceptual and performance responses during forty minutes of intermittent-sprint exercise in 33°C 50% rh. Based on the ability to evoke classic acclimation responses and reduced physiological strain during intermittent-sprint exercise in the heat compared to TA, PA was judged an effective method for conferring the heat acclimated phenotype. Due to the reduced heat stress in the initial stages this protocol may possess an advantage compared to traditional models of acclimation, in that it may permit better tolerance of initial heat exposure and maximise exposure time in the early stages of acclimation by reducing incidence of heat illness and limiting participant drop out. However, while evidence suggests the current design maintained physiological strain during the acclimation regime, the degree of strain may have been insufficient to evoke the minimum endogenous criteria for a sufficient period of time to elicit a maximal acclimation response and therefore, needs further optimisation.

Study 4

The aims of this study were to examine central and peripheral contributions to fatigue during intermittent-sprint exercise in 33°C, 50% relative humidity (rh) using transcranial magnetic stimulation (TMS) and femoral nerve stimulation (FNS) and to investigate
whether progressive heat acclimation or normothermic training could ameliorate fatigue during exercise of this type. When data for CISP 1 were pooled for all seventeen participants significant reductions were observed in MVC force, cortical (CVA) and peripheral voluntary activation (PVA), potentiated twitch force, maximal rate of force development, and half relaxation time. When data were analysed by group, MVC and potentiated twitch force were significantly reduced after intermittent sprinting whereas CVA showed a trend toward a decline but was not significantly different. The reduction in MVC and potentiated twitch force during intermittent sprinting remained after twelve days of progressive heat acclimation. Findings indicate that during forty minutes of intermittent-sprint exercise in the heat neuromuscular fatigue may be primarily peripheral in origin and progressive acclimation or training does not reduce the extent of fatigue during exercise of this type despite reduced physiological strain.

8.2. Mechanistic Overview

This thesis attempts to explain the physiological and perceptual responses to progressive heat acclimation compared to a traditional model at rest and during exercise while also considering the effect of progressive heat acclimation on performance, physiological and perceptual responses during an intermittent-sprint model of exercise that replicates field-based team-sports. Prior to the production of this thesis a large body of research had investigated heat acclimation using methods as classified by Taylor (2000) and examined the efficacy of these methods using an endurance model of exercise. An array of heat acclimation protocols differing in total number of days, duration of individual sessions, intensity of exercise and work done (section 2.5.3. & 2.5.4.), have resulted in varying adaptation to the heat (section 2.5.2., 2.5.3. & 2.5.4.). Further, studies use varying criteria to determine whether heat acclimation was conferred and thus there is difficulty in determining the efficacy of a protocol in a consistent fashion. Considering this, a review of literature for this thesis resulted in the identification of nine key criteria (section 2.5.) that can be used to assess the efficacy of a heat acclimation model. In the following sections the progressive protocol used in study three and four of this thesis will be evaluated.
against these criteria and existing acclimation protocols to determine its efficacy accepting that, due to the progressive nature of the protocol, exercise criteria have to be determined against data from the intermittent-sprint sessions.

**Figure 8.1.** Classification of the nine heat acclimation criteria that can be used to determine the efficacy of a heat acclimation protocol (decreased heart rate and core temperature in rest and exercise, increased sweat rate and plasma volume, reduced skin temperature, decreased perception of effort / thermal sensation and improved physical performance). $T_{re \text{ rest}}$, $T_{re \text{ exe}}$ = Rectal temperature in rest or exercise; $T_{sk}$ = skin temperature; PV = plasma volume; HR = heart rate; $S_{\text{th}}$ = sweating threshold; $S_{\text{rate}}$ = sweat rate; SGF = sweat gland function; RPE = rating of perceived exertion; $T_{\text{sen}}$ = thermal sensation; PPO = peak power output; WD = work done.

### 8.2.1. Progressive acclimation and thermoregulatory markers of acclimation

Functionally, heat acclimation enhances the capacity of an organism to resist heat stress by augmenting heat dissipation and elevating the upper body temperature that can be sustained in the heat (Horowitz and Kodesh, 2010). Such resistance is a consequence of systemic adaptation, a chronic process dependent on heat induced reprogramming of gene expression (Horowitz and Kodesh, 2010). Historically, a number of different heat
acclimation protocols, primarily considered constant work rate or isothermal, were used
to elicit systemic adaptation, synonymous with classic heat acclimation responses. Such
protocols display varying efficacy, but consistently augment heat dissipation through
decreased exercise and at rest (when reported) T_{re} (section 2.5.3. & 2.5.4.). In this thesis
(chapter six and seven), progressive acclimation was used to evoke acclimation responses
and, similar to existing protocols, reduced resting T_{re} in study four during acclimation (-
0.3°C) and prior to CISP 2 (-0.3°C), possibly due to lowering of the hypothalamic
thermoregulatory set point, reduced heat storage, cardiovascular adjustment and
attenuation of hyperpyrexic molecules (Garden et al., 1966; Buono et al., 1998,
Armstrong 1998; Shin et al., 2013). In study three, however, despite a similar reduction
during acclimation (-0.2°C) and greater reduction prior to CISP 2 (-0.4°C), resting T_{re} was
not statistically different across acclimation or CISPs for PA. In comparison, similar non
significant findings were reported for resting T_{re} in response to traditional acclimation (-
0.3°C) and prior to CISP 2 (-0.3°C). Considering the magnitude of the change in this thesis
compared to that reported by Kampmann (2008) in a review of studies (~ -0.4°C), this
may suggest sub-optimal stimulus from progressive acclimation. In contrast it may
indicate a type II error owing to the greater variability in resting T_{re} amongst participants
in study three and the greater number of groups in that study that necessitated more
comparisons during statistical analysis.

A further classic thermoregulatory adaptation conferred by existing heat acclimation
protocols is a reduced exercise T_{re} (section 2.5.2.2.) owing in part, to reduced resting T_{re}
and improved heat dissipation (Garden et al., 1966; Buono et al., 1998, Armstrong, 1998).
T_{re} during intermittent-sprint exercise was reduced in study three by both progressive (-
0.5°C) and traditional (-0.3°C) acclimation with the extent of adaptation from progressive
acclimation, not different to traditional methods, indicating the efficacy of this method. In
study four, however, exercise T_{re} tended to be reduced by progressive acclimation (-
0.3°C) but was not significantly different over time and not different between PA and TG.
That exercise T_{re} was reduced in study three but not four cannot be explained by (i)
training status, percent body fat or BSA between progressive acclimation participants in
study three compared to four nor (ii) difference in evoked T_{re} during exercise on day one,
six and twelve. Further, this discrepancy cannot be explained by the change in $T_{re}$ during intermittent-sprint exercise (Table 6.5. and 7.4.), but may be related to the lower physiological strain evident during CISP 1 in study four compared to study three. (Table 6.5. and 7.4.). Alternatively, the discrepancy may reflect insufficient power in study four to detect a difference as post hoc analysis suggested twelve participants would be required to detect a significant difference.

Although $T_{sk}$ was not assessed during acclimation, the effect of progressive acclimation on this measure can be determined from the intermittent-sprint exercise data. Twelve days of progressive acclimation significantly reduced $T_{sk}$ in study three (-0.70°C). In study four mean $T_{sk}$ during intermittent-sprint exercise after acclimation was also reduced post acclimation (-0.33°C). By comparison, traditional acclimation (study three) also lowered $T_{sk}$ (-0.95°C), but the extent of reduction was not different to progressive acclimation. The two-fold greater reduction in $T_{sk}$ in study three may be explained by the greater stimulus presented by the higher physiological strain experienced during CISP 1 in this study in both traditional and progressive acclimation groups. A high $T_{sk}$ in conjunction with high $T_{re}$, similar to the values reported in study three, has been shown to evoke the greatest skin blood flow response during exercise and subsequently, greater competition for cardiac output (Sawka et al., 2012). Consequently, the high $T_{sk}$ and $T_{re}$ in study three may have elevated skin blood flow, decreased the gradient for heat exchange and increased the cardiovascular strain in study three compared to four. Decreased $T_{sk}$ is reported in response to decreased hypothalamic setpoint for sweat onset coupled with an increased sweat rate and increased evaporation (Shvartz et al., 1973; Lorenzo and Minson, 2010). However, sweat rate, despite being increased during traditional acclimation and progressive acclimation, was not different during intermittent sprinting in study three or four. Given that core temperature and skin temperature decreased in response to heat acclimation it is likely that the threshold for the onset of sweating was reduced, i.e. a central adaptation. Therefore, it is possible that the heat acclimation protocols used were an insufficient stimulus to promote peripheral adaptation, accepting that whole body sweat rate is a crude measure and does not provide an actual measure of eccrine gland function. Such a suggestion is supported by the observation that the extent of sweating
induced during acclimation only approached those observed during the CISPs in the final days of acclimation.

8.2.2. Progressive acclimation and cardiovascular markers of acclimation

Improved cardiovascular stability that is reported with existing heat acclimation protocols (e.g. Shvartz et al., 1973; Patterson et al., 2004; Amorim et al., 2011) was also observed in this thesis in response to progressive heat acclimation. In study three, resting heart rate was reduced during progressive acclimation (-12 b.min\(^{-1}\)) and was not different to the reduction induced by traditional acclimation (-11 b.min\(^{-1}\)). However, this response did not persist to intermittent-sprint exercise. Prior to CISP 2, although there was a trend towards a reduction in resting heart rate (-9 and -11 b.min\(^{-1}\) in traditional and progressive, respectively), resting heart rate was not significantly reduced (Table 6.5.), but may be explained by the greater variability in this measure prior to intermittent-sprinting. In contrast, in study four progressive acclimation reduced resting heart rate by day twelve (-9 b.min\(^{-1}\), Table 7.3.) and this response was still evident prior to CISP 2 (-10 b.min\(^{-1}\), Table 7.4.).

In addition to decreased resting heart rate, progressive acclimation also reduced exercise heart rate during intermittent-sprint exercise. After twelve days of progressive acclimation, mean exercising heart rate during intermittent sprinting was reduced in both study three (-16 b.min\(^{-1}\)) and four (-17 b.min\(^{-1}\)) (Table 6.5. and 7.4.). This reduction was not different to that observed in traditional heat acclimation within study three (-17 b.min\(^{-1}\)) and in other work using existing heat acclimation protocols (Yamazaki and Hamasaki, 2003; Patterson et al., 2004), again supporting that progressive heat acclimation is at least comparable with other HA methods. In agreement with Horowitz and Kodesh, (2010), autonomic nervous system habituation and increased vagal activity may mediate the reduced heart rate, but centrally mediated effects including improved cardiac efficiency or decreased sympathetic activity may also contribute (Yamazaki and Hamasaki, 2003; Garrett et al., 2011).
Improved cardiovascular stability following heat acclimation has been attributed to an expansion of plasma volume and this response has been reported using a range of existing heat acclimation protocols (Nielsen et al., 1993, 1997; Patterson et al., 2004; Garrett et al., 2012). In the current thesis plasma volume displayed a trend toward expansion but the wide inter-individual responses and large variance prevented detection of a significant difference and may be symptomatic of the method used to determine plasma volume change (Weinstein et al., 1998).

8.2.3. Progressive acclimation and endocrine markers of acclimation

In response to heat acclimation plasma volume expansion occurs in tandem with interstitial fluid expansion (Patterson et al., 2004). As such, extracellular fluid expansion is considered the primary mechanism for plasma volume expansion that must therefore, be mediated by electrolyte retention, specifically, Na⁺ (Wendt et al., 2007). Retention of Na⁺ is mediated by the hormone aldosterone, concentrations of which have been shown to increase at rest and end exercise during heat acclimation (Armstrong et al., 1998; Garrett et al., 2012), but also not change (Sunderland et al., 2008), possibly due to the training status of participants, but also the protocol used. In the current thesis (study 3), plasma aldosterone levels were not altered by either progressive or traditional heat acclimation but tended to increase post exercise. Further, although plasma Na⁺ was not assessed, sweat Na⁺ was determined and, despite a trend toward a reduction in both progressive (-14%) and traditional (-15%) protocols, there was no significant difference during acclimation (Table 6.2.) or in CISP 1 compared to CISP 2 (Table 6.6.)

A central tenet of progressive acclimation is that successive increases in heat stress elevate physiological strain, thereby maximizing systemic adaptation. If such a notion is correct then physiological strain should not attenuate during a progressive compared to traditional acclimation and may be evidenced by examination of plasma cortisol concentrations. Plasma cortisol concentration has been shown to be increased in response to an exercise bout in the heat but, more importantly, is known to be
attenuated over the course of what was essentially a constant work rate protocol (Armstrong et al., 1989). In contrast, cortisol concentration has also been shown not to change in response to short-term intermittent and isothermal acclimation (Sunderland et al., 2008; Garrett et al., 2009). In the current work, plasma cortisol concentration was not different between progressive and traditional acclimation and was not different post exercise on day one, six or twelve during progressive acclimation but tended to increase. Further, the final increment in heat stress during progressive acclimation did not invoke an increased cortisol response, indicating no effect of increased strain in the final stage of the progressive heat acclimation protocol. Although other markers of heat adaptation were evident, current data suggests a greater heat stress increment may be required to maximise cortisol response and adaptation.

8.2.4. Progressive acclimation and temporal patterning of acclimation responses

The reprogrammed gene expression that underpins systemic adaptation in the heat acclimated phenotype displays biphasic temporal patterning that is evoked by sustained heat insult (Horowitz and Kodesh, 2010). Progressive heat acclimation, owing to the decreased heat stress in the initial phase of the protocol and stepwise increases laterally, could alter the extent or patterning of this genotypic response that may or may not affect the heat acclimated phenotype. However, in the initial stages of progressive acclimation resting $T_{re}$ in study three and four were reduced by day six, a response that was identical to that observed for both traditional acclimation and training. Further, despite resting heart rate in study four not being significantly reduced until day twelve, which may indicate alteration in temporal patterning, resting heart rate during progressive heat acclimation in study three was significantly reduced by day six. Again this response was identical to that observed during traditional acclimation. Taken together, these data would appear to suggest that there is no effect of the reduced heat stress of progressive heat acclimation on temporal patterning of the adaptation response.
As stated, the temporal patterning of gene expression in heat acclimation is biphasic, considered to comprise an initial transient stage to maintain DNA integrity and a sustained stage where signaling networks aligned to cytoprotection are enhanced (Horowitz et al., 2004). Such activity includes upregulation of genes associated with HSPs that confer thermotolerance, but are now suggested to play a role in systemic adaptation and the heat acclimated phenotype (Kuennen et al., 2011). The stimulus for upregulation of genes encoding for HSPs is heat insult and recent evidence suggests that extra cellular HSP72 concentration is significantly increased only in very hot ambient conditions where the exogenous heat stress evokes a minimum endogenous criteria of rapid and sustained increases in core temperature to > 38.5°C and enhanced sympathetic activity (Gibson et al., 2013). Therefore, given the reduced heat stress in the initial stages of the progressive compared to traditional protocol and the fact that during the final phase of progressive acclimation exercise $T_{re}$ was greater than 38.5°C for only 10 - 15 minutes of each session (day nine to twelve, study three and four), it is conceivable that progressive acclimation did not evoke sufficient strain to optimise upregulation of gene expression, HSP response and systemic adaptation. This may also explain non-significant findings in some other key markers of heat acclimation measures across study three and four.

In 1963, Lind and Bass observed a plateau in exercise $T_{re}$ and heart rate during acclimatization that was perceived to signal the attainment of optimal heat adaptation. Numerous studies have since reported similar findings with the overall conclusion that heat acclimation generally requires 10 - 14 days to acquire (Armstrong and Maresh, 1991). It is now known, however, that rather than reflecting optimal heat acclimation, plateaus in key markers of acclimation may be an artifact of constant work rate methods and the inherent decrease in physiological strain observed over the duration of the protocol (Taylor, 2000). To circumvent this inherent limitation and optimise adaptation, progressive heat acclimation uses increases in heat stress to augment physiological strain for the duration of the protocol. In the current thesis this manifested as consistently maintained exercise $T_{re}$ and heart rate over day one to twelve (Table 6.1. and 7.3.). In contrast, with traditional acclimation in the current work, the classic plateau in physiological responses was observed. Considering the responses to progressive
acclimation in the current thesis it is possible that this method may alter the temporal patterning of adaptation with the potential for sustained / greater adaptation as the protocol progresses. Two observations from the current data support this suggestion. Firstly, the reduction in resting $T_{re}$ at the end of progressive acclimation in study three (-0.2°C) was augmented prior to CISP 2 (-0.4°C). This indicates a potential lag effect of progressive acclimation whereby further physiological adaptation occurs after completion of the protocol during recovery. Positioning of the highest heat stress in the final stage of progressive acclimation may evoke this response and this concept is supported by Daanen et al., (2011) and Costa et al., (2014) who reported a similar response with a progressive type acclimation protocol. Further, with isothermal strain, Patterson et al., (2004) demonstrated an enhanced reduction in resting $T_{re}$ after twenty two days of acclimation (-0.37°C) compared to eight (-0.2°C) again suggesting physiological adaptation can be further augmented with an appropriate acclimation protocol. It is acknowledged, however that this response was absent in study four thereby limiting the ability to separate what may be a protocol mediated effect from a methodological artifact. Secondly, acknowledging that plasma volume was not significantly different in either study three or four and there were wide interindividual differences, it is interesting to note that after day six only in progressive acclimation did plasma volume show a tendency to increase further. Sustained and generalized expansion of plasma volume has been reported when heat acclimation was maintained for twenty two days using an isothermal model of acclimation (Patterson et al., 2004) and this indicates a similar potential with a progressive model.

8.2.5. Progressive acclimation and perceptual responses

Consistent with the elevated heat stress of the protocol, progressive acclimation produced significant increases in thermal sensation on day six and twelve of acclimation in both study three and four (Table 6.3. and 7.3.). In contrast, thermal sensation was significantly decreased by traditional acclimation and training. Sensory response is considered an integration of inputs from exercise and temperature stimuli, but thermal sensation is more influenced by peripheral thermal receptor input that central (Mower,
1976). Given that exercise intensity was the same in all conditions during acclimation in this thesis and that skin temperature responses are correlated to thermal sensation (Kamon et al., 1974) it is possible that altered thermal sensation reflected differences in $T_{sk}$ evoked by acclimation. During intermittent-sprint exercise, thermal sensation was significantly reduced by traditional, but also progressive acclimation, again possibly mediated by reductions in $T_{sk}$ during CISP 2. These data reinforce the efficacy of progressive acclimation, but it is also important to note that in study four, twelve days of training also evoked a reduction in thermal sensation. This may have been mediated by reductions in resting $T_{re}$ and thresholds for sweating and cutaneous vasodilation that may have been influenced by exercise intensity (Kampmann et al., 2008).

Mean RPE during acclimation was not altered by either traditional or progressive regimes (Table 6.3. and 7.3.). In contrast to thermal sensation, RPE therefore was generally unaffected by increases in heat stress with progressive acclimation, demonstrating the differing origins of this measure. During intermittent-sprint exercise after acclimation RPE was reduced by both progressive (-4 units) and traditional acclimation. However, in study four, mean RPE tended to decline but was not different in CISP 2 compared to CISP 1 which could reflect the maximal nature of the exercise. RPE is correlated with physiological markers during exercise (Edwards et al., 1972) and has been suggested to be derived from peripheral afferent stimuli (Marcora, 2009), emanating from group III and IV afferents that are abundant in skeletal muscle and sensitive to physiologic, metabolic and mechanical stress (Craig, 2002). As such, reduced physiological and metabolic strain derived from the combination of exercise and heat used during acclimation may have contributed to the reduced RPE. However, there is strong evidence to suggest perception of effort is strongly influenced, if not totally dependent, on centrally generated corticofugal motor commands that give rise to corollary discharges (Enoka and Stuart, 1992). That is, perception of effort is centrally generated by neural signals from the motor to sensory cortex (Marcora, 2009) that during exercise would reduce central drive contributing to fatigue.
8.2.6. Progressive acclimation, intermittent-sprint performance and neuromuscular fatigue

In the studies contained in chapters five to seven of this thesis, the mean reduction in PPO and WD over forty minutes of intermittent-sprint exercise in the heat was approximately 9%. By comparison, in the one temperate trial conducted (chapter five control condition) PPO and WD were both reduced by 6%. Based on the reliability of PPO in the CISP being 2.9% (chapter four) these data suggest a meaningful effect on performance when intermittent-sprint exercise is conducted over forty minutes, but also an additional meaningful effect of heat adding to the existing literature on this topic (Figure 8.2.). Although meaningful, the decrements in performance observed in this thesis did not consistently produce statistical significance. Chapter five (study two) demonstrated a significant ~ 8% decrement in performance while under heat stress. In chapter six, performance was different only after traditional (~ 19%), but not progressive acclimation (6%), despite a 2% improvement in PPO previously being identified as a statistically significant effect (Castle et al., 2011). In chapter seven (study four), analysis of CISP 1 data pooled for seventeen participants highlighted significant decrements in intermittent-sprint performance during exercise in the heat (4%), however, these did not persist when data were analysed by group across both CISPs. This finding, coupled with other non significant findings from earlier chapters of this thesis may suggest a loss of statistical power owing to the number of comparisons performed during ANOVA’s with a conservative Bonferroni correction. Consequently, while the type I error rate was well controlled increased type II error rate may have occurred (Field, 2009). Overall, taken together, these data would still support that intermittent-sprinting is impaired over forty minutes in the heat (Figure 8.2.).

Similar to existing studies (Morris et al., 1998, 2000), this thesis demonstrated strong significant negative correlations between PPO and $T_{re}$ that were generally not ameliorated by progressive acclimation, but were by traditional acclimation. This may be related to the sample size or individual heat tolerance levels of this group (chapter six) as previously discussed. Alternatively, these data could indicate that progressive heat acclimation has
limited effect on intermittent-sprint exercise in the heat. Further, it may be that the design of the CISP contributed to this result. Due to the maximal nature of the sprinting in the CISP the same level of fatigue may be induced post exercise even after an intervention such as heat acclimation. Further, due to the fixed duration of the protocol, the improvements induced by heat acclimation that may allow the athlete to exercise for longer and potentially impact performance, for example, during the second half of a match, do not have sufficient impact on the maximal sprint performance or work done in the initial forty minutes.

Figure 8.2. Percent change in sprint performance from control for existing studies examining the effect of heat on sprint performance with data for study two of this thesis included for comparison. PPO = peak power output; MPO = mean power output; WD = work done TDR = total distance run; TRT = total run time; ST = sprint time; * = significant difference from control.
Previous work has demonstrated that, when intermittent-sprint exercise similar in duration and work:rest ratios to field-based team-sports is completed in a hot environment, physiological strain is increased and performance decreased (Morris et al., 1998, 2000, 2005, Sunderland and Nevill, 2005, Figure 2.8.). Until now, our understanding of the central and peripheral mechanisms underpinning this fatigue has been limited, because (i) neuromuscular fatigue has not been assessed using activity patterns similar to field-based team-sports and we have to rely on findings from repeated-sprint ability protocols (Racinais et al., 2007; Perrey et al., 2010b); (ii) the twitch interpolation technique has been used to examine fatigue and does not provide insight to the site or real extent of central fatigue; (iii) for the one study that has used TMS to elucidate central mechanisms of fatigue (Girard et al., 2013a), a repeated-sprint ability protocol that was of a shorter duration and with fewer sprints than most field-based matches was used and the study was only conducted in a temperate environment. In chapter seven, the contribution of supraspinal fatigue to intermittent-sprint exercise in the heat using an ecologically valid team-sport protocol was determined using TMS. Contrary to that observed with repeated-sprint activity (Girard et al., 2013a), evidence of supraspinal fatigue was observed in addition to peripheral fatigue. However, when data were analysed between groups, despite a trend toward decreased voluntary activation the decrease was not significant. Heat acclimation did not ameliorate neuromuscular fatigue during intermittent-sprinting but, interestingly, data suggest that there was a trend toward improved maximal rate of relaxation and work done post acclimation. Although not significantly different, this may suggest a trend towards more work completed for the same level of fatigue indicating that an optimised heat acclimation protocol may improve performance during intermittent-sprinting in the heat.
8.3. Future Perspective and Directions for Future Research

8.3.1 Future perspectives

The heat acclimated phenotype can be evoked using a variety of protocols. Assessment of the validity of these protocols can be judged against the ability to elicit classic heat acclimation responses. Studies, however, differ in their interpretation of the combination of classical responses that constitute heat acclimation. Sawka et al., (1996) suggest three classical responses, those being lower heart rate, lower core temperature, and higher sweat rate during exercise-heat stress. Other research incorporates increased plasma volume, reduced skin temperature, decreased perception of effort / thermal sensation or comfort and improved physical performance (Pandolf et al., 1977; Horowitz and Kodesh, 2010). Therefore, the ability to assess objectively the efficacy of a heat acclimation protocol is compromised. As such, there is a potential need for the development of a benchmark set of heat acclimation responses that would serve as criteria against which all heat acclimation protocols may be judged. Attainment of all criteria by a protocol could identify a heat acclimation protocol that carries strong validity that perhaps, is judged as a ‘gold standard’ benchmark. From the review of literature completed for this thesis (section 2.5.) it is proposed that nine criteria constitute the benchmark set (Figure 8.1.). This would include; decreased resting and exercise heart rate and core temperature, decreased skin temperature, increased sweat rate and plasma volume, improved perceptual response and improved performance.

Theoretically, heat acclimation may be viewed from a dichotomous or continuous perspective. Dichotomy, however, presents polar perspectives and therefore, with respect to the ability of a protocol to elicit classic heat acclimation responses, may infer many protocols are ineffective. Rather, it is proposed that when assessing the efficacy of a heat acclimation protocol a continuum is more representative and appropriate, whereby a heat acclimation protocol, dependent upon the heat acclimation criteria
achieved against the benchmark standard, would reside on a particular point of the continuum (Figure 8.3).

**Figure 8.3.** Theoretical continuum of heat acclimation with existing studies and data from current thesis plotted. Existing studies taken from tables in section 2.5.3 & 2.5.4. Progressive heat acclimation represents pooled data from study three and four of this thesis. Position on continuum reflects the number of heat acclimation criteria achieved against the nine identified in section 2.5.

The concept of a continuum also serves as a useful model from which to consider an individual’s state of heat acclimation. Currently, heat acclimation protocols tend to adopt a “one size fits all approach” whereby athletes or individuals, irrespective of specialisation or physical state are subjected to the same protocol to evoke systemic adaptation. It is well known, however, that a multitude of factors modify the response to acclimation including aerobic fitness, hydration and genetics (Pandolf, 1977; Horowitz and Kodesh, 2010, Garett et al., 2012). Further, the work in study three and four of this thesis demonstrated that individuals display varying tolerance to the heat as displayed in earlier work (Moran et al., 2007). As such, individuals presenting for heat acclimation are essentially already at differing points on the heat acclimation continuum and this therefore, requires consideration when administering heat acclimation if responses are to be optimised. Progressive heat acclimation starts to address this issue as the reduced
heat stress may cater for a number of less heat tolerant individuals in the initial stages of acclimation, but further work is required to optimise the protocol and the evoked responses since both study 3 and 4 suggested adaptation was not maximised. This may include use of different heat stress doses in each progressive increment.

Numerous protocols have been used to evoke heat acclimation. Taylor, (2000) categorized these as constant work rate, controlled hyperthermia and self-regulated methods. Since this classification there has been an emergence of evidence examining alternate heat acclimation methods for application to an intermittent-sprint model of exercise (Sunderland et al., 2008; Petersen et al., 2010; Brade et al., 2013). In addition, the progressive model used in this study, although based on a constant work rate model, provides an alternative acclimation possibility and further useful insight to the field. Given these developments it is suggested that the classification of Taylor (2000) needs to be extended to reflect new emerging methods of conferring heat acclimation as demonstrated below (Figure 8.4.).

Figure 8.4. Classification of heat acclimation (Taylor, 2000). New emerging methods or variation on existing methods shown in red.
8.3.2. Directions for future research

In the present thesis progressive heat acclimation was used to confer the heat acclimated phenotype. From the data presented (chapter six and seven) this method was considered as effective as the more traditional constant work rate methods in evoking classic heat acclimation responses. In addition, the protocol may be beneficial for less heat tolerant individuals as the progressive nature of the heat stress has the potential to prevent drop out and early cessation of initial sessions due to excessive heat strain, thereby maximising exposure time. It is unknown, however, how the progressive nature of the protocol affects the time course and extent of cellular adaptation to heat. The heat shock protein response is a common, acute and transient reaction to heat stress that confers thermotolerance. Evidence now exits to suggest that thermotolerance and heat acclimation are part of the same continuum with the heat shock protein response important for cellular and systemic adaptation (Kueenen et al., 2011). Recent research proposes that extra cellular HSP72 concentration is significantly increased only in very hot ambient conditions where the exogenous heat stress evokes a minimum endogenous criteria of rapid and sustained increases in core temperature to > 38.5°C and enhanced sympathetic activity (Gibson et al., 2013). As such, it is unknown whether the progressive nature of the protocol and concomitant reduced physiological strain in the initial stages creates a suboptimal heat shock response and adaptation, or merely a different temporal patterning of the response with greater initial lag that is optimised in the latter stages by the stepwise increases in heat stress. The data presented in chapter six suggests the protocol, in contrast to traditional constant work rate methods, can elevate physiological strain and confer the acclimated phenotype, but whether this is optimal requires further examination at a cellular level. With a different structure, the progressive model may, evoke a greater response, but more research is required to elucidate the most appropriate sequence / duration and insult of each heat stress increment to facilitate possible shortening of the protocol to increase usability. Finally, isothermal strain heat acclimation is demonstrated as an effective method to confer heat acclimation (Patterson et al., 2004; Garrett et al., 2009, 2011). Whether progressive acclimation is as effective is currently unknown and further research comparing these protocols when matched for heat stress would add useful information to the field.
This thesis is the first to examine central and peripheral fatigue following intermittent-sprint exercise in the heat using TMS. Although it is generally considered repeated-sprint type exercise is limited by peripheral mechanisms, the protocols from which these data are derived use fewer sprints and are of shorter duration than field-based team sports and have not always been completed in the heat (Racinais et al., 2007; Perrey et al., 2010b; Girard et al., 2013a & b). In chapter seven in the current work, when data were pooled for all seventeen participants evidence of peripheral fatigue and decreased cortical voluntary activation existed after forty minutes of intermittent-sprint exercise in the heat. Decreased cerebral blood flow and cerebral oxygenation have been reported in hypoxia compared to normoxia and correlated to a twofold greater decrease in cortical voluntary activation despite similar levels of peripheral fatigue (Goodall et al., 2012a). Similarly, passive (Ross et al., 2012) and exercise induced (Nybo and Nielsen, 2001b) hyperthermia is known to decrease cerebral blood flow and therefore, theoretically cerebral oxygenation. As such, hyperthermia may have mediated the decline in cortical voluntary activation observed in the current study. However, no work has examined cerebral blood flow and oxygenation during intermittent-sprint exercise in the heat to ascertain whether hyperthermia may mediate cortical voluntary activation during this type of activity and research in this area would add useful mechanistic information to the field.

The duration of intermittent-sprinting in the heat in the current study may explain the decrements in cortical voluntary activation observed in this study, but not others. However, whether the duration of exposure to hyperthermia during intermittent-sprint exercise may elicit greater decrements in cortical voluntary activation or peripheral fatigue is currently unknown. The CISP protocol used in the current work provided insight to the effects of hyperthermia on neuromuscular fatigue over forty minutes of exercise, essentially one half of a field-based team-sport, but it is unknown whether the observed decrements in central and peripheral fatigue during this time would be exacerbated with a second half period. Given the increased competition for cardiac output between metabolic and thermoregulatory demands, combined with the exercise-induced dehydration and potential impact on cerebral blood flow, such a response is possible.
Recent research (Nybo et al., 2013), however, would not concur and suggests that hyperthermia does not mediate central fatigue. In this work (Nybo et al., 2013), however, central and peripheral fatigue were assessed before and following an actual football match in the heat where activity was essentially self-paced. Consequently, during the match in the heat a different pacing strategy was evident as total distance covered and high-intensity running was reduced. As such, it is possible the effect of heat stress on central fatigue may have been attenuated compared to a non self-paced laboratory based protocol of a similar duration.

Compared to direct stimulation of the motor nerve, supramaximal TMS is advantageous as it allows the site of central fatigue to be localised (Goodall et al., 2012b; Ross et al., 2012). However, with TMS it is not possible to dissociate between cortical and spinal contributions to evoked responses (Sidhu et al., 2012a). Modulation of spinal loop properties e.g. net synaptic excitatory input and excitability of the motor neuron pool may contribute to spinal mediated fatigue. During intermittent-sprint exercise, such factors have been assessed in temperate environments using brief protocols by H-reflex measures (Perrey et al., 2010b) and a reduction, but also no change, has been observed indicating lack of clarity with respect to spinal fatigue in this type of exercise. More recently, studies have also attempted to distinguish between cortical and spinal contributions to fatigue during sustained locomotor exercise using cervicomedullary stimulation of the descending tracts below the level of the motor cortex, in addition to TMS (Sidhu et al., 2012a, 2012b), but this technique has not been applied to intermittent-sprint exercise. As such, examination of spinal contributions to fatigue during prolonged intermittent-sprint exercise in the heat could be assessed using both H-reflex and cervicomedullary stimulation methods and such work would add useful insight to neuromuscular fatigue during intermittent-sprint exercise.

The mechanisms of fatigue in intermittent-sprint exercise show task dependency varying with the duration, intensity and type of contraction (Bishop, 2012). Recent research suggests that fatigue during activity of this type is mediated primarily by peripheral
mechanisms (Perrey et al., 2010b; Girard et al., 2013a) and therefore, understanding of the genesis of peripheral fatigue is important. Much research has relied upon the use of single twitch stimuli to ascertain central fatigue, but this method cannot be used to characterise the peripheral fatigue. Recent work has used the ratio of low and high frequency stimuli in short duration running sprints to ascertain low frequency fatigue and demonstrated that this type of peripheral fatigue predominates in sprint-running exercise (Perrey et al., 2010b). Low frequency fatigue indicates failure of excitation contraction coupling, relates to the reduced efficacy of the calcium cycle and is dependent on the intensity of exercise (Martin et al., 2010). Whether this type of fatigue persists when sprints are cycling based and performed in the heat in quantities, durations and work:rest ratios that simulate field-based team sports is currently unknown and requires further research.

8.4. Practical Application of Findings

The CISP, presented in study four, has been used in previous work to assess intermittent-sprint exercise performance (Castle et al., 2006, Maxwell et al., 2008; Castle et al., 2011). Prior to this thesis, the reliability of the CISP had not, however, been determined. From the experimental data presented in chapter four it is now known that the CISP is a reliable measure of intermittent-sprint performance. Given that cycling-based protocols provide valid assessment of repeated-sprinting in match-play (Bishop et al., 2001) the CISP may therefore, serve as a suitable protocol for examination of differing interventions and training on performance and metabolism in intermittent-sprint exercise and is more conducive to sensitive assessment of complex physiological variables compared to running-based models.

Data presented in chapter 5 highlights the need for heat acclimation for games players when competing in hot environments. However, the experimental data presented suggests that, while the overall heat load in acclimation should match expected
conditions the composition of thermal factors contributing to the total heat stress should not be a primary concern when planning the heat acclimation programme, particularly for durations upward of forty minutes.

The practical importance of the data presented in chapter six and seven is that progressive heat acclimation may provide a valid alternative model of acclimation for athletes in preparation for competition. While it is acknowledged that progressive acclimation did not induce statistical performance improvements during intermittent-sprint exercise, up to 2.7% and 3.8% improvements in PPO and work done were observed. In the context of the participants in the current thesis such differences equate to an increase of ~ 30 W or ~ 110 J. Coupled with the reduced physiological strain and improved thermal comfort this may represent a meaningful change in performance during this type of exercise in the heat and may confer advantages during self-paced match play over durations greater than forty minutes. The progressive acclimation protocol uses a constant work rate as its basis, but attempts to optimise heat acclimation responses through the use of successive heat stress increments. This gradual introduction to heat stress may prove beneficial to athletes that show evidence of heat intolerance. Further, as comparative evidence from hypoxia demonstrates a beneficial effect of environmental manipulating in a health-based population (MacKenzie et al., 2011) it is conceivable that progressive heat acclimation may be beneficial to, for example, an elderly population who display a marked decrease in the ability to cope with heat stress. As such, progressive heat acclimation may serve to individualise the heat acclimation process. With limited evidence for progressive heat acclimation, work in the current thesis serves as a basis for future research examining the optimisation of this method and further individualisation of the heat acclimation process through, for example, manipulation of the dose and increment of heat stress. Currently, Isothermal strain heat acclimation also proposes to optimise heat adaptation. Little is known, however, of the effect of the increasing workload associated with this method on training state and immune function response in an athletic population. In addition, such a method may not be viable for inducing heat acclimation in, for example, an elderly population where the increasing amount of work required to elicit and maintain a core temperature of 38.5°C may not be sustainable. With
respect to traditional protocols, the sudden exposure to high heat stress associated with this type of protocol may be too great an insult. Consequently, for both athletic and elderly populations, progressive heat acclimation may prove a viable alternative method of acclimation to the heat.

The use of TMS in conjunction with the interpolated twitch technique to examine fatigue during intermittent-sprint exercise in the heat provides useful practical insight to the genesis of neuromuscular fatigue during exercise of this type. Use of these techniques in a hot environment over durations that replicate field-based team-sports provide new knowledge that may permit better understanding of the factors contributing to fatigue during exercise of this type and how it may be ameliorated to improve or maintain performance. The work presented in chapter seven provides insight to the effect of heat acclimation on markers of central and peripheral fatigue. Although the evidence suggests heat acclimation did not ameliorate central and peripheral fatigue, the percent decrement in work done over time and the maximal rate of relaxation tended to be reduced after heat acclimation (chapter 7). This may suggest a potential benefit of heat acclimation to intermittent-sprint exercise that may be augmented if performance extends to a second half.

8.5. Conclusion

In conclusion, the data presented in chapter five, six and seven suggest that intermittent-sprint exercise performance is impaired when completed in the heat over a period of forty minutes. Similar to previous research that posits a possible hyperthermia induced central fatigue (Morris et al., 1998, 2000, 2005; Castle et al., 2011), in this thesis which is the first to examine neuromuscular fatigue during intermittent-sprinting using TMS, evidence of a reduction in central drive was present after forty minutes of intermittent sprinting in the heat when data were pooled for all participants. Further data analysis, however, revealed only a trend toward reduced central drive that, while possibly
meaningful based on the reliability of the measurement technique, indicated a predominance of peripheral fatigue during exercise of this type. Given that intermittent-sprint performance was reduced in the heat, the effect of heat acclimation in ameliorating this response was investigated. A progressive heat acclimation protocol was used that, based on the evidence presented in chapters six and seven, was deemed effective in conferring the heat acclimated phenotype. The progressive acclimation model reduced physiological strain and thermal comfort during intermittent-sprint exercise in the heat but, in contrast to traditional acclimation, did not ameliorate the reduction in performance and the significant negative correlation between power output and T_{re}. PA did, however, increase power output after acclimation by 2%, which, although not significant in this work and possibly not meaningful based on the reliability of the CISP, has been demonstrated significant in other work (Castle et al., 2011). Neuromuscular fatigue, measured using TMS following intermittent-sprint exercise in the heat, was not ameliorated by acclimation. Of note, however, was that the percent reduction in maximal rate of force development (MRFD) and maximal relaxation rate (MRR) was lower after PA, but did not change with TG. As such it is possible an acclimation induced reduction in physiological strain may have initiated a trend toward an improved mechanical property of muscle. As such, it may be that progressive heat acclimation, if optimised, could contribute to changes in mechanical properties of muscle that may improve peripheral fatigue during intermittent-sprinting. Future investigations should examine the endogenous strain and temporal patterning and dose of progressive heat acclimation to elucidate the optimal heat stress and sequencing to maximise the adaptations conferred by acclimation of this type.
CHAPTER IX. REFERENCES


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Please read the following carefully

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

- have a fever, suffer from fainting spells or dizziness
- have suspended training due to a joint or muscle injury
- have a known history of medical disorders, i.e. high blood pressure, heart or lung disease

and, if appropriate to the study design:

- have had hyper/hypothermia, heat exhaustion, or any other or cold disorder;
- have anaphylactic shock symptoms to needles, probes or other medical-type equipment;
- have a known history of infectious diseases (e.g. HIV, Hepatitis B);
- have a known history of rectal bleeding, anal fissures, haemorrhoids, or any other condition of the rectum.

Declaration

I…………………………………………………………….. hereby volunteer to be a subject in experiments/investigations during the period of …………………………………………………………………………20…….

My replies to the above questions are correct to the best of my belief and I understand that they will be treated with the strictest confidence. The experimenter has fully informed me of, and I have understood, the purposes of the experiment and possible risks involved.

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

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

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

Signature of Subject…………………………………………………Date……………………
Signature of Experimenter……………………………………………………Date …………………
APPENDIX 2. TMS specific health questionnaire

Participant Health Questionnaire

Title of Study: Progressive heat acclimation and central fatigue in high intensity intermittent sprint exercise in the heat

Principle Investigator: Mark Hayes

The following health questionnaire will help us to determine whether you are able to take part in the above titled study. Although slight, the methods used in this study carry some risk if there are underlying health conditions. Please answer each question as honestly as possible to enable us to ensure that the protocol is safe for you. The data collected will be kept strictly confidential, and your results will be completely anonymous in any subsequent publication or presentation.

What is your date of birth? ..............................................

When did you last see your doctor? ..............................................

Are you currently taking any medication?  Yes / No

If yes, please describe below:

........................................................................................................................................................................

To the best of your knowledge, please indicate Yes or No in answer to the questions overleaf:
1. Do you have any medically inserted metal plates or pins?
   Yes / No

2. Have you ever been diagnosed as having heart disease or a heart condition?
   Yes / No

3. Do you suffer from chest pains, heart palpitations or tightness of the chest?
   Yes / No

4. Do you feel pain in your chest when you undertake physical activity?
   Yes / No

5. Do you have a cardiac pacemaker?
   Yes / No

6. Have you had a neurosurgical procedure?
   Yes / No

7. Do you have known high blood pressure?
   Yes / No

8. Do you have known low blood pressure or do you often feel faint?
   Yes / No

9. Do you suffer from epilepsy?
   Yes / No

10. Have you ever had any type of a seizure?
    Yes / No

11. Have you ever had a brain injury, tumour or infection, including meningitis?
    Yes / No

12. Do you have any other brain problem or congenital brain defect?
    Yes / No

13. Have you ever had a head injury?
    Yes / No
14. Have you ever had a stroke?
   Yes / No

15. Have you had a cold or feverish illness in the last month?
   Yes / No

16. Do you ever lose balance because of dizziness, or do you ever lose consciousness?
   Yes / No

17. Have you suffered an upper respiratory tract infection in the last month?
   Yes / No

18. Do you have any known allergies? (Please specify)
   Yes / No

19. Have had hyper/hypothermia, heat exhaustion, or any other heat or cold disorder
   Yes / No

20. Have you had anaphylactic shock symptoms to needles, probes or other medical-type equipment.
   Yes / No

21. Have you had a history of infectious diseases (e.g. HIV, Hepatitis B);
   Yes / No

22. Have you ever had rectal bleeding, anal fissures, haemorrhoids, or any other condition of the rectum.
   Yes/No

23. Have you had exposure to a hot environment in the last 4 weeks
   Yes / No

If the answer to any of the above details is yes, please give further details below:

If you feel at all unwell because of temporary illness such as cold or fever please inform the investigator. If your health status changes so that you would subsequently answer Yes to any of the above questions, please notify the investigator immediately.

I have read and fully understand this health questionnaire. I confirm that to the best of my knowledge, the answers are correct and accurate. I know of no reason why I should not participate in this study, and I understand that I will be taking part at my own risk.
Participant name (please PRINT) .................................................................

Participant signature .................................................................

Date .................................................................

Witnessed by researcher (please PRINT) .................................................................

Researcher signature .................................................................

Date .................................................................