Heat acclimation with controlled heart rate: the effect of hydration on adaptation, cardiac function and exercise performance

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ABSTRACT

The effects of hydration status during heat acclimation on adaptation, and its influence on acute thermoregulatory and cardiovascular responses to exercise in heat acclimated individuals remains contentious. The aims of this thesis were to 1) characterise the responses to heat acclimation with controlled heart rate and the effect hydration status had on adaptive responses, 2) investigate the effectiveness of these interventions on exercise performance and, 3) determine the acute effects of maintaining euhydration or allowing progressive dehydration on central haemodynamics and thermoregulation during prolonged submaximal exercise. Chapter 4 reported responses to heat acclimation with both maintained euhydration and matched levels of dehydration in a counterbalanced cross-over study. Euhydrated acclimation increased sweat rate, lowered skin temperature and improved cycling time trial performance in the heat. These responses were not observed with dehydrated acclimation. Neither intervention lowered core temperature or increased plasma volume at rest or increased maximal aerobic cycling capacity in a temperate environment. Chapters 5 and 6 explored the haematological, thermal and haemodynamic responses to prolonged submaximal exercise following euhydrated and dehydrated heat acclimation, respectively. Responses were compared to pre-acclimation euhydrated and dehydrated trials with matched body mass deficits. Prior to both interventions, dehydration resulted in a reduction in cardiac output and mean arterial pressure. This was associated with hyperthermia, a decline in blood volume and increased heart rate impairing ventricular filling as end diastolic and stroke volumes were significantly lower than euhydration whilst end systolic volume remained similar. With acclimation, in the absence of an increased plasma or blood volume, stroke volume was not augmented by either intervention when euhydrated. Furthermore, with matched progressive dehydration, neither acclimation intervention altered the responses seen in pre-acclimation trials. These findings highlight the persistent effect of dehydration on the development of thermal and cardiovascular strain during exercise in the heat despite acclimation.

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List of Abbreviations

BV – Blood volume (ml)
Ca^{2+} – Calcium cation
CVP – central venous pressure (mmHg)
CO – Carbon monoxide
CO_2 – Carbon dioxide
DBP – Diastolic blood pressure
DEH – Dehydration
EDV – End diastolic volume (ml)
EF – Ejection fraction (%)
eNOS – Endothelial nitric-oxide synthase
ESV – End systolic volume (ml)
EUH –Euhydration
HA – Heat acclimation/acclimatisation
[Hb] – Haemoglobin concentration ($g \cdot dl^{-1}$)
Hb_{mass} – Haemoglobin mass (g)
Hct – Haematocrit (%)
HR – Heart rate ($beats \cdot min^{-1}$)
HSP – Heat shock protein
IVRT – Iso-volumetric relaxation time
kJ – Kilojoules (kJ)
LV – Left ventricle
MAP – Mean arterial pressure (mmHg)
Na^+ – Sodium cation
O_2 – Oxygen
PV – Plasma volume (ml)
$\dot{Q}$ – Cardiac output ($L \cdot min^{-1}$)
RCV – Red cell volume (RCV)
RPE – Rating of perceived exertion
SBP – Systolic blood pressure (mmHg)
SV – Stroke volume (ml)
SVR – Systemic vascular resistance ($mmHg \cdot L \cdot min^{-1}$)
T_C – Core body temperature (°C)
T_{sk} – Average skin temperature (°C)
TT – Time trial
USG – Urine specific gravity
$\dot{V}O_2$ – Oxygen uptake ($L \cdot min^{-1}$)

CHAPTER 1

Introduction

1.1 – Background

During dynamic exercise metabolically liberated heat leads to temperature elevations across multiple internal tissues of the body. However, when skin temperature (T_{sk}) is already high, such as during exercise in a hot environment, even slight elevations in core temperature (T_C) in the region of 3-4°C could be potentially fatal (Crandall & González-Alonso, 2010). In order to maintain T_C within the narrow limits required for optimal physiological function during exercise, various adjustments occur to achieve adequate heat exchange between tissues and the environment, mainly via convection and evaporation. To meet the increased blood flow demands of locomotor muscles, other metabolically active organs and the skin for thermoregulation, alterations in vascular tone occur to increase blood flow, cardiac output ($\dot{Q}$) and, to a lesser extent, redistribute blood from visceral organs to the periphery (Crandall & González-Alonso, 2010; Rowell, 1993). Increased sudomotor activity also aids heat transfer from the skin surface along a vapour pressure gradient. However, if this lost body water is not replaced during prolonged exercise, dehydration occurs. During strenuous exercise the strain placed on the circulatory system by heat stress and dehydration leads to significant alterations in cardiovascular, thermoregulatory and metabolic function that contribute to impaired exercise performance and early fatigue.

The increased demand for blood flow during moderate exercise and heat stress is typically met by increases in heart rate (HR) while stroke volume (SV) may be maintained or slightly elevated (Rowell, 1974). However, the ability of the circulatory system to increase $\dot{Q}$ and meet the demands of thermoregulation and aerobic metabolism are highly dependent on the environmental conditions, hydration status and the duration and intensity of exercise (Crandall, 2008; González-Alonso, 2007). For instance, exercise and whole-body heat stress is associated with a fall in SV compared to the same work in a temperate environment (Rowell, Marx, Bruce, Conn, & Kusumi, 1966). The concomitant hypovolemia and hyperthermia that occurs with exercise induced dehydration exacerbates the cardiovascular strain that occurs during heat stress. Excessive body water losses have the potential to

lower SV during exercise to such a degree that $\dot{Q}$ and mean arterial pressure (MAP) may become compromised (González-Alonso, Mora-Rodríguez, Below, & Coyle, 1995, 1997). These impairments in thermoregulatory and cardiovascular function during exercising heat stress are related to the deficit in total body water (Montain & Coyle, 1992b). The interplay between thermoregulatory adjustments to exercise and heat stress and dehydration appear to result in an impaired filling of the left ventricle (LV). Despite this generally accepted notion, the relationship between LV volumes during prolonged dynamic exercise and heat stress is relatively unexplored.

Repeated exposures to high internal and external heat loads may lead to several adaptive responses that act to lower subsequent physiological strain for a given exercising heat stress. These include a lower resting HR and T_C, a lower T_C, HR, oxygen uptake ($\dot{V}O_2$) and glycogen utilisation during exercise in the heat at a given workload and an increase in plasma volume (PV), sweating rate and faster onset of sweating (Periard, Travers, Racinais, & Sawka, 2016). These adaptations may be brought about via exposure to artificially or naturally occurring environments. Heat adapted individuals are better able to tolerate submaximal exercise in the heat (Nielsen et al., 1993) and demonstrate improved self-paced exercise in hot conditions (Garrett, Creasy, Rehrer, Patterson, & Cotter, 2012; Karlsen et al., 2015; Keiser et al., 2015; Lorenzo, Halliwell, Sawka, & Minson, 2010). In contrast, whether adaptations to exercising heat stress confers any benefit to performance in cool or temperate environments remains an issue of contention.

It is believed the most important adaptations to exercise and heat stress are of cardiovascular origin. Decreases in exercising T_C and HR typically occur in tandem with an expansion of PV prior to increases in sweat output (Mitchell et al., 1976; Senay, Mitchell, & Wyndham, 1976). An elevated PV theoretically increases ventricular filling, and together with a reduction in HR, SV is enhanced following heat adaptation (Nielsen et al., 1993; Rowell, Kraning II, Kennedy, & Evans, 1967). However, some have observed transient decreases in PV as the number of heat exposures increases (Shapiro, Hubbard, Kimbrough, & Pandolf, 1981) and this phenomenon has been

proposed to be an experimental artefact (Taylor, 2014). Instead, interventions that maintain internal heat load and stimulate fluid regulatory responses via dehydration have been shown to result in a sustained expansion of PV (Patterson, Stocks, & Taylor, 2004b). Despite this, very few investigations have directly assessed the influence of hydration status on adaptation of individuals and findings remain equivocal (Garrett et al., 2014; Neal, Massey, Tipton, Young, & Corbett, 2016b). It is also relatively unclear if the acute development of dehydration off-sets the cardiovascular and thermoregulatory adjustments to heat adaptation or whether these adaptations dampen the marked physiological strain that occurs with dehydration. Therefore, the direct effects of heat adaptation and hydration on human cardiovascular function during exercise heat stress remains poorly understood.

Recently, exercising at a maintained target HR in the heat has been proposed to sustain internal whole-body heat load (Periard, Racinais, & Sawka, 2015). This technique may match the cardiovascular and thermal adjustments to exercise in the heat with and without dehydration and therefore potentially offers a practical and easily implemented method of heat adaptation for individuals. However, to date very few studies have utilised this experimental approach (Keiser et al., 2015; Pethick et al., 2018; Philp, Buchheit, Kitic, Minson, & Fell, 2017) and the responses to such an intervention are yet to be fully described.

1.2 – Thesis aims

The aims of this thesis were to i) characterise the responses to HA with controlled HR and the effects hydration status have on the magnitude and rate of adaptations, ii) to investigate the effectiveness of these interventions on maximal aerobic capacity in temperate conditions and self-paced exercise performance in the heat, iii) to characterise the LV volumes and central haemodynamic responses to dynamic sub-maximal exercise in the heat, and iv) determine the effects of acute exercise induced dehydration following HA on central haemodynamics and thermoregulation.

This thesis comprises of one large scientific study. The data collected from this study was used to test several unique hypotheses. Each of these aspects are broken down into experimental chapters. In Chapter 4, a counterbalanced cross-over study was conducted to determine the responses to two medium-term (10-day) exercise HA interventions with controlled HR. Interventions differed in the hydration strategy employed throughout the acclimation period. The adaptive responses to each adaptation period as well their effects on maximal aerobic capacity in a temperate environment and self-paced exercise performance in the heat were explored. In Chapter 5, the influence of euhydrated HA on the haematological, thermal and haemodynamic responses to prolonged exercise and heat stress, with and without dehydration, were determined. Finally, Chapter 6 explored the effect dehydrated HA had upon these responses to determine whether a medium-term period of HA with fluid restriction influenced the effects of acute dehydration during prolonged exercise and heat stress.

CHAPTER 2

Review of literature

The following Chapter reviews the literature that is relevant to the studies described above. The review describes the cardiovascular and thermoregulatory adjustments to different physiological stressors. The haemodynamic responses to passive heating are outlined before the circulatory demands of exercise with and without heat stress and or dehydration are described. In later sections the adaptive responses to HA are discussed in relation to the various methods that have been employed over the last decade to improve work capacity, exercise performance, and thermal tolerance. How these interventions might be optimised to provide a practicable means of improving adaptations to hot environments, and how these adaptations might alter cardiovascular stability during subsequent exercising heat stress with and without dehydration is discussed. Finally, the experimental aims and hypotheses are presented.

The peer reviewed publications included in this review were sourced via electronic databases that included but were not limited to: MEDLINE, PubMed, Google Scholar and Brunel SUMMON. Search strategies regularly included the following keywords: *'acclimation', 'acclimatisation', 'adaptation', 'blood', 'blood flow', 'cardiac', 'cardiovascular' 'controlled hyperthermia' 'dehydration', 'exercise', 'fluid', 'function', 'haemodynamic', 'heat strain', 'heat stress', 'hydration', 'hyperthermia', 'performance', 'plasma', 'thermoregulation', 'ventricle'* and *'volume'*. In cases where full text publications were not available via these databases, additional searches were performed elsewhere using ResearchGate, Qatar National Library and SciHub. Additional resources included narrative and systematic reviews and where studies of interest were found, original texts were sourced using the databases mentioned. Articles found and included in this review contain data obtained from human and animal studies and are highlighted where appropriate. As many of the typical acute and chronic adjustments to exercise and heat stress are well established, historical experimental evidence for such phenomena is included. In general, discussion of key expected outcome measures and magnitudes of change described in the sections below is afforded to more recent studies where detailed experimental methodology and statistical analyses have been provided.

2.1 – Defining thermal stress and strain

As highlighted in Chapter 1, when organisms are subjected to conditions of high exogenous thermal load or are producing large amounts of metabolic heat (e.g. during high intensity dynamic exercise) various physiological responses are necessary to maintain homeostasis. The acute responses that support physiological homeostasis under a particular stress is termed accommodation (Taylor, 2014) and are highly dependent on physical and environmental factors such as exercise intensity, environmental temperature and humidity. Therefore, to determine the adaptive effects to repeated stressors, it is important to define heat stress and strain.

Stress is any effector that may disrupt homeostasis. In experimental procedures external stressors such as ambient heat and humidity can be tightly controlled, are time-dependent and are termed forcing functions (Taylor, 2014). Heat stress itself may be defined simply as the elevation of temperature above normal ambient conditions. Strain refers to the physiological responses to stress and the extent that homeostasis is disturbed. During exercise in the heat, physiological strain is typified by changes in mean arterial pressure (MAP), central venous pressure (CVP), BV, plasma osmolality and mean body temperature (Taylor, 2014). Under conditions of environmental and or exercising stress, effector organs are controlled to restore the internal environment, such as increases in HR, SV, sweat rate or skin blood flow. These responses may be stress specific (Adolph, 1955) but can perhaps also be considered non-specific (Selye, 1973). Following heat adaptation, overall physiological strain may be less pronounced for a given thermal stress, such as a lower body temperature and cardiovascular strain. Conversely other effector organs may display a higher level of strain, such as increased sweating rates although this does not imply a greater whole-body strain and each of these adjustments confers positive and beneficial responses to environmental heat stress. In this thesis, heat stress will be used to describe a broad range of temperatures above typical ambient conditions while strain will describe the physiological responses to

these external stressors. Exercise heat stress refers to increases in metabolic heat production that occurs in warm or hot environments.

2.2 – Left ventricular function

Cardiac function encompasses several phases of filling and emptying of blood from the cardiac atria to the ventricles and the pulmonary and systemic circulations over a single complete cycle that generates a volume of blood (the SV). This volume serves all active tissues of the systemic circulation and is highly dependent upon the volume of blood in the central circulation and the ability of the heart to mechanically generate the necessary pressure to eject blood to the systemic circulation at a sufficient rate. A single cycle consists of myocardial contraction (systole) and relaxation (diastole) that each result in emptying and filling of the LV, respectively. For this to occur, various pressure and volume shifts must take place in a coordinated sequence. This is made possible by the elegant anatomy of the heart allowing it to generate the necessary pressure for systolic ejection and aid diastolic filling.

The following sections of this literature review will describe a typical cardiac cycle and how this is made possible due to the anatomical arrangements of cardiac myocytes. The literature pertinent to LV function during heat stress and dehydration will be evaluated in relation to how these stressors affect the filling and distribution of blood from the heart and the influence this has on thermoregulation and exercise capacity. Finally, the adaptive effects of heat acclimation will be explored and how these may relate to changes in cardiac function in humans during exercise in the heat.

A typical cardiac cycle is displayed in Figure 2.1. The systolic phase is initiated during a brief iso-volumetric contraction, during which time shortening of endocardial fibres and stretching of the epicardial fibres results in a rapid increase in intra-ventricular pressure without a change in LV volume (Sengupta et al., 2006a). Intra-ventricular pressure continues to rise and once this exceeds aortic pressure (~80 mmHg), the aortic valve is opened, and blood is ejected into the circulation and the volume of the LV decreases.

Approximately 150-200 ms after the QRS complex of electrical excitation of the ventricle, repolarisation occurs. This causes the level of ventricular active tension and hence the rate of blood ejection to decrease. Ventricular pressure falls slightly below that of the outflow tract pressure. Blood briefly continues to flow outward due to kinetic energy propelling blood into the aorta and once this energy is lower than that of the outflow tract, blood begins to flow backward toward regions of lower pressure in the ventricles, catching cusps of the aortic valve causing its abrupt closure (Klabunde, 2012; Tortora & Derricksen, 2012). Rebound of blood on the closed cusps of the aortic valve produces the dicrotic wave of the aortic pressure curve (Figure 2.1). The volume ejected during systole in the healthy resting human is approximately 60% of the end diastolic volume (EDV; Klabunde, 2012; Tortora & Derrickson, 2012). Ventricular pressure following systole reaches approximately 5 mmHg and promotes the efficient filling of the LV during iso-volumetric relaxation. This is where fibres relax with no change in LV volume (Sengupta et al., 2007).

Reduced intra-ventricular pressure creates the required gradient between the left atrium and LV, opening the mitral valve and blood fills the LV. Contraction of the left atrium (atrial kick) pumps additional blood into the ventricle, helping to increase subsequent SV by ~25% in healthy humans (Alpert, Petersen, & Godtfredsen, 1988). The order of events does not vary significantly during periods of enhanced demand for blood flow however, end diastolic, systolic and SV can all be influenced by several factors acting upon the LV.

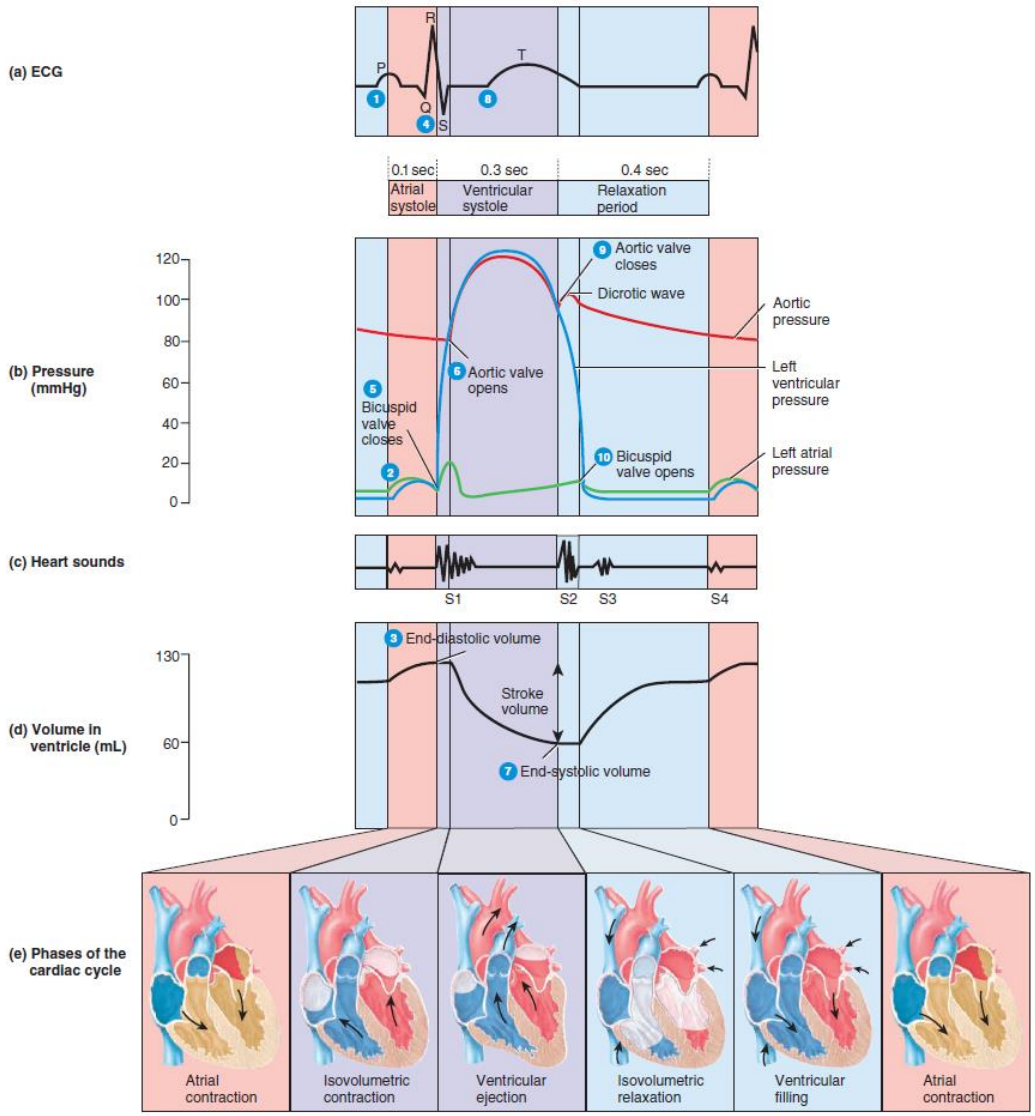


Figure 2.1: The typical timings and magnitudes of a) an electrocardiogram trace, b) pressure changes in the heart, c) auscultations, d) LV volumes, and e) phases of a single cardiac cycle. Reproduced from Tortora & Derrickson, (2012).

2.2.1 – Preload, afterload and their effects on normal left ventricular function

Cardiac function changes in response to alterations in preload (right atrial and CVP) and afterload (aortic blood pressure; Rowell, 1993). The relationships between preload, afterload and myocardial function are displayed in Figure 2.2. In a supine resting position, a CVP of ~5 mmHg fills the LV (Rowell, 1986, 1993). However, during exercise CVP increases with exercise intensity to ~11 mmHg as blood flow is promoted back to the heart and this is paired with

similar increases in SV (Higginbotham et al., 1986). The increased SV has been linked to the stretching of cardiac myofibres during diastole and their associated increased tensional recoil (Figure 2.2). This phenomenon is known as the Frank-Starling mechanism (Patterson, Piper, & Starling, 1914). Similarly, altered afterload will affect SV since the resistance that the LV has to work against to eject blood varies (Sonnenblick, 1962). A higher afterload reduces force production of LV myofibres as more energy during iso-volumetric contraction is needed to overcome the higher aortic pressure before blood may be ejected into the outflow tract. As seen in Figure 2.2, progressive increases in afterload inhibit myofibre shortening during the ejection phase for a given myofibre length and therefore decreases SV.

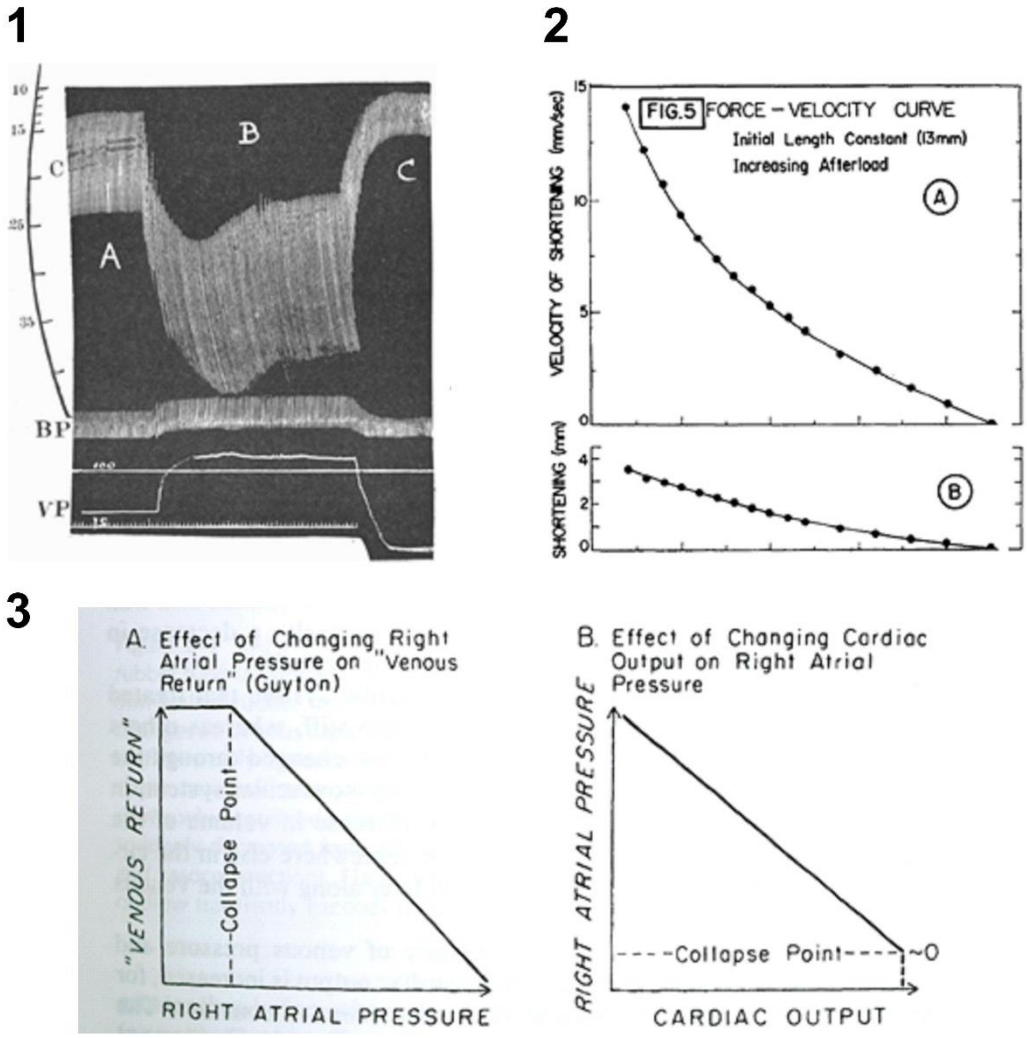


Figure 2.2: Panel 1: Frank-Starling mechanism displayed using a dog lung preparation. With increased diastolic pressure (VP) LV SV increases (B; reproduced from Patterson et al., 1914). Panel 2: Afterload-shortening relationship. Increasing afterload at a given myofibre length results in a reduction in myofibre shortening (B) and velocity of shortening (A; reproduced from Sonnenblick, 1962). Panel 3: Venous return curves (A, left) showing that manipulation of right atrial pressure by pumping blood into the circulation increases venous return. Increasing cardiac output (B, right) lowers right atrial pressure which is in turn caused by the effect of blood flow on peripheral vascular volume (reproduced from Rowell, 1993 using data from Guyton et al., 1973; Levy, 1979).

At the systemic level, the experiments of Guyton and colleagues (Guyton et al., 1973; Levy, 1979) demonstrated how $\dot{Q}$ and therefore venous return determines CVP. By pumping blood out of the right atrium and lowering its pressure, the greater the venous return. This demonstrates that a change in atrial pressure acts on blood flow in a retrograde direction to alter the flow of

blood from the LV, through the systemic circulation and into the right atrium (Figure 2.2-3, left). Alternatively, pacing of the heart to increase $\dot{Q}$ (Figure 2.2-3, right) is limited by the fall in right atrial pressure and therefore pre-load of the LV since blood is transferred into the peripheral vasculature. Together, this demonstrates that preload and afterload affect the ability of the heart to eject blood into the peripheral circulation.

2.2.2 – Neural control of the left ventricle

In addition to preload and afterload, the force of contraction by the LV is also influenced by sympathetic and parasympathetic branches of the autonomic nervous system. This section will briefly describe the neural control of the myocardium and further highlight the homeostatic mechanisms that may play an integrated role in physiological responses to various stressors (Taylor, 2014).

In cardiac myocytes, contractions are tightly regulated by the excitation-contraction coupling pathway (Parks & Howlett, 2013). This pathway converts an electrical stimulus from the sinoatrial node into a mechanical contraction. A wave of excitation triggers inward movement of calcium ions (Ca^{2+}) into the myocyte through the sarcolemma, releasing further Ca^{2+} from the sarcoplasmic reticulum (calcium-induced calcium release; Bers, 2002) . The gain of the excitation-contraction coupling depends on several factors including; increased sarcoplasmic reticulum Ca^{2+} load, β-adrenergic stimulation and a decrease in temperature (Ginsburg & Bers, 2004; Shutt & Howlett, 2008; Viatchenko-Karpinski & Györke, 2001). Ca^{2+} binds to troponin C of the troponin-myosin complex resulting in a crossbridge formation and myocyte contraction (Bers, 2002). Relaxation of the myocyte occurs when Ca^{2+} is removed into the sarcoplasmic reticulum via the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) while a small amount is removed from the cell by a Na^{+}/ Ca^{2+} exchanger (Reuter et al., 2005). Phospholambans regulate re-uptake of Ca^{2+} by inhibiting SERCA.

The resting healthy human HR is ~60 beats·min^{-1} and is regulated by the predominant activity of the parasympathetic nervous system and its neurotransmitter release of acetylcholine. This acts to slow otherwise basal intrinsic pacemaker activity that regulates HR ~100 beats·min^{-1} (Jose, 1966). However, sympathetic nervous activity releases adrenaline and noradrenaline which stimulate β-adrenergic receptors in the myocardium. This results in the increase in the positive chronotropic and inotropic effects (i.e. increased rate and force of contraction, respectively) and positive lusitropic effects (relaxation; Klabunde, 2012).

Increases in inotropic and lusitropic states are directly related to the magnitude of sympathetic stimulation. Despite this, direct β-adrenergic stimulation of the cardiac sympathetic nerves would only result in a small increase in SV, since a concomitant fall in right atrial pressure would occur, particularly at higher $\dot{Q}$, thus reducing EDV (Barnes, Bower, & Rink, 1986). Together these paragraphs demonstrate that ventricular preload, afterload and myofibre contractility by sympathetic stimulation all act to affect $\dot{Q}$. This is achieved by enhancing the force of contraction as well as the rate of contraction and relaxation. However, in conditions where severe passive heat exposure or haemorrhage occurs and CVP reaches ~0 mmHg (Crandall et al., 2008), it is impossible for sympathetic activity to increase $\dot{Q}$ further and arterial pressure is compromised. In the next section of this literature review, the mechanical contractile properties of the LV are briefly explored and how this affects the filling and emptying of the heart during a cardiac cycle is addressed. Subsequent sections shall then explore the limits of cardiovascular function, control and therefore distribution of blood flow during stressors such as exercise, dehydration and exogenous thermal load.

2.2.3 – Basic anatomical and mechanical properties of the left ventricle

SV is in an important factor during exercise and as outlined in subsequent sections may be a limiting factor in maintaining blood perfusion to the systemic vasculature. Particularly at high exercise intensities or when the level of heat stress is uncompensable. Not only is this determined by preload, afterload and

contractility, but also by the mechanical movement of the LV during contraction and relaxation. The following sections will briefly describe the anatomy and architecture of the LV and how it determines its mechanical action across the cardiac cycle.

The myocardium is made up of several layers (Greenbaum, Ho, Gibson, Becker, & Anderson, 1981) with differences in fibre orientation between each. A description of the anatomical orientation of myofibres within each layer and the global anatomy of the LV are outlined in elegant detail in the following manuscripts (Beladen, Călin, Roşca, Ginghină, & Popescu, 2014; Buckberg, Nanda, Nguyen, & Kocica, 2018; Sengupta et al., 2006b). Briefly, in the subendocardial region fibres are orientated in an oblique helical arrangement and this gradually changes to a left handed helix in the subepicardial region (Sengupta et al., 2006b). Myocardial fibres in the mid LV wall are mainly oriented in the circumferential direction, whereas epicardial fibres spiral obliquely toward the apex the endocardial fibres spiral obliquely toward the base of the ventricle (Beladen et al., 2014). Therefore, the fibres are arranged in an angled helix throughout the LV.

Since each cardiac myocyte can only contract along its short axis (Spotnitz, 2000), movement of the LV during contraction and relaxation is determined by the myofiber arrangement in the LV wall. Therefore, in systole the LV apex undergoes counter-clockwise rotation about its longitudinal axis, while the opposite clockwise rotation occurs at the base during LV ejection (Sengupta et al., 2006a). This results in a net twisting or ‘wringing’ motion of the LV which serves to increase mechanical efficiency as blood is ejected towards the aortic outflow tract (Sengupta et al., 2007).

Systolic twisting of the LV is a key factor in its mechanical performance when ejecting blood and generates increased intraventricular pressure with lower transmural strain and O_2 consumption (Beyar & Seideman, 1985). Systolic LV twist is followed by rapid untwisting in early diastole as elastic potential that is stored in the shortened myofibres of the myocardium is released. Most of this untwisting occurs during iso-volmetric relaxation and the apex is the major

source of this recoil (Notomi et al., 2006). This rapid untwist also produces a large decrease in LV pressure, creating an early diastolic intraventricular pressure gradient contributing to diastolic suction of blood into the LV (Notomi et al., 2006). Untwisting rate correlates with the iso-volumetric relaxation and early diastolic intraventricular pressure gradient (Notomi et al., 2006). Furthermore, this untwisting appears to occur prior to the peak intraventricular pressure gradient and filling velocities in diastole (Notomi et al., 2006). Therefore, under any conditions where the rate of untwist is affected, LV filling may be significantly impaired potentially altering EDV and SV.

2.2.4 – Summary

The above sections provide an overview of the anatomical and mechanical properties of the LV. While direct measurements of mechanical function were not performed in this thesis, an understanding of the contribution and limitations of such properties to various stressors provides a global overview of the factors at play in production of a SV. Furthermore, relatively little is known regarding the adaptive responses that alter cardiovascular adjustments to the stressors of exogenous heat-stress induced hyperthermia and dehydration. A positive adaptive response to a given stressor is a reduction in overall physiological strain. The next sections characterise separate and combined stressors from passive heating to combinations of exercise, high ambient temperatures and dehydration. The thermoregulatory and cardiovascular demands will be discussed before potential adaptations that serve to lower physiological strain will be highlighted. In doing so gaps in the literature will be identified therefore forming the basis of the experiments undertaken.

2.3 – The haemodynamic and thermoregulatory challenge of exercise, heat stress and dehydration

2.3.1 – Passive heat stress

Passive heat stress results in redistribution of blood from central circulations towards the periphery (Crandall et al., 2008). This together with much larger increases in $\dot{Q}$ places a significant physiological strain upon the cardiovascular system that is only seconded by exercise. Humans exposed to passive whole-body heat stress may experience average T_{sk} upwards of ~40°C (Minson, Wladkowski, Cardell, Pawelczyk, & Kenney, 1998; Pearson et al., 2011; Rowell, Brengelmann, & Murray, 1969a; Stöhr et al., 2011b). The demands to maintain MAP in the face of increased skin blood flow requirements must be met by an increase in $\dot{Q}$ along with redistribution of BV from the compliant vascular beds of the renal and splanchnic circulations. The circulatory responses to heat stress have been extensively researched for over half a century and the degree of this remarkable repartitioning is summarised in Figure 2.3 and Table 2.1. Central blood volume under passive heat stress can see ~350 and ~650 $ml \cdot min^{-1}$ reductions in renal and splanchnic circulations, respectively (Minson et al., 1998; Rowell, Brengelmann, Blackmon, & Murray, 1970; Rowell et al., 1969a; Rowell, Detry, Profant, & Wyss, 1971). This is coupled with increased arm and leg blood flows in the region of 600 $ml \cdot min^{-1}$ (Naylor et al., 2011) and 0.9-1.4 $L \cdot min^{-1}$ (Chiesa, Trangmar, & Gonzalez-Alonso, 2016; Pearson et al., 2011), respectively, with maximal skin blood flow estimated to be in the region of 7.8 $L \cdot min^{-1}$ (Rowell, 1974). However, reported absolute increases in skin blood flow measured via occlusion plethysmography are less clear cut. Inherent error is associated with the assumption that flow is isolated to the cutaneous circulation with changes in transverse sectional area of the limb (Whitney, 1953), thus overestimating skin blood flow changes.

In the absence of an active muscle pump, CVP and right atrial pressure approach 0 mmHg (Crandall et al., 2008; Rowell et al., 1969a), reducing filling pressure of the heart. Decreases in MAP have been reported to be in the

region of -2-6 mmHg (Crandall et al., 2008; Minson et al., 1998; Wilson et al., 2009) and modulation of the baroreceptor set point is shifted to the lower prevailing blood pressure during heat stress (Crandall, 2008). Such a shift decreases the functional reserve of carotid baroreceptors to buffer against further decreases in pressure, which may contribute to orthostatic intolerance with heat stress (Crandall, 2000). Despite the drastic reduction in filling pressures, SV is either maintained (Nelson et al., 2011; Stöhr et al., 2011b) or has a tendency to slightly increase (Rowell et al., 1969a; Wilson et al., 2009; Wilson et al., 2007). Therefore, increases in $\dot{Q}$ in the region of 1.6 to 7.1 $L \cdot min^{-1}$ (Ganio et al., 2012; Minson et al., 1998; Nelson et al., 2011; Pearson et al., 2011; Rowell et al., 1970; Rowell et al., 1969a; Rowell et al., 1971; Stöhr et al., 2011b), depending on the level of heat stress, is achieved predominantly via elevations in HR.

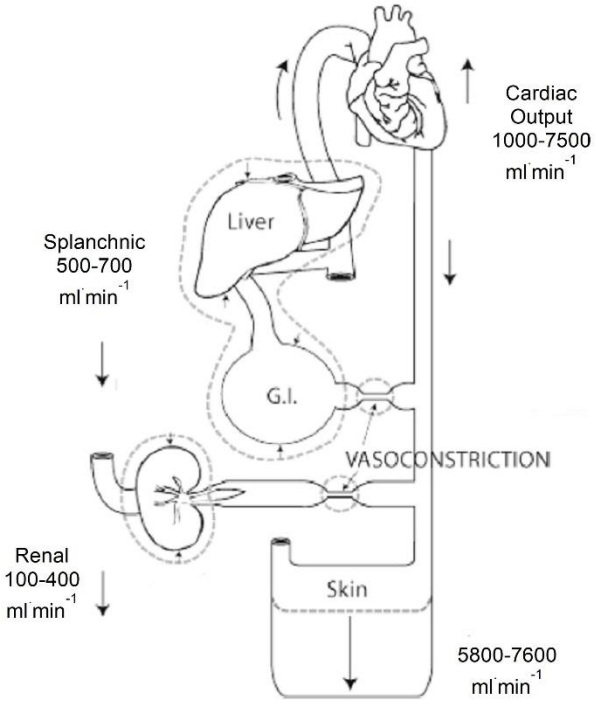


Figure 2.3: Repartitioning of blood volume from central circulations in various degrees of passive heat stress. This is achieved by changes in vascular tone and increases in $\dot{Q}$ despite reductions in vascular filling (Redrawn from Kenney et al., 2014; data from a series of studies, see text for details).

Table 2.1: Haemodynamic responses to elevated core and skin temperatures during passive heat stress in the supine position. Increases in cardiac output are achieved primarily via increases in heart rate. (Data from various studies, see text for details).

	Normothermia	Passive Heat stress
Average T_{sk} (°C)	34.4 ± 1.1	39.5 ± 1.7
T_C (°C)	37.0 ± 0.1	38.3 ± 0.5
$\dot{Q}$ ($L \cdot min^{-1}$)	6.5 ± 0.9	9.7 ± 1.8
HR ($beats \cdot min^{-1}$)	62 ± 55	109 ± 27
SV (ml)	105 ± 15	107 ± 1
MAP (mmHg)	93 ± 9	90 ± 10
CVP (mmHg)	5.5 ± 0.7	0.2

Increases in HR are achieved via a variety of mechanisms that consist of direct intrinsic effects of higher temperature upon the sinoatrial node (Garrey & Townsend, 1948; Gorman & Proppe, 1984; Jose, Stitt, & Collison, 1970), baroreceptor-mediated feedback where reductions in MAP and CVP occur (Crandall, 2000; Wilson & Crandall, 2011) and increases in circulating catecholamines (Rowell, 1990). These alterations indicate that heat stress has an inotropic effect on the heart that improves contractile function in the face of reduced preload. This has important implications since, as highlighted above, heat stress reduces filling pressure and creates a left-ward shift of the Frank-Starling curve. Therefore, by moving the operating point to the steeper portion of the curve, any further reductions in filling pressure will result in greater changes to SV (Wilson et al., 2009). However cardiac diastolic and systolic functions are maintained and improved respectively (Brothers et al., 2009), along with an increased peak twisting velocity (Stöhr et al., 2011b) and ejection fraction of the LV (Crandall et al., 2008). This enhanced contractility of the heart along with increases in HR act to increase $\dot{Q}$ even with more severe levels of heat stress. Furthermore, the increased systolic function and twisting velocity is maintained when heat stress is coupled with dehydration in the region of ~3.5% body mass loss. This indicates that an enhanced

contractility remains and reductions in SV occur as a result of a reduced LV filling (Stöhr et al., 2011b) at least at rest and during single leg knee-extensor exercise.

2.3.2 – Exercise and heat stress

Regulatory demands of skin and muscle blood flow during the paired stressors of high ambient temperatures and whole body exercise places the largest challenge upon the cardiovascular system it can face (Rowell, 1993). Muscle and skin require substantial fractions of the $\dot{Q}$ for both oxidative metabolism and thermoregulation. The haemodynamic responses to exercise with or without heat stress can vary greatly depending on a wide range of factors. These include; the type of exercise undertaken and therefore the active muscle mass and pressure changes across the circulation, the intensity and duration of exercise, the degree and type of heat stress (whole body, skin or isolated limb) and hydration status. Debate remains as to whether this physiological strain is a direct result of competition between vascular beds for the respective perfusion necessary for locomotive skeletal muscle blood flow and thermoregulation or whether changes in flow of these circulations represents a commensalistic relationship (Kenney et al., 2014). Nevertheless, exercise in hot environments results in significant cardiovascular strain (Nielsen, Savard, Richter, Hargreaves, & Saltin, 1990; Periard, Cramer, Chapman, Caillaud, & Thompson, 2011; Rowell et al., 1966; Rowell, Murray, Brengelmann, & Kraning, 1969b; Trinity, Pahnhe, Lee, & Coyle, 2010) and impairs the capacity to perform dynamic exercise (Ely, Cheuvront, Kenefick, & Sawka, 2010; Ely, Cheuvront, Roberts, & Montain, 2007; Galloway & Maughan, 1997; González-Alonso & Calbet, 2003; Periard et al., 2011), with initial body temperature being inversely related to time to exhaustion with uncompensable heat stress (González-Alonso, Mora-Rodríguez, & Coyle, 1999a). The following sections shall identify the limitations to skin and locomotor muscle blood flow imposed by heat and dehydration.

Heat stress and exercise each place the cardiovascular system under significant strain. When the metabolic heat production of exercise is paired

with exogenous heat stress however, this additional thermoregulatory strain is significant. In a classic study conducted by Rowell et al., (1966) this miss-match between thermoregulatory and O_2 demands to greater exercise intensities in the heat in untrained un-acclimatised individuals manifested as a reduced $\dot{Q}$ during moderate to high intensity exercise (Figure 2.8)

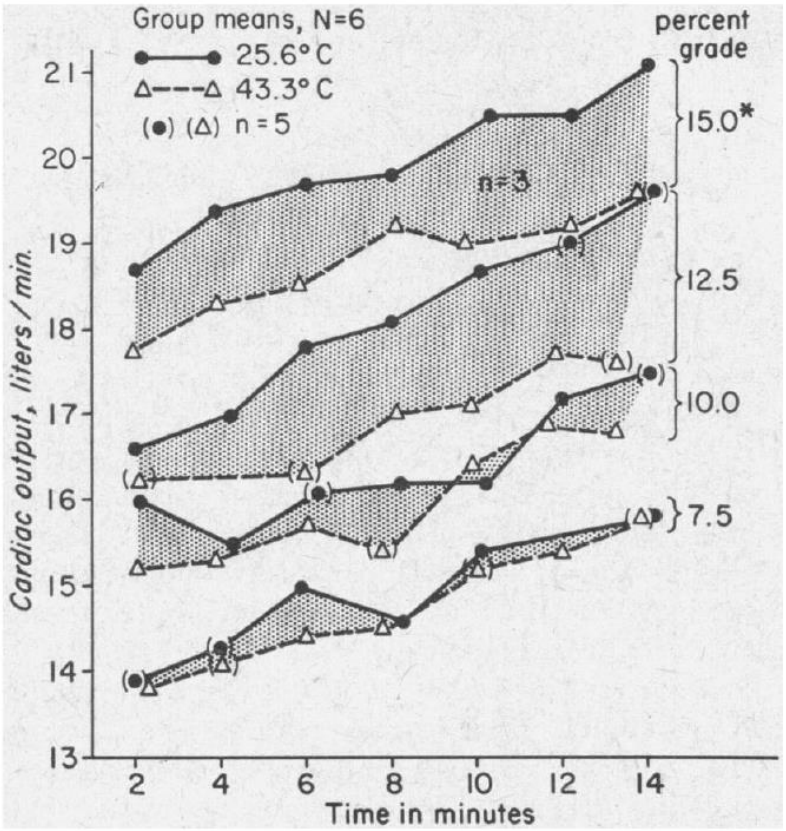


Figure 2.4: Cardiac outputs of untrained men walking on a treadmill at different grades of incline in temperate (circles) and hot (triangles) conditions. At higher exercise intensities there is a greater demand for peripheral blood flow for thermoregulatory and metabolic needs. This compromises cardiac filling and SV resulting in significantly elevated HR and decreased mean arterial pressure. Reproduced from Rowell et al. (1966).

A lower $\dot{Q}$ during exercise in the heat is possibly due to thermoregulatory requirements for skin blood flow while skeletal muscle perfusion remains unchanged (Nielsen et al., 1993). This gives rise to substantial decreases in central blood volume and ultimately SV (Rowell et al., 1966; Rowell et al., 1969b). A moderate increase in metabolic demand during small muscle mass exercise (Pearson et al., 2011; Savard, Nielsen, Laszynska, Larsen, & Saltin, 1988; Stöhr et al., 2011b), treadmill walking (Nielsen et al., 1990; Rowell et

al., 1966) and cycling exercise (Nielsen et al., 1993; Trinity et al., 2010) in the heat has been shown to be met by maintained or increased $\dot{Q}$. In the face of a diminished EDV, this maintenance of $\dot{Q}$ is achieved by significant increases in HR. However, at higher intensities of exercise the significantly elevated HR limits filling time of the LV and therefore the ability to increase $\dot{Q}$ (Fritzsche, Switzer, Hodgkinson, & Coyle, 1999; Trinity et al., 2010). This limited cardiac capacity under an increased adrenergic state (Rowell, 1990) with increased sympathetic nerve activity (Crandall, Etzel, & Farr, 1999; Gagnon, Schlader, & Crandall, 2015; Low, Keller, Wingo, Brothers, & Crandall, 2011) limits the perfusion and oxygen uptake of the exercising limbs during high intensity exercise (González-Alonso & Calbet, 2003; Rowell, 1974).

The limitation of the cardiovascular system is further highlighted when heat stress is combined with dehydration. Excessive fluid losses through sweating result in significant reductions in PV and the declines in SV and $\dot{Q}$ during steady state exercise are linearly related to body water deficits (Montain & Coyle, 1992b). In a series of studies, González-Alonso et al., (1998; 1995, 1997) investigated the separate and combined effects of dehydration and exercising heat stress on central and peripheral haemodynamics (Figure 2.5). Significantly greater reductions in SV were observed when dehydration occurred alongside hyperthermia (González-Alonso et al., 1995, 1997), reducing muscle blood flow (González-Alonso et al., 1998). Interestingly, when exercising in a cold environment the decline in SV is attenuated (González-Alonso, mora-Rodríguez, & Coyle, 2000a) and expansion of the plasma compartment via dextran infusion fully restores this decline (González-Alonso et al., 1997). Furthermore, supine exercise in the heat in the hypohydrated state also reverses the detrimental effects of dehydration and hyperthermia during upright exercise, permitting better maintenance of arterial pressure, SV and $\dot{Q}$ (González-Alonso et al., 1999a). Together, this suggests that dehydration and hyperthermia lead to significant temperature related elevations in HR and a reduction in central BV that limits cardiac filling during exercise and hence, $\dot{Q}$.

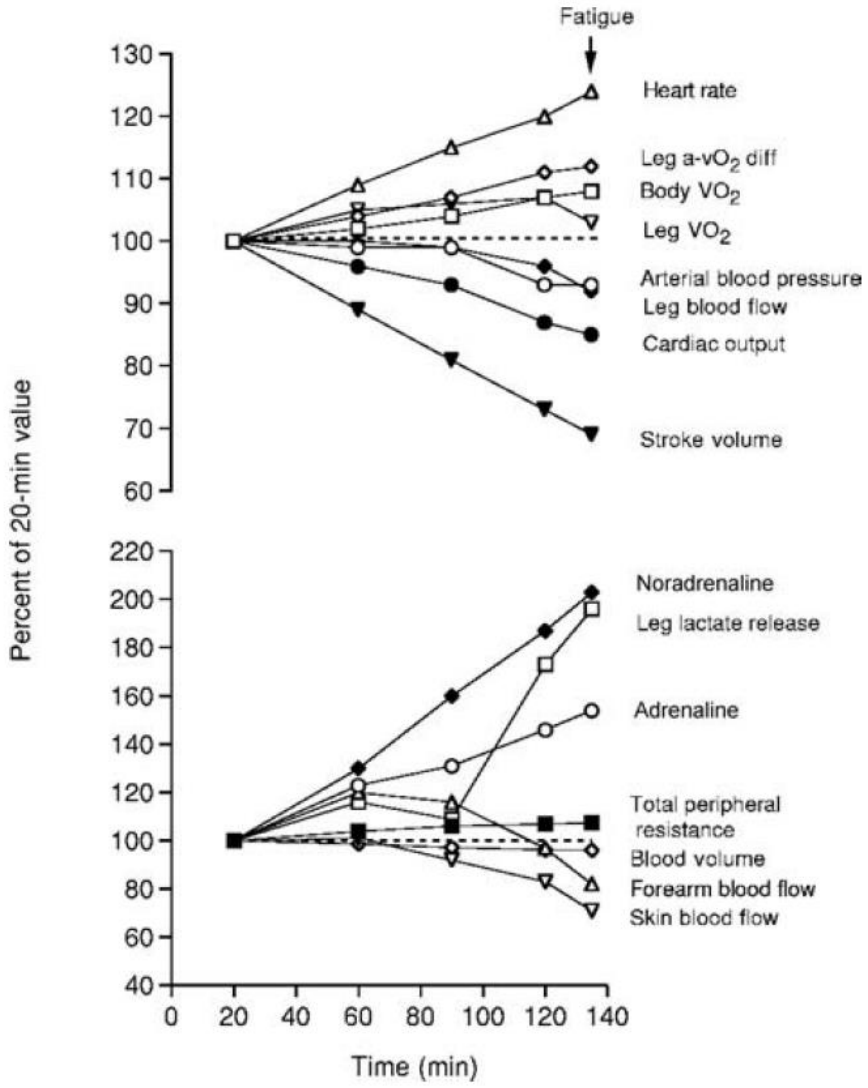


Figure 2.5: Effects of progressive dehydration and hyperthermia on cardiovascular haemodynamics, metabolism and circulating catecholamines. Reproduced from González-Alonso (2007) using data from González-Alonso et al. (1997) and (1998).

The effects of heat stress with and without dehydration on cardiac mechanics are less well understood. However, research to date suggests a relatively minor contribution of enhanced mechanical function during more pronounced cardiovascular challenges. Previous studies have identified increases in systolic and diastolic twist to compensate for the decrease in venous return that is associated with passive elevations in T_C between 0.8-1°C (Brothers et al., 2009; Nelson et al., 2010a; Nelson et al., 2010b). Stöhr, González-Alonso, and Shave (2011c) exposed healthy males to passive heat stress using a

water perfused suit. LV function was assessed during single-leg extensions following increases in T_{sk} alone, moderate increases in T_C and T_{sk} and severe increases in T_C (~2°C). LV twist was enhanced during exercise with moderate heat stress and plateaued with severe heat stress. This occurred mainly through enhanced basal rotation. Interestingly however, SV was maintained while ejection fraction was enhanced. As highlighted previously, the level of limb hyperaemia during small muscle mass exercise with heat stress is significantly augmented (Pearson et al., 2011). This increased vascular conductance together with the lowered MAP with severe heat stress may reduce afterload and hence, serve to maintain $\dot{Q}$ without further increases in twist mechanics (Stöhr et al., 2011b).

At rest, left ventricular twist, untwist and strain have been shown to be reduced following long-duration endurance exercise that led to a ~4.5% reduction in body mass (Nottin et al., 2009), The authors associated these reductions to prolonged exercise rather than to changes in LV mechanics occurring in response to dehydration. Stöhr et al. (2011a) investigated the effects of dehydration at rest and during small muscle mass exercise and observed contrasting results. Body mass deficits of ~3.5% resulted in significant increases in peak systolic longitudinal strain and strain rate, while diastolic longitudinal strain was also enhanced (Stöhr et al., 2011a). This occurred in the face of a declining EDV. Furthermore, many studies that investigate the mechanical responses to reduced preload simultaneously increase HR (Brothers et al., 2009; Nelson et al., 2010a; Rickards et al., 2015; Stöhr et al., 2011a). Significant BV reductions without changes in HR have been shown to reduce SV, EDV and LV strain, possibly via altered geometry of the LV (Lord et al., 2018). Therefore, it appears that the reductions in SV and $\dot{Q}$ during passive heating, heat stress and dehydration occur independently of twist mechanics and are likely due to reductions in preload (Stöhr et al., 2011a).

2.3.3 – Summary

The previous sections have highlighted the potential for heat stress, exercise and dehydration to result in significant thermoregulatory and cardiovascular strain. With significant increases in T_C during exercise in a hot environment, HR is elevated while SV may be decreased compared to exercise in temperate conditions, via a combination of increased cutaneous blood flow and impaired ventricular filling (Rowell et al., 1966; Rowell et al., 1969b; González-Alonso et al., 1999a; González-Alonso et al., 2000; Trinity et al., 2010). With progressive dehydration, augmented increases in T_C and HR occur while BV is significantly decreased. As exercise progresses, thermoregulatory and cardiovascular function is significantly impaired despite the presence of an elevated sympathetic activity, and there is a steady fall in SV, $\dot{Q}$, MAP and muscle blood flows (González-Alonso et al., 1998). Therefore, the significant physiological strain brought about by heat stress and dehydration impairs adequate blood flow for aerobic metabolism and heat exchange, alters relative exercise intensity and impairs exercise performance (Nybo et al., 2001; Périard et al., 2011).

The next section provides an overview of what is considered the most effective means of mitigating impairments in work capacity and exercise performance in the heat; heat acclimation/acclimatisation. The adaptations that contribute to lowering physiological strain is addressed and their effects on altering thermoregulatory and cardiovascular function is highlighted.

2.4 – Heat acclimation and acclimatisation

In the previous sections of this thesis the differences in thermal stress and hence physiological strain have been elucidated. While it is clear that exercise performance in the heat can be significantly impaired, the following section shall explore heat adaptation in both human and animal models. Moreover, these adaptations mean that individuals may improve their ability to tolerate environmental heat stress and subsequently, improve performances in the heat. Various methods for achieving the repeated increase in thermal strain

required to induce adaptation exist and each technique shall be discussed in relation to the adaptations they produce. In doing so, this section will highlight the responses that are yet to be fully elucidated in human heat adaptation.

2.4.1 – Heat stress as an adaptive impulse

Humans as homeotherms need to regulate T_C to within a narrow optimal limit. Acute responses to elevated environmental temperatures are relatively well counteracted; however, should thermal stress be large enough, then significant strain results. The level of thermal strain is referred to as a stimulus. Adaptation occurs if the stimulus is of sufficient frequency (overload) that ensures a regular challenge to homeostasis. This is a widely accepted theory of training and has been applied to heat adaptation for over half a century (Adolph, 1955; Bass, Kleeman, Quinn, Henschel, & Hegnauer, 1955). Thermal stress can be imposed upon individuals by increasing heat production or limiting the avenues of heat exchange between the body and the environment (i.e. exogenous heat). The level of strain can then be quantified by physiological responses (the effector response). This signifies acute physiological accommodation to thermal stress, whereas habituation results in a phenotypic adaptation that reduces strain caused by a given stressor. Changes in an effector response activation threshold for thermal stress are therefore indicative of systemic thermoregulatory adaptation (Fox, Goldsmith, Kidd, & Lewis, 1963a, 1963b; Nadel, Pandolf, Roberts, & Stolwijk, 1974). These adaptations modify thermo-effector function to respond at a greater magnitude and in some instances, with a greater sensitivity to deviations from a thermoneutral zone (Fox et al., 1963a; Nadel et al., 1974). Several methods exist for providing thermal stress and hence, physiological strain. However, not all lend to a continued adaptive drive since a constant external stimulus may result in a transiently decreased physiological impact as adaptation results in physiological habituation (Taylor, 2014). Instead, it has been suggested that optimal adaptation occurs through the application of a constant internal thermal stimulus (Taylor, 2014). This concept has been applied to heat adaptation throughout history, yet some maintain that much of the research methods used do not account for physiological habituation

(Taylor, 2014) and therefore, human adaptive responses are not yet fully understood. The next section will describe the existing methods of adapting to cope with heat stress and relates them to their effectiveness to drive physiological change.

2.4.2 – Methods of adapting to heat stress

The methods for heat adaptation are outlined briefly in Table 2.2 below. Adaptive methods can be broadly broken down into two main approaches; namely acclimatisation, which consists of exposure to a natural climate that is either similar or identical to the one at which a competition or task is to be completed, or acclimation, which is the use of artificially simulated environments that best replicate the conditions of a future destination. While both may be used to induce similar adaptations (Wenger, 1988), it is generally accepted that acclimatisation is not a cost effective or time efficient method of preparing for exercise or competition in the heat (Taylor & Cotter, 2006). Moreover, acclimation confers greater control of the environmental milieu and more accurate monitoring of the mechanisms that drive adaptation and is therefore widely used in empirical research. In this thesis heat acclimation shall be carried out using tightly controlled laboratory trials to determine the adaptations of the cardiovascular system in trained individuals. As such, this literature review shall focus on research that conducts heat acclimation and shall address acclimatisation where relevant to the aims of this thesis for the sake of completeness. From here on, heat acclimation and acclimatisation will be referred to as HA.

Within the literature, periods of HA vary quite substantially with interventions consisting of between 3–24 consecutive daily exposures. While it is considered longer HA interventions may result in a more complete adaptation, shorter interventions may provide a practical and cost-effective means of mitigating thermal strain during competition in hot environments. Intervention durations are categorised as short-term (≤ 5 days), medium-term (6-14 days) and long-term (≥ 15 days) within the literature (Tyler, Reeve, Hodges, & Cheung, 2016).

Traditionally, the indicator that one is better able to tolerate an elevated ambient temperature and humidity is an increase in work capacity. Early war-driven research focused on physical performance of soldiers in hot desert environments. One observation of the classic work by Adolph (1947) was that soldiers were less able to tolerate desert marching compared to cooler ambient conditions. Likewise, industry drove research into environmental ergonomics highlighting that work must be slowed or temporarily ceased to avoid heat stress (e.g. Ladell, 1955) and large-scale interventions saw increases in work capacity and decreases in the incidences of heat related illnesses in miners (Wyndham, 1967; Wyndham, Williams, Morrison, Heyns, & Siebert, 1968b). HA regimens improve feelings of thermal comfort (Gonzalez & Gagge, 1976) and increase submaximal exercise performances in the heat via increases in sweat rate, skin blood flow, PV and fluid balance, reduced cardiovascular strain and metabolic rate and acquired thermal tolerance (Febbraio et al., 1994; Lorenzo et al., 2010; Maloyan, Palmon, & Horowitz, 1999; Nielsen et al., 1993; Patterson, Stocks, & Taylor, 2014; Sawka & Coyle, 1999; Yamazaki & Hamasaki, 2003). Table 2.3 summarises these adaptations and also highlights observed adaptive responses that are to date limited to animal models. The following sections will expand upon these adaptations, how they lend to improved thermoregulation, performance and thermal tolerance, and the stimuli behind each.

Table 2.2: Methods of heat adaptation and examples of their use. Methodological categories are adapted from Taylor & Cotter (2006).

Method of adaptation	Description	Examples
Natural acclimatisation	Natural acclimatisation is associated with long term residence in, or travel to a hot climate. Seasonal adaptations are evident in residents of regions with markedly varying climate. Behavioural interventions to modify the microclimate can minimise exposure (e.g. clothing) and thus any seasonal adaptations that might be seen. This method is rarely feasible for the travelling athlete.	• Shapiro et al. (1981) • Inoue et al. (1995)
Passive heat acclimation	Exogenous heat is used to evoke adaptation with minimal contribution of metabolic heat production. Various methods to apply heat exist and some examples include; water baths, saunas, climate chambers and water-perfused or vapour-barrier suits. These passive techniques have also been used following exercise to maintain elevated body temperatures above resting levels. It is generally considered that passive HA is less effective at improving work or exercise capacity than techniques employing exercise and heat stress.	• Fox et al. (1963a) • Fox et al. (1964) • Beaudin et al. (2012) • Stanley et al. (2015) • Zurawlew et al. (2016) • Mee et al. (2018)

Table 2.2: Continued

Method of adaptation	Description	Examples
Exercise-training heat adaptation	The elevated and sustained levels of internal temperature via exercise in temperate environmental conditions. Endurance trained individuals display adaptations that are typical to heat acclimation (e.g. enhanced cardiovascular stability, higher heat tolerance, increased sweating rate and an earlier onset of sweating). Trained individuals also display a more rapid attainment of adaptation in subsequent heat acclimation. Note: elevations in whole-body temperature must occur for sufficient stimulation to induce heat adaptation. Shown to be less effective than combined exercise-heat acclimation, even when core temperature is matched.	• Greenleaf (1964) • Shvartz et al. (1973) • Hanane et al. (1977) • Nadel (1979) • Bradford et al. (2015)
Exercise-heat acclimation	Artificial heat adaptation that uses exercise in closely controlled combinations of temperature, wind speed, humidity and radiant heat. Exercise can also be closely regulated by use of ergometers and measuring physiological responses. Such regimens can be further classified as: • Constant workload • Self-regulated • Controlled hyperthermia • Controlled heart rate	• Pandolf (1998) • Houmard et al. (1990) • Chinevere et al. (2008) • Lorenzo et al. (2010) • Garrett et al. (2012) • Patterson et al. (2014) • Keiser et al. (2015) • Neal et al. (2016b) • Pethick et al. (2018)

Table 2.3: Physiological adaptations and consequences of the heat acclimated phenotype. Observations from numerous studies. Adapted from Sawka et al., (2011) and Périard et al., (2015); see text for details.

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

2.4.3 – Sweating and skin blood flow

Integral adaptations to HA are the enhanced sweating and skin blood flow responses to exercise and thermal stress. The changes in these parameters to HA significantly promote heat loss and hence improve thermoregulatory capacity. HA increases sweat rate while simultaneously lowering the solute content of sweat (Chinevere et al., 2008; Dill, Hall, & Edwards, 1938; Robinson, Turell, Belding, & Horvath, 1943). Moreover, the onset of whole body sweating occurs earlier and at a lower absolute T_C in acclimated individuals (Nadel et al., 1974; Roberts, Wenger, Stolwijk, & Nadel, 1977). Evaporative sweat losses represent a primary avenue of heat dissipation and therefore adaptation to thermal sweating is one of the most prominent effects of HA (Gonzalez & Gagge, 1976; Nielsen, Strange, Christensen, Warberg, & Saltin, 1997). Sweat secretion is directly related to thermal balance. The more rapid onset of thermoregulatory sweating is closely related to smaller changes in mean body temperature and not due to a predetermined T_C threshold (Patterson, Stocks, & Taylor, 2004a) and as such sweating sensitivity is increased following heat acclimation. Sweat rate and sensitivity changes occur at the level of the gland (Buono, Ball, & Kolkhorst, 2007; Buono, Martha, & Heaney, 2009; Fox et al., 1964; Inoue, Havenith, Kenney, Loomis, & Buskirk, 1999). These changes include an increased cholinergic sensitivity of eccrine glands and glandular hypertrophy (Sato & Sato, 1983). Additionally, data taken from patas monkeys highlight an increase in gland efficiency following acclimation, since larger glands produce more sweat for a given length of the secretory coil (Sato, Owen, Meatthes, Sato, & Gisolfi, 1990).

A further adaptive response of eccrine glands is that greater solute absorption occurs within the duct following HA, thus a more dilute sweat is secreted onto the skin (Chinevere et al., 2008; Dill et al., 1938; Ogawa, Asayama, & Miyagawa, 1982). The consequences of this are twofold; firstly, increased sodium reabsorption prevents its depletion and therefore facilitates extracellular fluid expansion during post-exercise recovery (Patterson et al., 2014) and second, the water vapour pressure at the skin is lowered (Bulmer & Forwell, 1956). Taken together, these adaptive responses increase the

evaporative cooling capacity of the skin for a given humidity and convective airflow.

Adaptations also occur within the cutaneous circulation to improve the sensitivity and responsiveness of redistributions of blood flow with heat stress. Enhanced evaporative heat losses reduce T_{sk} and therefore skin blood flow requirements, thereby contributing to a maintenance of central BV (Eichna, Park, Nelson, Horvath, & Palmes, 1950; Rowell et al., 1967). Despite this, the circulatory challenges to exercise in the heat are still profound and skin blood flows in the acclimated individual have been reported to be maintained (Nielsen et al., 1993; Regan, Macfarlane, & Taylor, 1996), enhanced (Fox et al., 1963b; Fujii et al., 2012; Roberts et al., 1977) or lowered (Rowell et al., 1967; Wyndham, 1951) compared to acute unacclimated responses. These discrepancies are possibly due to differences between the levels of thermal stress and hence, the degrees of physiological strain making it difficult to compare absolute changes in skin blood flow. However, this can be offset by determining the threshold and sensitivity of thermoeffector function. For instance, the threshold for cutaneous vasodilation relative to an absolute T_C and mean body temperature is reduced following acclimation (Goto et al., 2010; Roberts et al., 1977; Yamazaki & Hamasaki, 2003) by ~0.4°C, with this threshold occurring before that of thermoregulatory sweating (Fox et al., 1963b). Additionally, recent research has also identified functional adaptations of the cutaneous vasculature following HA (Lorenzo & Minson, 2010). The authors infused acetylcholine using microdialysis and observed significantly greater levels of cutaneous vascular conductance following acclimation in trained cyclists compared to their pre-acclimation levels. Further research into these adaptations is warranted, but it was proposed that this peripheral adaptation occurred via increases in the number and sensitivity of muscarinic receptors, a decrease in cholinesterase activity thereby increasing the vascular responsiveness to acetylcholine, or changes to the pathway of vasodilation within smooth muscle or endothelial cells (Lorenzo & Minson, 2010). Taken together, these findings imply that the responsiveness of the cutaneous vasculature stems from functional adaptations that occur during

heat acclimation rather than a structural limitation determining maximal vasodilatory capacity.

2.4.4 – Haematological and body fluid adjustments

Typically, HA interventions result in a ~4% expansion in PV (Tyler et al., 2016) although the magnitude of responses reported in the literature has ranged from -2 to +16% (Schmit et al., 2015; Sunderland, Morris, & Nevill, 2008). A potential benefit of increased PV is that during subsequent acute exercise-induced dehydration, a preferential defence of this intravascular volume may occur. This view stems from the observation that PV increased at the expense of interstitial fluid in subjects who did not instead exhibit a concomitant increase in total body water (Senay et al., 1976). The increased intravascular protein, along with a more diluted sweat (Nadel et al., 1974) might be expected to cause this selective expansion. However, more recent research has identified a ubiquitous expansion of the entire extracellular compartment when the level of thermal strain from repeated heat exposures was maintained (Patterson et al., 2004b). Furthermore, when the same group measured body fluid compartments during exercise and recovery following HA, they noted that the intravascular compartment was not selectively defended (Patterson et al., 2014). Instead, greater relative plasma losses are seen following HA with controlled hyperthermia resulting in a significantly larger haemoconcentration.

PV expansion has typically been seen as a transient phenomenon (Bass et al., 1955; Shapiro et al., 1981; Wyndham et al., 1968a). The initial expansion observed after 4-5 days of repeated heat exposures has been strongly associated with the improvements in cardiovascular stability that occur within a similar time frame, prior to increases in evaporative heat losses. Therefore, this almost bi-phasic response is seen as a necessary outcome of adaptation. However, others using long-term HA with controlled hyperthermia have not observed a transient return of PV towards basal values (Patterson et al., 2004b, 2014) and this has led to the suggestion that this reduction instead appears to be an experimental artefact (Periard et al., 2015; Taylor, 2014). The mechanisms behind such an expansion are not entirely clear but may be

due to increased extracellular fluid via increased electrolyte retention and oncotic effects of intravascular protein (Patterson et al., 2004b, 2014). Interestingly, rapid restoration of the plasma compartment may respond to help to defend against post-exercise hypotension (Halliwill, Sieck, Romero, Buck, & Ely, 2014; Patterson et al., 2014). An immediate restoration of the plasma compartment begins when exercise in the heat is ceased (Harrison, Edwards, Graveney, Cochrane, & Davies, 1981). This may be associated with reductions in skeletal muscle blood flow modulating release of accumulated intramuscular water (Nielsen, Sjogaard, Ugelvig, Knudsen, & Dohlmann, 1986). However, this expansion is more rapid following HA and the higher post-exercise plasma sodium content appears to facilitate this response without affecting fluid homeostasis via reduced sweat sodium content and increased loss of PV (haemoconcentration; Patterson et al., 2014). Despite a potentially greater relative loss of PV following HA, a larger intravascular compartment has been attributed to a lowering of the specific heat capacity of blood, decreasing the necessary skin blood flow response, and an improvement in ventricular filling (Sawka et al., 2011). This conveys a particular benefit to individuals exercising in the heat with adequate fluid replacement following an acclimation period and is discussed later in further detail.

Dehydration adversely affects thermoregulation and leads to increases in cardiovascular strain (González-Alonso, 1998; Montain, Sawka, Latzka, & Valeri, 1998; Sawka & Coyle, 1999). T_C is higher during exercise in temperate and hot conditions when hypohydrated compared to euhydrated and the more severe the water deficit, the greater the level of hyperthermia (Montain & Coyle, 1992b). Un-replaced fluid through excessive sweating also leads to the development of plasma hyperosmolality and hypovolemia and as highlighted previously, this can impair the ability to lose heat through evaporation (Sawka & Coyle, 1999). Based upon such evidence it is generally accepted that exercise training should be undertaken well-hydrated with adequate fluid replacement (Bergeron et al., 2012). Despite this more recent evidence has begun to suggest that HA may confer a protective effect against impaired thermoregulatory and fluid regulatory processes during exercise in the heat.

As highlighted above, following exercise a selective expansion of the intravascular space occurs due to interactions of the renin-angiotensin-aldosterone system. Permissive dehydration will stress this system whereas a state of euhydration will blunt the release of aldosterone while exercising in hot ambient conditions (Kenefick et al., 2007) since a lower circulatory volume is a primary determinant of Aldosterone secretion (Nose, Mack, Shi, & Nadel, 1988a). Aldosterone acts to retain sodium and water at the distal renal tubules (Buono et al., 2007; Funder, 1993; Good, 2007) and is therefore an important mediator in the rapid expansion of PV following exercise (Nagashima, Wu, Kavouras, & Mack, 2001). Furthermore, HA has been shown to attenuate the effects of hyperosmolarity on impaired sweating and skin blood flow responses to dehydration (Takamata, Yoshida, Nishida, & Morimoto, 2001) and fitter individuals exhibit lower levels of thermal and cardiovascular strain when exercising while hypohydrated (Merry, Ainslie, & Cotter, 2010). Therefore, permissive dehydration during HA may facilitate adaptations by increasing fluid-electrolyte retention, PV expansion (Patterson et al., 2004b, 2014) and cardiovascular responses to exercise heat stress, which may prove particularly advantageous to short term regimens (Garrett et al., 2014; Garrett, Rehrer, & Patterson, 2011).

Despite this proposed benefit, very few investigations have directly assessed the effect of hydration strategy throughout HA on adaptation (Garrett et al., 2012; Neal et al., 2016b; Schleh, Ruby, & Dumke, 2018), while others have assessed responses between groups (Pethick et al., 2018). Furthermore, despite early research suggesting a greater PV expansion to short-term dehydrated exercise HA with controlled hyperthermia (Garrett et al., 2014), subsequent findings have been equivocal. Other short- and medium-term interventions have observed no additional benefit of fluid restriction on adaptations (Neal et al., 2016b; Schleh et al., 2018) while others have observed no increases in PV of trained individuals performing dehydrating exercise in the heat (Neal, Corbett, Massey, & Tipton, 2016a). Consequently, the acute and adaptive haematological responses to HA and dehydration remains unclear. Further research appropriately addressing the possibility that dehydration may confer any additional benefit to adaptation is warranted while

the possibility that HA may negate the detrimental effects of dehydration is yet to be fully elucidated.

2.4.5 – Cardiovascular stability

Exercise in the heat is associated with an increased level of cardiovascular strain and for a given $\dot{Q}$, HR is elevated. Despite high levels of initial physiological strain, a reduction in HR for a given exercise intensity is seen within 4-5 days of HA and this adaptation appears virtually complete within 7 days of repeated heat exposures (Pandolf, 1998). As highlighted previously, aerobic training can reduce physiological strain and improve exercise tolerance in the heat, but these benefits are nominal when compared to HA interventions. For example (Cohen & Gisolfi, 1982) asked women to complete 4 hours of exercise in a hot-dry (45°C dry bulb) environment both before and after an 11-week training programme in temperate conditions. They then attempted the tolerance test again after an 8-day HA regimen. Exercise training reduced T_C (~0.3°C) and increased exercise tolerance by nearly 2 hours while HR and T_{sk} were significantly lowered. These responses were however relatively modest when compared to the much larger reductions in T_C, HR and improved exercise tolerance that occurred following just 8-days of HA (Figure 2.6).

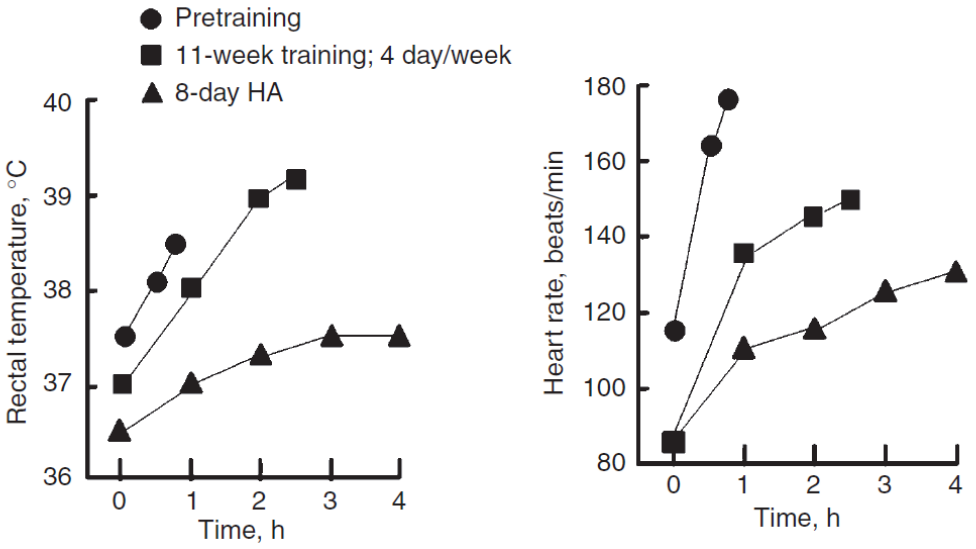


Figure 2.6: Effects of an aerobic training programme and subsequent heat acclimation on core temperature, heart rate and exercise tolerance during exercise-heat stress in women. Reproduced from Sawka et al. (2011) using data from Cohen and Gisolfi (1982).

It has long been considered that the key early adjustments to HA are of cardiovascular origin (Fox et al., 1963b; Ladell, 1951; Senay, 1986; Senay et al., 1976; Taylor, Henschel, & Keys, 1943; Wyndham, Rogers, Senay, & Mitchell, 1976). Indeed, one of the earliest observations with HA alongside increased tolerance to work in the heat was a lowered HR and increased PV (Bass et al., 1955; Bazett, Sunderman, Doupe, & Scott, 1940; Horvath & Shelley, 1946; Robinson et al., 1943). As previously highlighted, these observations were typically accompanied by a decrease in T_C and increase in SV prior to improvements in evaporative heat loss (Mitchell et al., 1976; Senay et al., 1976). Therefore, it is generally accepted that the main adaptive precursor necessary for the decrease in cardiovascular strain with HA is an expansion of PV (Ladell, 1951; Sawka, Toner, Francesconi, & Pandolf, 1983c; Wyndham et al., 1976). An increased PV, and therefore BV will aid ventricular filling and thus permit a lower HR and higher SV for a given $\dot{Q}$. However, a decrease in exercising HR may also be reflective of decreases in thermal strain with HA (Shapiro et al., 1981). Therefore, while improvements in cardiac function have been displayed in the animal model (Cohen et al., 2007; Levi et al., 1993), data in humans is limited regarding the integrated responses of lowered physiological strain to heat stress (Taylor, 2014). To date, research

investigating the changes in central cardiovascular function to HA has resulted in conflicting results and has not directly determined cardiac function (i.e. LV volumes) during exercise to elucidate the mechanisms increasing SV.

In an early study by Rowell et al. (1967), SV was increased and HR lowered, resulting in no change in $\dot{Q}$ following HA to dry heat. While responses of participants varied slightly, the overall increase in SV was attributed to greater filling time afforded by the reduction in HR rather than an increase in central BV (Rowell et al., 1967). A later study conducted in hot-humid heat observed varying responses over 8 days of HA, where SV initially increased, but later fell along with HR such that $\dot{Q}$ was unchanged (Wyndham et al., 1976). Two studies conducted by Nielsen and colleagues (1993; 1997) investigated the haemodynamic responses to exercise following exercise-HA in both dry (Nielsen et al., 1993) and humid heat (Nielsen et al., 1997). Using dye-dilution, the authors observed that $\dot{Q}$ and SV were significantly elevated by ~2 $L \cdot min^{-1}$ and ~20 ml respectively immediately prior to exhaustion to exercise in dry heat. With no changes in leg blood flow this additional $\dot{Q}$ appeared to be directed to the upper body tissues, at least in part to the cutaneous vasculature as forearm blood flow increased by approximately ~3 $ml \cdot 100ml^{-1} \cdot min^{-1}$. This was accompanied by an almost doubling of exercise time to exhaustion in hot, dry heat (40°C, 10% relative humidity; Nielsen et al., 1993). However, these responses differed markedly in humid heat (35°C, 87% relative humidity; Nielsen et al., 1997). In the study in humid heat $\dot{Q}$, SV and forearm blood flow remained unchanged following HA. Furthermore, in the humid conditions evaporative sweat losses remained unchanged while sweat rate increased through acclimation. This resulted in an increased amount of sweat dripping from the skin and was associated with unchanged T_C responses following acclimation. This indicates that the effects of HA were minimal due to limited possible evaporative heat loss with high environmental humidity (Nielsen et al., 1997). An interesting observation from these studies is the similar expansion of PV (~9-13%) during heat acclimation. These similarities imply that while an increased PV, lowered HR an T_C may lend to improved cardiovascular stability, the magnitude of these responses is largely dependent on the type and degree of heat stress.

Considering increases in SV and Q̇ with HA may depend on the environmental biophysics permitting evaporative heat exchange and therefore, reduced thermal strain, the question arises as to what degree dehydration may also potentially offset improvements in cardiovascular stability? Several investigators have studied the effects of acute hypohydration on thermoregulatory and haemodynamic responses to exercise in the heat. Most have induced body mass deficits via overnight heat exposure and/or fluid restriction (Buskirk, Iampietro, & Bass, 1958; Sawka et al., 1983c) and diuretics (Ikegawa et al., 2011). These have resulted in large (~5%) decreases in body mass (Buskirk et al., 1958; Sawka et al., 1983c) or iso-osmotic hypovolemia (Ikegawa et al., 2011), neither of which offer a typical reflection of individuals undertaking endurance exercise in the heat. Nevertheless, the findings offer some insight as to the effects of acute hypovolemia on responses to HA. Sawka et al. (1983c) reported similar dehydrated T_C responses between pre- and post-acclimation trials. In contrast, HR was ~20 beats·min^{-1} lower and was ascribed to a smaller reduction in PV with an overnight dehydration of ~5% of body mass that may have permitted an increased LV filling (Sawka et al., 1983c). In a more recent study by Ikegawa et al. (2011) absolute PV was returned to pre-acclimation levels prior to 30 min sub-maximal exercise in 30°C and 50% relative humidity heat. This was associated with a similar SV and Q̇, while esophageal temperature was slightly lowered and forearm blood flow and sweating sensitivity were enhanced. However, HR and T_C were higher and Q̇ was still significantly lower than euhydrated responses (Ikegawa et al., 2011). Together, these findings suggest the potential for acute dehydration to, at least in part, offset the beneficial thermoregulatory and cardiovascular responses to HA. However, the thermal and cardiovascular responses to progressive dehydration following HA remains unknown.

2.4.6 – Thermal tolerance

Cellular adaptations that occur as a result of non-lethal heat exposure that subsequently allows an organism to survive an otherwise lethal heat exposure is known as acquired thermal tolerance (Moseley, 1997). These cellular

adaptations include the up-regulation of heat shock proteins (HSPs) and such responses are seen following exposure to a variety of stressors, including heat, hypoxia and oxidative stress. HSPs play key regulatory roles in protein transport across cell membranes, re-folding of denatured proteins and preventing initiation of the apoptotic cascade observed in response to acute cellular stress (Ely, Lovering, Horowitz, & Minson, 2014; Goldberg, 2003). HSPs are grouped by their molecular weight and the HSP-70 and -90 families appear highly inducible by heat stress and have been extensively investigated in animal and human models. In human subjects, basal levels of HSP-70 in peripheral blood mononucleocytes fluctuate with circadian variation in T_C (Sandstrom et al., 2009) and has been shown to increase following exercise-HA (Maloyan et al., 1999; Sandstrom, Siegler, Lovell, Madden, & McNaughton, 2008; Yamada, Amorim, Moseley, Robergs, & Schneider, 2007). Similar increases have also been observed in HSP-90 following a 10-day HA regimen (McClung et al., 2008). Additionally, attenuating oxidative stress via quercetin supplementation during HA blunts the accumulation of HSP-70, and thus thermal tolerance and HA appear to occur in concert as both may be governed by a heat shock response (Kuennen et al., 2011).

Further evidence for the relationship to thermal tolerance and HA can be found in the direct contributions each protein may have on physiological adaptations to repeated heat exposures (Table 2.3). For instance, it has been suggested that HSP-90 might act upon mineralocorticoid receptors of the kidney and sweat glands to limit Na^+ loss and therefore leads to an expanded PV and more dilute sweat (Ely et al., 2014). HSP-90 acts as a regulator on steroid hormone receptors and its inhibition during aldosterone administration abolishes the vasodilation of rabbit renal arterioles (Uhrenholt et al., 2003). Furthermore, HSP-90 is a co-factor in producing endothelial nitric oxide synthase (eNOS) (Brouet, Sonveaux, Dessy, Balligand, & Feron, 2001), acting as a chaperone to both eNOS and its phosphorylation sites. eNOS leads to the production of nitric oxide in the endothelium which is involved in vascular control. Nitric oxide diffuses the underlying smooth muscle cells leading to vasodilation (Laughlin et al., 2012). In addition to chemical mediators such as HSP-90, eNOS is produced by shear stress and or

intracellular calcium signalling by mechanical forces of shear stress from blood flow in the endothelium releasing other chemical factors such as acetyl choline, substance P and noradrenaline (Balligand, Feron, & Dessy, 2009).

Exercise training increases endothelium dependent vasodilation and eNOS activity in skeletal muscle arteries and arterioles that perfuse non-working limbs such as the arms during cycling (Clarkson et al., 1999; Goto et al., 2003; Higashi et al., 1999). Therefore the level of blood flow perfusing inactive limbs, provided exercise is of sufficient duration and intensity, through the conduit arteries results in greater rates of shear stress in order to provide sufficient thermoregulatory blood flow to the skin (Birk et al., 2012; Goto et al., 2003; Ooue et al., 2008; Tanaka et al., 2006). Cutaneous vasodilation during sustained dynamic exercise is largely mediated via eNOS derived nitric oxide (McNamara, Keen, Simmons, Alexander, & Wong, 2014). During exercise, larger skin blood flows in the heat result in greater rates of shear stress in the conduit arteries (Simmons et al., 2011). However, the level of shear stress of the cutaneous circulation is typically lower during exercise compared to passive heating at a given T_C (Kenney & Johnson, 1992). Despite this, pulse pressure and therefore circumferential vascular wall strain also results in the release of eNOS independently of shear stress (Laughlin, Newcomer, & Bender, 2008). Therefore, elevated thermoregulatory skin blood flow may lead to favourable vascular adaptations, even in the indirectly active or non-active limbs. Taken together this implies that elevated levels of shear stress and vascular strain are implicated in vascular adaptations to enhance more rapid vasodilation during dynamic intense exercise. An intervention that elevates and controls cardiovascular responses during exercise in the heat might be expected to illicit significant vascular adaptations, possibly via the mechanisms outlined above.

In the rat model, HA also leads to mechanical and metabolic performance adaptations of the heart. In addition to a lower HR during exercise at a particular relative intensity, in the rat model LV compliance, and systolic pressure generation are increased while myocardial O_2 consumption may be decreased following HA (Levy, Hasin, Navon, & Horowitz, 1997). With an

expansion of the plasma compartment and hence enhanced venous return, these cardiac adaptations take place to match the systemic changes that occur with acclimation. Less metabolic and mechanical energy is therefore required to generate a given $\dot{Q}$ and therefore a feature of heat acclimation is increased work efficiency of the heart (Horowitz, 2002).

2.5 – Heat acclimation with controlled heart rate

In order for HA interventions to maximise adaptation whilst supporting athletic preparation for competition, methods must be easily practicable and facilitate exercise training where possible. As such various methodologies are used to improve exercise performance in hot environments (Table 2.2). These include passive heat exposures (Zurawlew et al., 2016) or conducting regular training in additional clothing (Ely et al., 2018) or moderately warm environments (Bradford et al., 2015). However, the effects of such interventions on adaptation and performance has been mixed. As previously highlighted, controlled hyperthermia is considered an effective intervention that maximises adaptations to HA (Taylor, 2014). Studies employing this technique have used alterations in workload within fixed environmental conditions to maintain a target T_C as thermoregulatory adaptations progress. Interestingly however, despite these increases in exercising workload, HR tends to be maintained at a relatively low level due to the intermittent nature of the protocol which is governed by maintenance of a target T_C. Table 2.4 highlights these responses from studies where changes across HA are reported.

Five of the six studies in Table 2.4 reported an increase in workload across HA while average exercising HR was either maintained or slightly decreased. This relationship typically appears to have persisted between short-term (5 day) to medium-term (10 day) interventions as well as between maintained euhydration and dehydration via fluid restriction protocols (Garrett et al., 2014; Neal et al., 2016b). In all cases, average exercising T_C was successfully maintained throughout acclimation. This data give rise to the possibility that exercising HR, at a level more commensurate with aerobic exercise training, may be used as a simple metric to monitor exercising heat stress to HA.

Table 2.4: Select studies employing controlled hyperthermia HA where thermoregulatory, cardiovascular and exercise responses were reported. Similarities or changes in a given parameter are representative of statistical differences between daily exposures within a given intervention. See relevant study for details.

Study	Participants	Environmental conditions	Duration	T_C maintained?	HR maintained?	Workload maintained?
Garrett et al. (2012)	8 males	40°C & 60% RH	5 days 90 min·d^{-1}	Yes	Yes	No (increased)
Garrett et al. (2014)	9 males	40°C & 60% RH	5 days 90 min·d^{-1}	Yes	Yes	No (increased)
Gibson et al. (2015a)	16 males	40°C & 39% RH	10 days 90 min·d^{-1}	Yes	Yes	No (increased)
Mee et al. (2015)	8 males & 8 females	40°C & 40% RH	10 days 90 min·d^{-1}	Yes	Yes	No (increased)
Neal et al. (2016b)	8 males	40°C & 50% RH	8 days 90 min·d^{-1}	Yes	No (decreased)	No (increased)
Neal et al. (2016a)	10 males	40°C & 50% RH	5 days 90 min·d^{-1}	Yes	No (decreased)	Yes

RH Relative humidity (%).

Regulating HA via a maintained exercising HR is a relatively recent suggestion (Periard et al., 2015). This proposal relates to the close relationship between exercising heat stress and cardiovascular strain which may alter relative exercise intensity (Wingo, Ganio, & Cureton, 2012). Exercise at a controlled HR in the heat may therefore maintain relative exercise intensity and the level of heat strain during HA exposures (Periard et al., 2015). Recent studies to date utilising HA with controlled HR have either compared responses to control exercise in cool conditions (Keiser et al., 2015; Philp et al., 2017) or compared the effects of hydration between independent groups (Pethick et al., 2018), while none have characterised the time course or magnitude of responses to such an intervention. An aim of this thesis was therefore to explore adaptive responses to HA with controlled HR and demonstrate the influence, if any, of hydration strategy during exposures on adaptations within individuals.

2.6 – The ergogenic effect of heat acclimation

Endurance exercise performance appears to be optimal at ambient temperatures between 10-14°C, above which progressive decrements have been observed (Ely et al., 2007; Galloway & Maughan, 1997). This suggests that the relative contribution of heat stress to affecting performance operates along a continuum of exogenous thermal loads, that can result in large variations in T_{sk}. Therefore, adaptations that reduce physiological strain and improve performance following repeated exposure to a significantly hot environment may confer some benefit to cooler climates where some thermal burden may still be limiting (Corbett, Neal, Lunt, & Tipton, 2014). HA interventions have previously been shown to lower oxygen uptake at a sub-maximal workload (Sawka, Pandolf, Avellini, & Shapiro, 1983b; Young, Sawka, Levine, Cadarette, & Pandolf, 1985) and increase lactate threshold (Lorenzo et al., 2010; Neal et al., 2016a) during exercise in cool conditions. As such, a relatively short period of combined stressors of exercise and heat stress may offer a potentially ergogenic performance enhancement in cooler conditions. These improvements may prove particularly beneficial for athletes

approaching competition. Therefore, the ergogenic potential of HA has begun to receive further attention in the scientific literature.

To date several investigations have observed improvements to various performance parameters in temperate environments. Perhaps most notable of these is the laboratory study of well-trained cyclists conducted by Lorenzo and colleagues (2010). The investigators observed a 6% increase in 60 min time-trial performance and 5% increases in power output at lactate threshold and $\dot{V}O_{2max}$ during tests conducted in 13°C and ~30% relative humidity. The authors suggested that the ~200 ml increase in PV may have contributed to these performance responses as lactate clearance may be improved and sub-maximal and maximal SV and thus $\dot{Q}$ may be enhanced (Lorenzo et al., 2010). However, an increased PV may result in a reduction in haemoglobin concentration (Coyle, Hopper, & Coggan, 1990) and therefore confer no improvement in $\dot{V}O_{2max}$ (Kanstrup & Ekblom, 1984). One response that is often unaccounted for however is whether HA may result in changes in red cell volume (RCV) or haemoglobin mass. Findings to date have reported varied responses to HA (Bazett et al., 1940; Gibson et al., 2015a; Karlsen et al., 2015; Keiser et al., 2015; Patterson et al., 2014; Scoon, Hopkins, Mayhew, & Cotter, 2007). Recently Karlsen et al. (2015) observed a ~7% increase in haemoglobin mass with HA that was not observed in a matched control group undergoing similar training in cool conditions. However, this did not translate to an improvement in $\dot{V}O_{2max}$ or time-trial performance in cool conditions (Karlsen et al., 2015). Given very few studies to date have directly determined the intra-individual influence of heat adaptation compared to typical temperate exercise training on performance in cool conditions, there is a need to appropriately match exercising stimuli to better differentiate the effect of training *per se* on possible performance effects (Corbett et al., 2014; Nybo & Lundby, 2016). In this regard, Keiser et al. (2015) used a counterbalanced cross-over design to compare HA to temperate exercise training. They observed no effect of HA on RCV while PV was expanded to a similar extent to that observed by Lorenzo and colleagues (2010). However, neither temperate nor HA interventions altered $\dot{V}O_{2max}$ in 18°C, while only HA improved $\dot{V}O_{2max}$ and TT performance in the heat (Keiser et al., 2015).

Together, given the variety of physiological adjustments to HA that may have an ergogenic potential in cool or temperate conditions and the wide range of conditions that may pose a thermally limiting challenge further study in this area is warranted (Corbett et al., 2014).

2.7 – Overall summary

Exercise combined with high exogenous thermal stress (i.e. environmental temperature) lead to significant cardiovascular adjustments that help offset challenges in thermoregulatory homeostasis. The extent of these haemodynamic responses is highly dependent on exercise intensity, degree of thermal stress and hydration status, which together can place the cardiovascular system under significant strain. Repeated exercising heat exposures lead to several positive adaptations, many of which may directly or indirectly alter cardiac function, thereby contributing to the generally observed reduced physiological strain after HA. Some of these responses are summarised in Figure 2.7, which highlight the proposed mechanisms behind improved cardiovascular stability. Exercise at a controlled HR may be a safe and practical method of inducing HA which results in an improved exercise performance in the heat, but the responses to such an intervention are yet to be fully documented. Furthermore, the additional stimulus of dehydration to exercise and heat stress throughout HA may result in an enhanced fluid regulatory response and therefore larger increases in PV. However, few studies to date have appropriately determined this effect against a euhydrated intervention and so far, conflicting responses have been observed. Finally, while a reduction in HR and increase in PV may augment cardiac filling, supporting an increase in SV, there are little data on the effect of HA on LV volumes during exercise in the heat in humans. What influence progressive dehydration has upon thermal, haematological and haemodynamic responses to exercise and heat stress in humans remains poorly understood. Finally, an increased PV may augment maximal $\dot{Q}$ in temperate conditions. However, whether this translates to an increase in $\dot{V}O_{2max}$ and hence, an ergogenic effect of HA, is unclear.

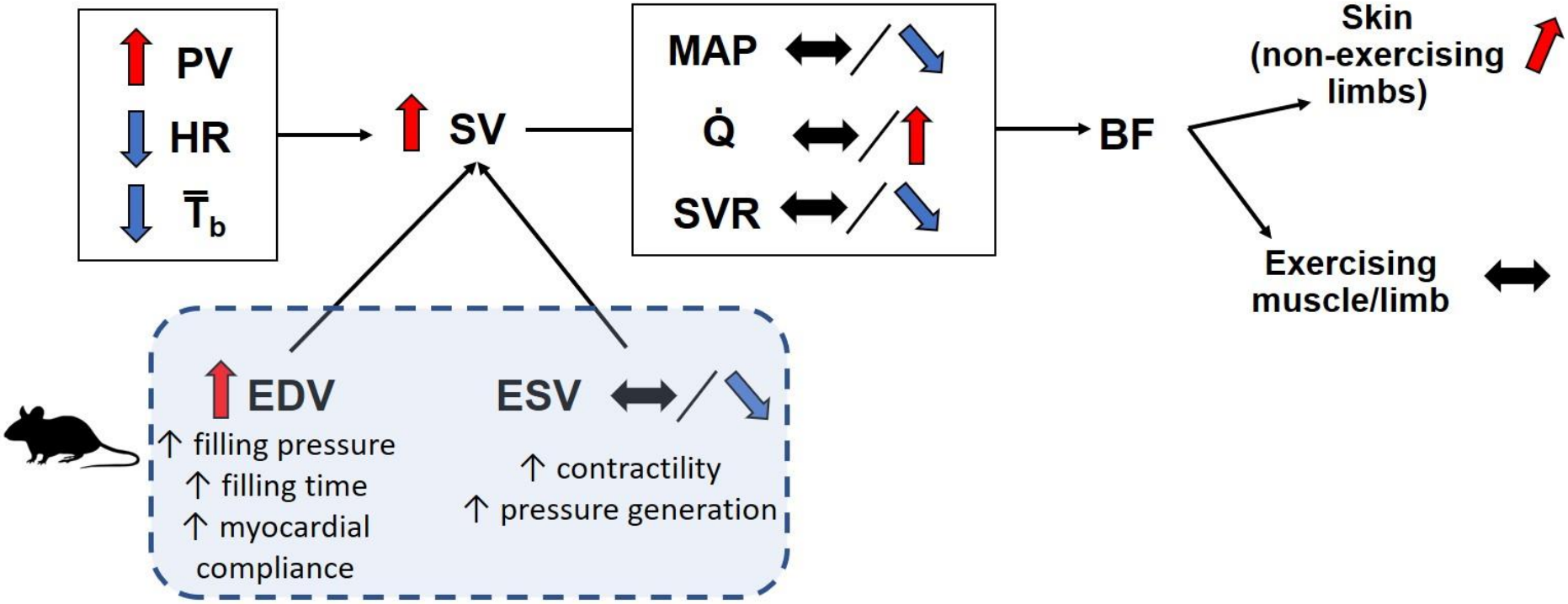


Figure 2.7: Schematic outline of the possible effect of heat acclimation on cardiovascular function during euhydrated dynamic submaximal upright exercise in hot-dry heat. An increase in PV and lowered HR and mean body temperature ($\overline{T}_b$) may contribute to improve LV filling; increasing SV. This may in-turn contribute to a maintained or increased $\dot{Q}$ at a comparative time-point during exercise. Increased systemic blood flow (BF) may be directed to the cutaneous circulation of non-exercising limbs. However, direct mechanistic evidence for altered LV volumes and function is limited to the rodent model. In addition, SV estimates in humans are typically made via invasive/non-invasive measurements of $\dot{Q}$.

2.8 – Thesis aims and hypotheses

2.8.1 – Study 1: Heat acclimation with controlled heart rate: the influence of hydration status on induction of adaptations, maximal aerobic capacity and self-paced exercise performance

Study aims: To characterise the adaptive responses to 10-days of HA with controlled HR with both maintained euhydration and daily exercise-induced dehydration via altered fluid ingestion. A further aim was to determine the influence each intervention has upon $\dot{V}O_{2max}$ in temperate conditions and self-paced exercise performance in the heat.

Research hypotheses: 1) Both maintained euhydration and dehydration via altered fluid ingestion throughout HA with controlled HR would lead to similar adaptations typical of the heat acclimated phenotype and, 2) these interventions would not significantly increase $\dot{V}O_{2max}$ in temperate conditions but, 3) would improve self-paced exercise performance in the heat.

2.8.2 – Study 2: Effects of heat acclimation with controlled heart rate on thermal, haematological and haemodynamic responses at rest and during prolonged exercise in the heat with altered hydration

Study aims: This study aimed to expand upon the descriptive adaptations that occur throughout euhydrated HA with controlled HR outlined in the previous study by determining the effects such an intervention has on thermoregulatory and cardiovascular function during prolonged constant load exercise heat stress. A further aim was to explore the influence of acute progressive exercise-induced dehydration and heat stress on thermal and cardiac function.

Research hypotheses: 1) euhydrated HA with controlled HR would lead to a lowered HR and increased SV during constant workload exercise in the heat and, 2) improve thermal responses to exercise while euhydration was maintained whereas, 3) acute dehydration would blunt otherwise improved

thermoregulatory and cardiovascular responses following HA, associated in part to a reduced BV and LV filling.

2.8.3 – Study 3: Effect of controlled heart rate heat acclimation with dehydration on thermoregulatory and cardiovascular function during prolonged exercise in the heat

Study aims: To build upon the observations of the previous study following euhydrated HA by determining the thermal, haematological and haemodynamic responses to prolonged exercise with maintained euhydration and progressive dehydration following dehydrated HA.

Research hypotheses: 1) dehydrated HA with controlled HR would improve thermoregulatory and cardiovascular responses to a period of prolonged exercise in the heat while euhydration was maintained. However, following HA, 2) acute progressive dehydration would result in significant haemoconcentration and hyperthermia and would be accompanied by reductions in SV and $\dot{Q}$ compared to euhydration and would be 3) similar to responses observed during dehydrating exercise prior to HA.

CHAPTER 3

General Methodology

3.1 – Introduction

This thesis comprises of one large experimental intervention that is separated into experimental chapters. This section outlines the general methodologies used in each of the following experimental chapters. In cases where specific/modified procedures were used, full descriptions are given in the relevant methodology sections.

3.2 – Pre-testing procedures

3.2.1 – Ethical approval

Prior to all experimentation, a research proposal was submitted to and approved by the Chief Medical Officer's Scientific Sub-committee at Aspetar, followed by approval from Anti-Doping Lab Qatar Research Ethics Committee (Approval number: F2015000105, Appendix I). The primary investigator also completed relevant training in Research Integrity (Brunel Graduate School) and gained certification in Protecting Human Research Participants (National Institute of Health certification number: 1603491) and Biomedical Research Ethics training from the Collaborative Institutional Training Initiative Program (CITI; ID no. 24744425) prior to experimentation as required by the Supreme Council of Health, Qatar. All studies conformed to the standards laid out in the Declaration of Helsinki.

3.2.2 – Participants

All participants undertaking the studies were active males aged 18-40 and were non-native residents of Qatar. Most hailed from North America and western Europe, while two participants were Caucasian of South African nationality. Participants had resided in the Middle East for an average of 2.1 years (range 0.3 – 5.6 years) before volunteering. Following confirmation of ethical approval, subjects were recruited via a combination of verbal communication, internet-based advertising and short presentations. Each subject provided written informed consent (Appendix II) prior to

commencement of any experimentation and was verbally informed of their right to withdraw from experimentation at any time without reason or penalty. Females were not recruited to take part in these experiments for the following reasons. Firstly, despite evidence that males and females exhibit similar thermoregulatory responses to exercise and/or heat stress when controlling for fitness and body morphology (Charkoudian & Stachenfeld, 2014; Notley, Park, Tagami, Ohnishi, & Taylor, 2017), females may require long-term HA interventions to fully develop reductions in cardiovascular and thermoregulatory strain (Mee et al., 2015). Secondly, many of the experiments performed required exercise in minimal clothing while some of the procedures involved may be considered invasive. Therefore, given the purposes of the experimentation (i.e. time-course of adaptation and not sex-differences with HA) and the cultural sensitivities of the region females were not recruited.

Participants completed two HA interventions that differed in fluid intake during exercise and heat stress. Further details are provided in sections 3.4 and Chapter 4. No additional laboratory intervention was performed in temperate conditions as one of the main aims of the thesis was to identify what differences in adaptive responses, if any, may be achieved via fluid restriction during exercise HA. Previous investigations have recruited additional participants matched for fitness and body morphology or conducted a work-matched period in cool conditions to account for the effect of training on adaptations observed. However, it is generally considered that such levels of experimental control do not account for differences in relative exercise intensity (Corbett et al., 2016; Périard et al. 2015). For these reasons and considering logistical constraints, a temperate control group was not included in this thesis.

3.2.3 – Pre-participation screening

Prior to enrolment in research studies, participants completed a health questionnaire (Appendix III) to ensure they were free from injury, illness and disease. Further pre-experimental screening was performed before experimental trials (Chapters 5 and 6) in the form of a resting visual

echocardiographic assessment of the LV. This was to screen for the presence of any ventricular wall asymmetry and a preserved ejection fraction (EF).

Due to the high average ambient temperatures in Qatar (Latitude: 25° North) where average peak daily temperatures range from 22-42°C for coldest and warmest months, respectively (Climate and Weather averages, Doha, Qatar), participants conducting HA interventions in the summer months were encouraged to minimise outdoor exercising exposures to once per week for a minimum of three weeks prior to their first visit to the laboratory. Over this period participants were invited to attend the laboratory to continue training and familiarise themselves with the equipment and procedures used in Chapters 4 through 6. Exercise training sessions over this period consisted of a mixture of self-paced interval training (i.e. 4 x 8 min, 3 x 10 min or 2 x 20 min efforts) on the same ergometer used in cycling time-trials, and submaximal cycling for a minimum of 60 min on the ergometer used during HA (see sections 3.3.9 and 3.4 for further details). Anecdotally, all participants also self-reported typical deliberate avoidance of outdoor exercise during summer months and instead stated the use of indoor ergometers, cross training (e.g. swimming), travel from the region or a combination of the above.

3.2.4 – Dietary food and fluid intake

Participants were provided with a food and fluid intake diary (Appendix IV) upon enrolment to each study. In the 24 h prior to an experimental visit, participants were asked to record their food and fluid intake. Participants were asked to keep their diaries so that prior to any corresponding experimental trial that followed the interventions in Chapters 4, 5 and 6, they could replicate their fluid and macronutrient intakes as best as possible. Relevant timings of dietary control are provided in each experimental Chapter.

3.3 – Experimental procedures

3.3.1 – Anthropometry

Participant stature was assessed to the nearest centimetre on a stadiometer, ensuring correct upright posture witsh the head in the Frankfort plane. Nude body mass was also assessed to the nearest 0.1 kg (SECA 798, Germany) privately by the participant in a toilet cubicle.

3.3.2 – Hydration status

Hydration status was assessed via measures of a stable nude body mass and urine specific gravity (USG). Participants urinated into a cup on arrival to the laboratory. 0.3 ml was then pipetted onto the prism surface of a digital USG refractometer (PAL-10S, ATAGO, Tokyo, Japan). USG refers to the density (mass per volume) of urine compared to pure water (Armstrong, 2005) and along with urine osmolality has been shown to be more sensitive to slight changes in hydration status than blood measurements (Armstrong et al., 1994). The American College of Sports Medicine (2014) regard a USG ≤ 1.020 as being indicative of euhydration. Participants were prompted to consume additional fluid before experimental trials began where necessary (i.e. USG ≥ 1.021).

3.3.3 – Environmental conditions

Environmental conditions varied according to the experimental trial undertaken. All resting and exercising trials conducted in hot conditions were undertaken in a closed-loop environmental chamber (TEMI 1000, Sanwood environmental chambers co., Taiwan). Temperature and relative humidity set-points were controlled within a narrow range from monitoring of inputted and exiting ambient air, respectively. Prior to all experimental testing and following a ~40 min period to stabilise environmental conditions, set-points were adjusted according to a second calibrated sensor near the middle of the chamber to achieve the desired conditions. Excess carbon dioxide was removed via a regenerative carbon dioxide scrubber system. Exercise trials in

temperate conditions were conducted in a separate custom-built chamber (LowOxygen Systems, LOXY International Ltd, Berlin, Germany). The chamber was closed while cooled ambient air was circulated by the ventilation system at a set environmental temperature (19°C). Relative humidity of the chamber was not controlled and was recorded manually via a hand-held heat stress monitor throughout experiments (Kestrel meter 5400, Kestrel, PA, USA). During all experimental exercise trials participants were clad in socks and cycling shorts. Cycling shoes and trainers were worn during exercise on upright and semi-recumbent cycling ergometers, respectively.

3.3.4 – Pulmonary gas exchange and ventilation

Breath-by-breath pulmonary gas exchange and ventilation was measured using a metabolic cart (Oxycon Pro, Jaeger, Germany) that was calibrated according to the manufacturer's instructions prior to each test. Ambient temperature, relative humidity and barometric pressure were determined and applied for the correction of measured values. Then, a turbine and flow meter used for ventilatory volumes were calibrated for volume and flow using the manual smooth repeated filling and emptying of a 3 L syringe. Finally, gas analysers were calibrated for O_2 and CO_2 using a two-point calibration between room air and a hypoxic gas mixture of known composition (16% O_2 and 5% CO_2). The turbine and sampling line was inserted into a face-mask of a standardised size and known dead space within each participant. During exercise testing participants were encouraged to avoid talking, coughing and swallowing where possible. All recorded parameters were smoothed to 5 s averages and exported to computer software (Microsoft Excel, Windows, United States) for further analysis.

3.3.5 – Skin and core temperatures

T_{sk} was continuously measured and logged using iButton temperature sensors/data loggers (iButton^{TM}, Maxim Integrated Products, Sunnyvale, CA, USA). Four sensors were placed on the skin surface and held in place using a thin strip of non-porous tape (Opsite Flexifix, Smith&nephew Medical Ltd.

Hull, U.K.) to allow convective airflow across that area of skin thus preventing a micro-climate forming around the sensor. Each participant used the same sensors throughout all experimentation which were located on the chest, forearm, thigh and calf, respectively. Area-weighted mean T_{sk} was then calculated using the equation of Ramanathan (1964):

$$\bar{T}_{sk} = 0.3\,T_{chest} + 0.3\,T_{arm} + 0.2\,T_{thigh} + 0.2\,T_{shin}$$

Where T is temperature and 0.3 and 0.2 are surface-area weighted factors.

Rectal temperature was used to indicate deep body tissue temperature (T_C) and was obtained by self-inserting a thermistor a distance of 15 cm beyond the anal sphincter. Each thermistor was a sterilised re-usable indwelling temperature probe attached to a precision digital thermometer capable of measuring to the nearest 0.1°C (DM 852, Ellab A/S, Hillerød, Denmark). Participants were assigned three specific thermistors for the duration of each study. Where comparisons were to be made between specific experimental trials, care was taken to ensure the same thermistor was used.

3.3.6 – Whole body sweating rate

Whole body sweat rate was calculated using the changes in pre- to post-exercise dry nude body mass to the nearest 100 g. Changes in nude mass were corrected for exercise duration, urine output and fluid intake using the following equation:

$$Sweat\ rate\ (L{\cdot}h^{-1}) = \frac{\left(mass_{pre} - mass_{post}\right) + fluid\ intake - urine\ output}{exercise\ duration}$$

Where mass is nude dry body mass before and after exercise, fluid intake and urine output are total volumes between mass measurements (in kilograms) and exercise duration is in hours (i.e. total time in minutes/60).

3.3.7 – Fluid intake and composition

Fluid intake during experimental procedures and HA was prescribed to the nearest millilitre. Fluid was in the form of an electrolyte drink with a standardised concentration (0.1% solution; HIGH5 ZERO, H5 Ltd, Bardon, UK). Total calculated fluid intake was divided into equal aliquots and provided at discrete 15-min intervals during exercise. During self-paced exercise performance trials fluid intake was permitted *ad libitum* in the form of plain water. All fluids were stored in the main laboratory (~22°C) prior to consumption and therefore were not equilibrated to hot or cool environments.

3.3.8 – Heart rate

Resting HR was recorded while participants laid in a supine position. Prior to recordings, participants were instructed to remain quiet and rest for a period of 2 min, after which a 60 s recording of HR was made via a chest strap and monitor (RS800CX, T31-Coded Transmitter, Polar Electro, Kempele, Finland). Exercising measurements of HR varied according to the experimental trial. During upright cycling ergometry, HR was recorded via radio-frequency receiver inherent to the ergometer. During semi-recumbent cycling ergometry, HR was recorded via a 3-lead electrocardiogram inherent to a portable ultrasound device.

3.3.9 – Maximal exercise tests

Upright cycling

Responses to submaximal and maximal upright cycling were determined on an electronically braked cycle ergometer (Lode Excalibur Sport, Lode, Groningen, The Netherlands) controlled via software in a computer terminal (Lode Ergometry Manager 9, Lode, Groningen, The Netherlands). Saddle and handlebar positions were adjusted for comfort and recorded to standardise position for all subsequent tests. The test consisted of 5 submaximal stages followed by ramp-incremental changes in workload to volitional fatigue (Figure 3.1). Each submaximal stage lasted 4 min to ensure an exercising steady-

state was achieved. Initial exercise intensity was 90 W and was followed by a step-increment in workload of 30 W until the final stage (210 W). At the end of this stage, resistance increased by the order of 1 W every 2 s until exhaustion despite strong verbal encouragement. Participants were encouraged to maintain a self-selected cadence (e.g. 90 revolutions per minute) throughout. Feedback was restricted to notification of the final minute of each stage and standardised verbal encouragement during ramp-incremental exercise that did not contain information regarding current performance. HR was recorded by the ergometer software while breath-by-breath measurements were recorded on a metabolic cart. Care was taken to ensure measurement parameters were time-aligned. HR, pulmonary and ventilatory parameters were averaged over the final minute of each stage. $\dot{V}O_{2max}$ was defined as the highest minute average value recorded prior to exhaustion.

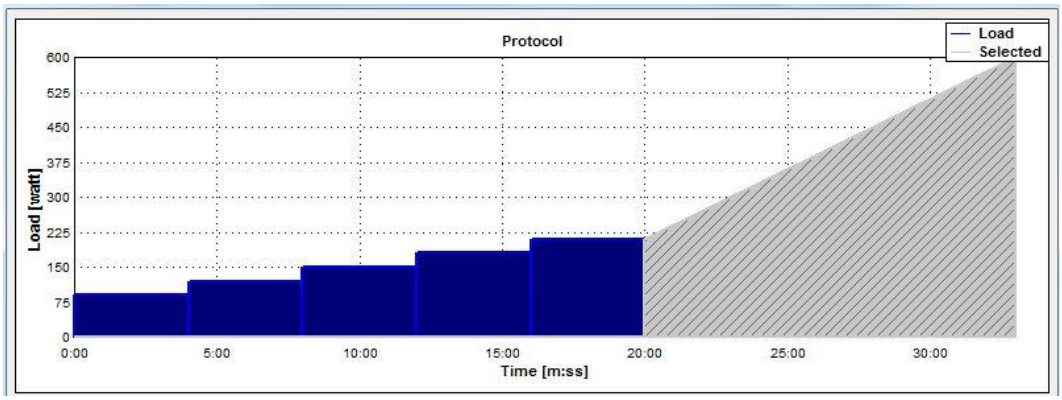


Figure 3.1: Programmed maximal-incremental protocol for upright cycling exercise. Submaximal and maximal recordings used to determine relative workloads and HR targets in subsequent experiments.

Semi-recumbent cycling

Semi-recumbent cycling was conducted to obtain images of the LV during exercise in Chapters 5 and 6 (see section 3.6 for details). In order to standardise relative exercise intensity during cardiac measurements, a step-incremental test to exhaustion was performed on a semi-recumbent ergometer (Ergoselect, Ergoline GMbH, Germany; Figure 3.2). A fan adjacent to the ergometer provided constant airflow of 3 $m \cdot s^{-1}$ directed towards the chest of participants throughout each test. Participants cycled at a standardised cadence of 80 revolutions per minute. Workload began at 60 W

and increased by 30 W every 3 min until exhaustion. $\dot{V}O_2$ was measured throughout and was averaged over the last 30 s of each stage so as to minimise a possible under-prediction of oxygen uptake during periods of non-steady-state exercise. The power output and $\dot{V}O_2$ at several submaximal stages were then used to calculate workloads via linear regression.

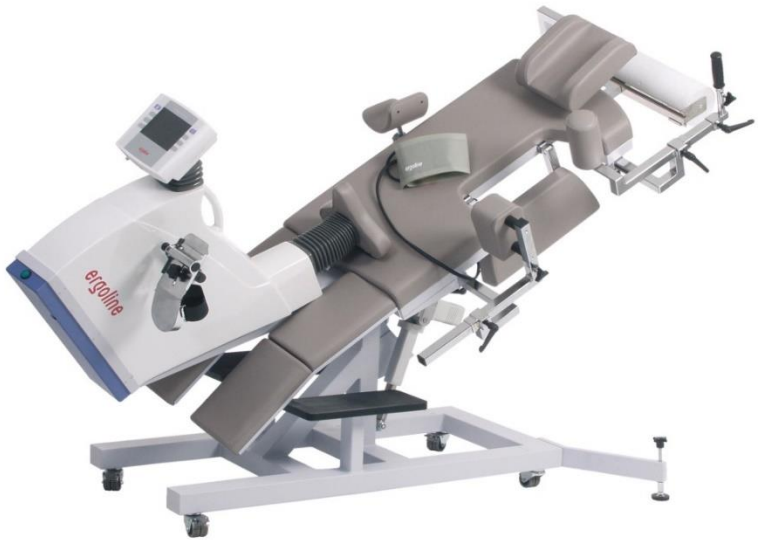


Figure 3.2: The Ergoline Ergoselect semi-recumbent cycle-ergometer that was used during pre-experimental maximal exercise testing and for all experiments involving echocardiographic assessments.

Self-paced cycling time-trials

In Chapter 4, participants performed a 30-min cycling time-trial in hot-humid conditions (35°C, 60% relative humidity). Performance trials were conducted on a cycle ergometer (Schoberer Rad Meßtechnik; SRM, Jülich, Germany) using computer software (SRM Training System V6.42.18). The ergometer determines the primary outcome measure (i.e. power output, W) via mechanical deformation of strain gauges within the crank. To minimise differences in power meters and fluctuations of the zero offset (± 4%; SRM), a single ergometer was used for all participants of this study. The ergometer was calibrated immediately prior to start of all performance trials according to manufacturer's instructions, following a minimum of 30 min exposure to the

desired ambient conditions. Briefly, in a standardised gear, rotation of the flywheel was initiated, and the crank arm was placed in a horizontal position with no load on the drive chain. The power control was then zeroed and set, and the ergometer display was covered in tape.

The ergometer was placed in 'open end' mode and a 30-min timer was displayed on a monitor in front of participants. A fan was placed adjacent to the ergometer with a constant wind flow of 3 $m \cdot s^{-1}$. Participants were instructed to exercise at the highest sustainable output throughout the trial, which began following a verbal countdown. Feedback was limited to time remaining. Cadence, power output and HR were recorded at 2 Hz throughout. Saddle and handlebar configurations were recorded and standardised for all procedures.

3.3.10 – Perceptual measures

At various time-points throughout experimental trials subjective ratings of perceived exertion, thermal sensation and comfort were obtained by asking the participant to point to an arbitrary scale. Participants were familiarised with the scales prior to exercise testing.

Rating of perceived exertion

Ratings of perceived exertion were obtained by using the 15-point Borg scale (Borg, 1982) ranging from 6 ('very, very light') to 20 ('very, very hard').

Thermal comfort

Thermal comfort was assessed using a 7 point Bedford scale (Bedford, 1936) ranging from 1 ('much too cold') to 7 ('much too hot').

3.4 – Heat acclimation

Both HA interventions implemented in this thesis involved repeated exposures to an artificial environmental stress (i.e. heat acclimation). Each intervention involved 10 consecutive daily exercising heat exposures, lasting 90 min per

day, in an environmental chamber where environmental conditions were hot and dry (40°C and 40% relative humidity). Each exposure was designed to maintain the adaptive stimulus to HA via exercising at a target HR. An example of an exercising heat exposure is displayed in Figure 3.3. Participants completed a customised exercise task that was dictated by computer software. Initial exercise workload was conducted at a power output corresponding to 65% $\dot{V}O_{2max}$ (measured in cool conditions) for a period of 15 min. This period was chosen to sufficiently raise HR and promote the onset of sweating while preventing the initial increases in T_C being dampened by cardiovascular strain. Thereafter, workload was automatically adjusted to maintain HR within a narrow range that was associated with the same relative intensity (i.e. the HR at 65% $\dot{V}O_{2max}$). Each exposure was conducted at a similar time of day to avoid circadian variations in resting T_C (Waterhouse et al., 2005).

Figure 3.3: Example of 90 min exercise HA exposure. Initial workload (green line) is fixed for 15 min. Thereafter, automatic adjustments are made to maintain exercising HR (red line) within a narrow range (see text for details). Participants were instructed to maintain a steady cadence (blue line) throughout.

Lode Ergometry Manager software samples HR at 30 s intervals and adjusts power output according to the current difference between actual HR and target HR. The adjustments vary in magnitude, whereby:

- If current HR 15 beats·min^{-1} ≥ target HR, then workload = workload – (workload/8)
- If current HR 1 – 14 beats·min^{-1} ≥ target HR, then workload = workload – (workload/16)
- If current HR ≤ 5 beats·min^{-1} below target HR, then workload = workload
- If current HR ≤ 6-14 beats·min^{-1} below target HR, then workload = workload + (workload/16)
- If current HR ≥ 15 beats·min^{-1} below target HR, then workload = workload + (workload/8)

To account for the conservative nature of this algorithm, target HR was set at 2 beats·min^{-1} higher than the desired value.

On arrival to the laboratory each day, participants provided a urine sample and measurement of nude body mass. Once clothed, participants laid supine in the main laboratory and rested for a period of 10 min. Resting measurements were obtained using the procedures outlined above at the end of this period. On days 1, 5 and 10 of each intervention a blood sample was obtained while remaining in the supine position via venepuncture of an antecubital vein. Following measurements participants moved to the environmental chamber and mounted the cycle ergometer.

T_C, T_{sk}, HR, ambient temperature and relative humidity was recorded every 5 min during exercise. Perceptual measurements were recorded every 10 min throughout each exposure. Fluid was provided in the form of six equal boluses, the first of which was consumed following the commencement of exercise, and then every 15 min thereafter. Volumes equalled either 90% or 10% of expected sweat losses depending on the intervention. This was to either i) ensure body mass was maintained within 1% of resting values without increasing, therefore ensuring euhydration, or ii) result in progressive exercise

induced dehydration. Relative changes in body mass and hourly sweat rate were determined immediately after exercise as described previously. Prescribed fluid intake was then calculated for the subsequent heat exposure to account for changes in sweating rate with HA and therefore standardise the changes in body mass each day. Immediately following exercise on days 1, 5 and 10, participants transferred to a bed adjacent to the ergometer and rested in a supine position while a post-exercise venous blood sample was collected. This was to avoid complications of blood sampling via venepuncture during cycling exercise.

3.5 – Blood sampling

Venous sampling was achieved via a combination of venous cannulation or venepuncture. Blood samples were collected to determine changes in BV, PV and red cell volume (RCV), as well as plasma electrolyte and metabolite concentrations. Cannulation of the cephalic vein (Figure 3.4) was conducted by an experienced clinician in experimental trials in Chapters 5 and 6. This involved application of a tourniquet to the proximal arm and a brief sustained muscular contraction of the wrist flexors to encourage venous engorgement. Cannulas were placed in the antecubital fossa to minimise discomfort during exercise. The skin surface was cleaned by rough application of an alcohol based anti-septic swab and the vein palpated prior to insertion of a 20 G needle and cannula (BD Nexiva closed IV catheter system, BD, Utah, USA). Identification of the vessel in question was achieved by flashback of blood through the introducer needle. The cannula was then advanced into the vein and the needle was withdrawn. Successful placement of the cannula in the vein was confirmed by secondary flashback to the flush site. 10 ml of sterile saline was then used to flush each port of the cannula before the ports were cleaned with an alcohol swab. Finally, the indwelling cannula was secured to the skin using an IV film dressing. The cannula was then flushed with further saline following sampling to maintain patency. Prior to all cannula blood sampling, a small amount of whole blood (~2 ml) was drawn into a syringe and discarded to ensure the proceeding sample was not diluted by previously infused saline.

Conversely, venepuncture procedures involved a single-use butterfly needle (BD Eclipse, 21 g, BD, Utah, USA). Once inserted into the vein, vacutainers were introduced to the adapter for the vacuum withdrawal of venous blood. Once blood samples are collected, the needle was withdrawn under pressure to the puncture site to help prevent haematoma with surgical gauze. All venepunctures were conducted in the supine position either following a 10 min resting period or immediately after cycling exercise.

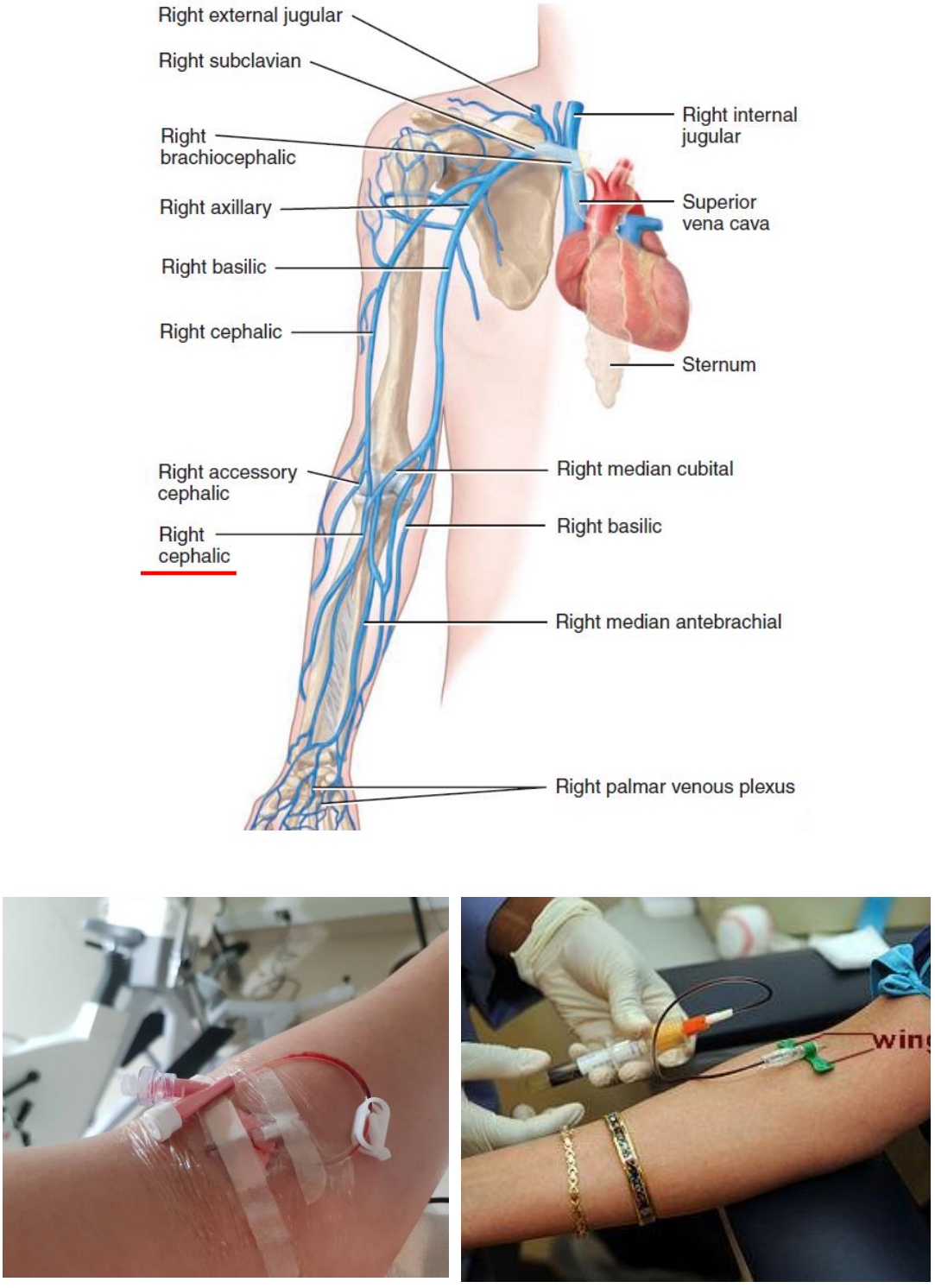


Figure 3.4: Diagrammatic representation of the anatomical location of the cephalic vein (top; from Tortora and Derrickson, 2012) and examples of an indwelling venous cannula (bottom left) and venepuncture (bottom right) introduced at the antecubital fossa.

3.6 – Echocardiography

3.6.1 - Overview

In this thesis, transthoracic echocardiography was used to determine LV volumes at different time points during exercise with altered hydration and heat acclimated states in Chapters 5 and 6. Transthoracic echocardiography is a key tool in clinical and research settings because it has a relatively low cost and is a safe, non-invasive method of imaging cardiac function (Lang et al., 2006; McGowan & Cleland, 2003). It is beneficial over other imaging techniques such as radionucleotide angiography and transoesophageal echocardiography, as it is less invasive and can be used to obtain the standardised imaging planes necessary for research (Lang et al., 2015). The following paragraphs briefly describe the physical underpinning of echocardiography, the reliability and effectiveness of this tool in cardiac imaging and the specific methodology used for image acquisition and analysis of left ventricular function in this thesis.

Sound is a mechanical vibration that is propagated through a medium. For instance, when it propagates through the air at an appropriate frequency (range: 20 Hz – 20 KHz) it is audible to humans as sound (Feigenbaum, Armstrong, & Ryan, 2005). However, when this wave of propagation has a frequency greater than 20 KHz it is referred to as ultrasound, which is significantly beyond the human audible range. Some animals are capable of harnessing ultrasound naturally to detect the location of objects and the same principles apply when using echocardiography to image the structure and function of cardiac tissue. The measurement of ultrasound for use in echocardiography is possible due to two key discoveries in the field of physics. First is that the frequency of acoustic waves vary due to the relative motion between the source and the observer (i.e. the Doppler Effect; Doppler, 1842). Second is the ability to create ultrasonic waves, which occurred in 1880 with the discovery of piezoelectricity by the Curie brothers (Feigenbaum et al., 2005; Mould, 2006). They showed that crystals of quartz, among other things, generated electrical polarisation when placed under mechanical stress

(Mould, 2006). Modern ultrasound devices utilise piezoelectric crystals that are located within a handheld transducer, which propagates short pulses of ultrasonic waves beneath the cutaneous tissue. Upon application of a specific voltage to the crystals they vibrate at a particular resonance frequency, emitting ultrasound waves. These waves propagate through the different tissues within the body and particles within the tissues oscillate in parallel to the line of propagation, creating a longitudinal wave that is reflected back to the transducer (Feigenbaum et al., 2005). The creation of the ultrasound pulse is achieved by an alternating current applied to the piezoelectric crystals; therefore, once the ultrasound energy is emitted it is followed by a period of quiescence while some of the beam is reflected back to the transducer (the dead time; Feigenbaum et al., 2005). If one assumes a constant speed of sound through all tissues of 1540 $m{\cdot}s^{-1}$, the distance between interfaces can then be calculated using the following formula:

$$d = tc/2$$

Where *d* is the distance of the reflecting surface, *t* is the time taken between transmission and reception, *c* is the velocity of the sound wave and the number 2 represents the fact that the pulse wave travels twice (once in each direction; Myers & Clough, 2014). The reflections between two interfaces (e.g. the endocardium and the left ventricular cavity) are then received by the transducer. The transducer converts the impact of the mechanical vibration that is the reflection into an electrical signal. This signal is registered on the oscilloscope of the echograph, indicating the position of the two targets relative to the transducer. The signal is then converted into a greyscale image and according to the amplitude of each reflection, is displayed as varying levels of brightness on the display monitor within a 90° sector (Feigenbaum et al., 2005).

One particular purpose of echocardiography is to obtain high image quality to discern between small structures. This is important for sonographers particularly if the purpose of a given examination is to quantify the movement of heart valves or tissues (Feigenbaum et al., 2005; Helle-Valle et al., 2005)

and determine anatomical landmarks necessary to correctly identify the imaging plane. To optimise an image, the correct balance between spatial and temporal resolution needs to be found. Spatial resolution refers to the ability to differentiate between small structures and the smallest discernible distance between them that can be measured. This too has two factors: axial and lateral resolution (Feigenbaum et al., 2005). Axial resolution refers to structures lying along the same axis as the ultrasound beam, while lateral resolution allows differentiation of objects that are side by side relative to the beam (figure 3.5).

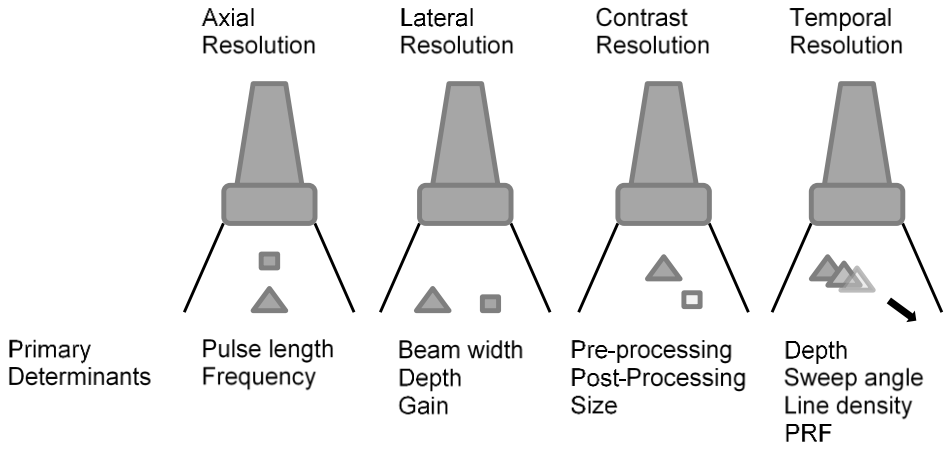


Figure 3.5: Different types of resolution. Note there is a trade-off of axial and temporal resolution since a higher frequency allows a greater likelihood that the position of two targets can be resolved. While this is beneficial for tracking moving targets (e.g. heart valves; temporal resolution), the depth of the beam is significantly diminished. PRF: Pulse repetition frequency. (Redrawn from Feigenbaum et al, 2005).

A higher frequency ultrasound wave enhances the spatial resolution, allowing for more accurate image acquisition. Likewise, a higher pulse repetition frequency of the transducer permits a more accurate 2-dimensional image to be obtained particularly at larger sector widths. However, as ultrasound propagates through a medium the beam intensity decreases at a specific rate depending on the frequency and depth (Feigenbaum et al., 2005). Therefore, this limits the depth at which tissue can be accurately represented and images are optimised by adjusting the emitted frequency, sector width, imaging depth and frame rates.

3.6.2 – Image acquisition procedures

In this thesis, echocardiography was used to assess the mass and diastolic and systolic volumes of the left ventricle in two experimental studies (Chapters 5 and 6). Both studies investigated the effects of HA on LV function and volumes at rest and during semi-recumbent cycling exercise in a hot humid environment with altered hydration. Echocardiographic image acquisition and analysis was conducted by a single sonographer according to the latest guidelines for assessment of global LV function from the American Society of Echocardiography and European Association of Cardiovascular Imaging (Lang et al., 2015; Lang et al., 2006). Images were recorded on a commercially available ultrasound machine (CX50 POC, Philips Healthcare, The Netherlands) using a S5-1 5 MHz sector array probe (Figure 3.6).

Figure 3.6: Philips CX50 portable ultrasound system and transducer that were used in experimental studies of Chapters 5 and 6.

At rest and during semi-recumbent cycling exercise, a minimum of 3 consecutive cardiac cycles were recorded at end expiration. In doing so, lateral displacement of cardiac tissue through respiratory swing was minimised and obliteration of the acoustic window by the lung tissue avoided. Acquired images were saved for off-line analysis using manufacturer specific software (QLAB, Version 10, Philips Electronics, Netherlands). Throughout

imaging HR was recorded via a 3-lead ECG inherent to the ultrasound machine. Data were averaged over 3 consecutive cardiac cycles where possible. Two-dimensional echocardiographic images were used for the calculation of LV systolic and diastolic dimensions and volumes. The specific methods of these imaging techniques and analyses are outlined below.

Left ventricular volumes

LV volumes were calculated using the modified Simpsons bi-plane method of disc summation from 2D apical four- and two-chamber views. This is the recommended method for volumetric analysis (Lang et al., 2015; Lang et al., 2006). Participants rested and exercised on a cycle ergometer that is modified to allow adoption of a recumbent position (Figure 3.2). During image acquisition the apparatus rotates about the longitudinal axis while cushioning and handles above the participant's head stabilise the torso throughout sampling. This movement enables the participant to be placed into the left lateral decubitus position at rest and during exercise while abduction of the left arm widens the intercostal space for clear imaging of the parasternal and apical views (Figure 3.7). Correct orientation of the apical four-chamber view was confirmed by noting that the insertion of the septal leaflet of the tricuspid valve is several millimetres more apical than the insertion of the mitral leaflet. Care was taken to ensure recording of a true apex by identifying the relatively thin walls and lack of motion while preventing foreshortening of the ventricle (Feigenbaum et al., 2005), whilst the inter-ventricular and inter-atrial septum was kept as vertical as possible. From this position the apical two-chamber was obtained by clockwise rotation of the transducer so that the right atrium and ventricle were completely excluded and only the left atrium, ventricle and mitral valve were visible (Feigenbaum et al., 2005).

Figure 3.7: Example of echocardiographic assessment of the LV in the left-lateral decubitus position.

The Simpson's bi-plane method is based on a standardised segmentation of the left ventricle (Lang et al., 2015; Lang et al., 2006) from which both lateral and the inferior and anterior borders of the heart can be observed (Figure 3.8). The endocardial border is outlined in each view at end-systole and end-diastole by tracing the blood-tissue interface (Figure 3.9). At the mitral valve level the contour is closed by connecting the two sections of the mitral ring with a horizontal line (Feigenbaum et al., 2005; Lang et al., 2015; Lang et al., 2006). The left ventricular volume is then calculated by the summation of a series of elliptical disks, the height of each is a fraction of the left ventricle long axis (Lang et al., 2006).

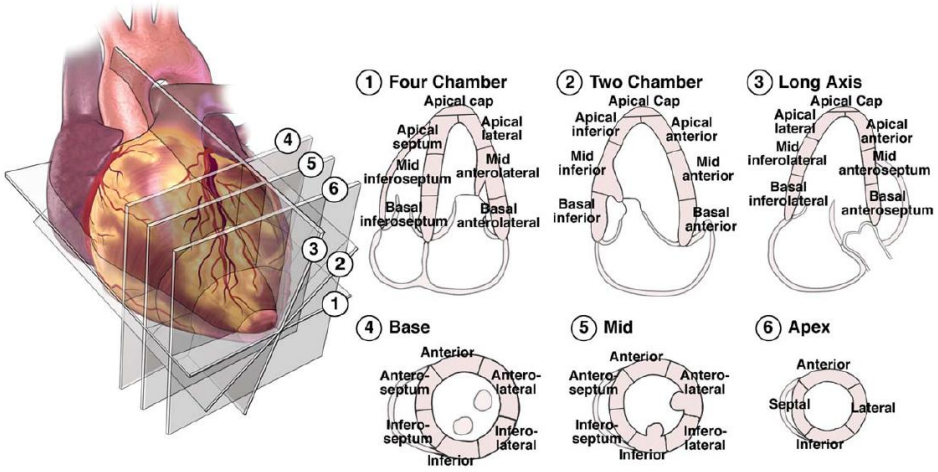


Figure 3.8: Seventeen segmentation model of the left ventricle as defined by the American Society of Echocardiography and European Association of Cardiovascular Imaging (from Lang et al., 2015). Planes 1 and 2 are used to determine intra-ventricular volumes.

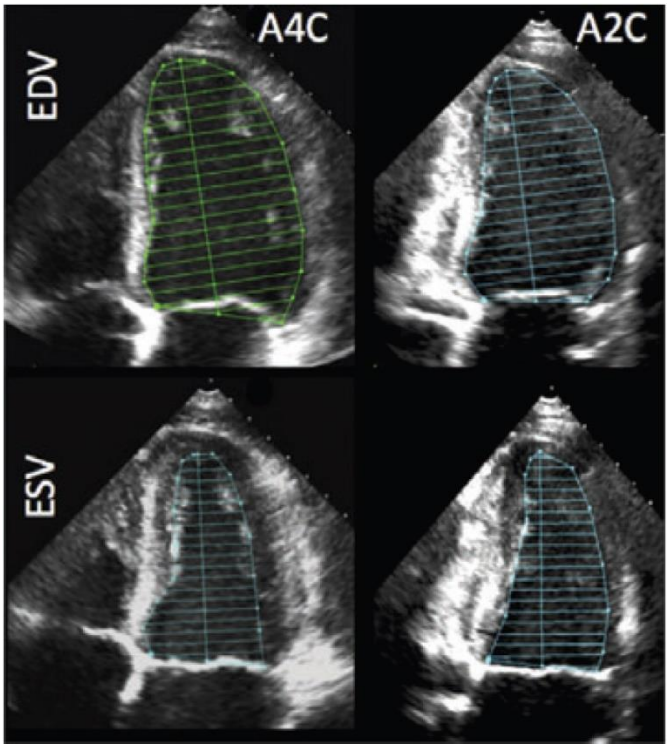


Figure 3.9: Biplane disk summation or Simpson's rule for determining LV volume. The apical four-chamber (A4C, left) and apical two-chamber (A2C, right) views are observed at end-diastole and end-systole for their respective volumes (EDV, top and ESV, bottom). From this, calculation of SV volume, ejection fraction and when incorporated with inherent electrocardiogram, cardiac output can be made. See text for details (from Lang et al., 2015).

Other methodologies exist for estimating LV volumes and are based on the assumption that the ventricle has the shape of a bullet or truncated ellipsoid (Quinones et al., 1981; Teichholz, Kreulen, Herman, & Gorlin, 1976). Previous work at Brunel University London has used the Teichholz (1976) method during exercise (Stöhr et al., 2011b; Stöhr et al., 2011c) since it requires less time to obtain the required images and was therefore more suitable during incremental exercise in participants with no ventricular contraction asymmetry (Stöhr et al., 2011c). To minimise data loss from difficulties in imaging, volumes were also calculated using the method of Teichholz et al. (1976). This method involves motion mode (M-Mode) imaging of the parasternal long-axis. Care was taken to ensure the image was oriented so that the posterior wall was as horizontal, and the transducer was as vertical as possible. An M-mode trace with a vertical beam through the mitral valve leaflets is then used to determine intra-ventricular volumes at peak systole and diastole (Teichholz et al., 1976). As highlighted, this technique makes several assumptions of LV geometry and the 2-dimensional plane of imaging makes it possible to foreshorten the LV (Figure 3.10). These limitations may alter the agreement between LV volumes determined by this technique and the Simpson's bi-plane method (see section 3.10.1). Therefore, the latter method was primarily used wherever possible in this thesis. In cases where apical images were of insufficient quality, data from the Teicholz method was used for all trials and time points within a participant (n=1, Chapter 6) to prevent possible confounding differences in imaging techniques altering results.

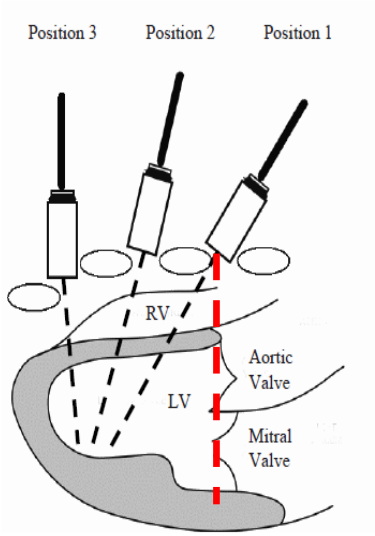


Figure 3.10: Example possible cross-sections from an M-Mode trace at different transducer positions and angles of the LV parasternal long-axis view. Geometrical assumption that the LV is a truncated ellipsoid in method of Teicholz et al., (1976) results in significant under- and over-estimation of LV volumes if view is off-axis and transducer beam does not pass vertically through mitral valve leaflets (red dashed line; redrawn from van Dalen et al. 2008).

Left ventricular mass

LV mass was calculated using the length-area method of Schiller et al. (1989). This was to non-invasively determine if either of the HA interventions used in Chapters 5 or 6 resulted in a structural remodelling of the LV. LV mass is calculated at rest using images of the parasternal short-axis and apical 4-chamber views (Figure 3.11).

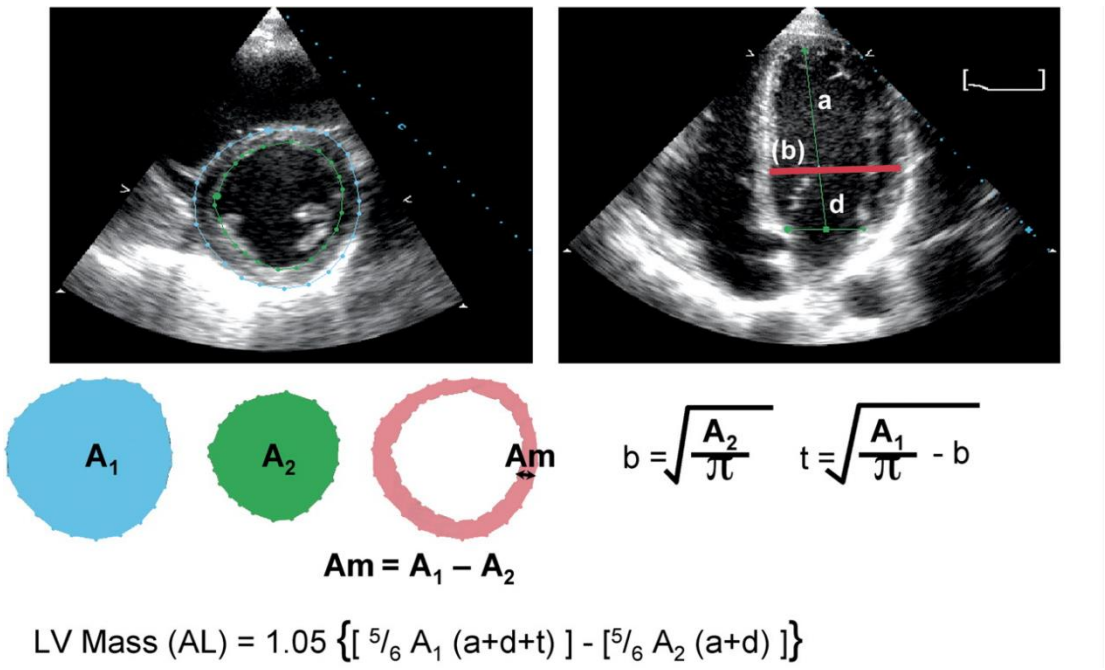


Figure 3.11: Method of estimating LV mass (in g) based on the area-length (AL) formula from the short-axis (top left) and apical four-chamber (top right) 2-dimensional views. A_1 is the total LV area, A2 is the LV cavity area and AM is the myocardial area. a is the semi-major axis from widest minor axis radius to the apex, b is the short-axis radius (back-calculated from the short-axis cavity area) and d is the truncated semi-major axis from the widest short-axis diameter to mitral annulus plane. Assuming a circular area, the radius (b) is computed and mean wall thickness (t) is derived from the short-axis epicardial and cavity areas (Schiller et al., 1989). Reproduced from Lang et al., (2006).

3.7 – Haemodynamics

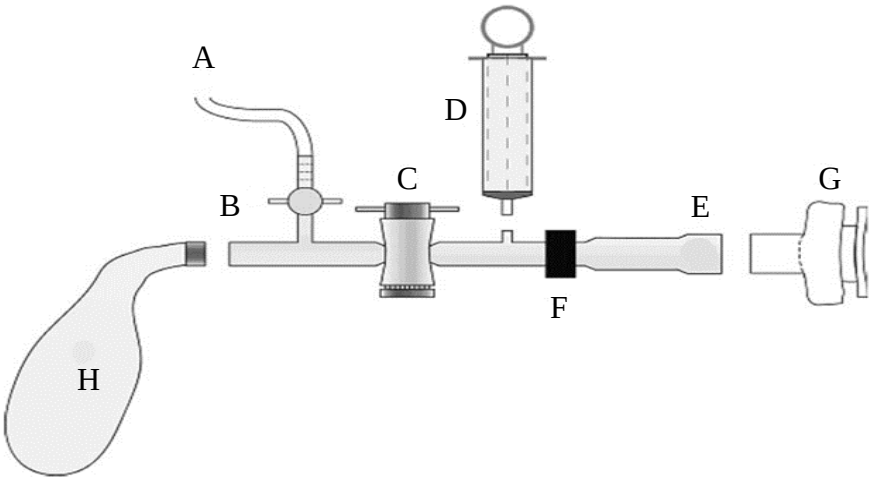
$\dot{Q}$ was determined by multiplying the echocardiographic estimated SV against HR. Systolic and diastolic blood pressure was measured manually using a sphygmomanometer with the cuff placed around the left arm of the participant while in a semi-recumbent position. Mean arterial pressure (MAP) was determined as ((2 X DBP) + SBP) / 3 where DBP and SBP are diastolic and systolic blood pressures, respectively. Systemic vascular resistance (SVR) was calculated as MAP/$\dot{Q}$ and expressed as peripheral resistance units of $mmHg \cdot L \cdot min^{-1}$.

3.8 – Haematology

3.8.1 – Haemoglobin mass

On two occasions prior to, and once ~24 h following each HA intervention in this thesis, participants had their haemoglobin mass (Hb_{mass}) determined via the carbon monoxide (CO) rebreathing technique as previously described by Schmidt and Prommer (2005). This method is widely accepted as the most time efficient way of accurately determining total Hb_{mass} in patients and athletes compared to the alternative O_2 and CO mixture rebreathing method (Heinicke et al., 2001). Participants sat quietly in a chair throughout the procedure. Participants placed on a nose clip and exhaled fully to residual volume immediately prior to connecting their mouth to the spirometer displayed in Figure 3.12. Briefly, participants then inhaled a whole CO-bolus administered in a single breath from a prefilled syringe (D). CO dosage was 1.2 $ml \cdot kg^{-1}$ of participant's body mass, rounded up to the nearest 10 ml for accurate measurement within the administering syringe. Dosages were kept constant within participants throughout all experiments. At the same time the dose was administered, valve C was opened to allow the O_2 reservoir to be inhaled (H). This allows the entire dosage to be inhaled immediately and, given the high affinity of CO for haemoglobin, a large part of the dose is absorbed by the blood within a few seconds. To further aid this process participants maintained end-inspiration for 10 s, after which they continued normal tidal ventilation from the spirometer for a further 1 min 50 s (Schmidt & Prommer, 2005). To verify no gas escaped from the spirometer or the connection between the mouth and mouthpiece, a portable CO analyser (Draeger PAC7000, Draeger, Luebeck, Germany) with parts-per-million sensitivity was continuously passed around the device throughout the procedure. After 2 min, the participant fully exhaled to residual volume to near-fully inflate the reservoir bag, which was then be closed off (C). Analysis of this expired gas allows for the quantification of the volume of CO taken up by the body. It is estimated that residual volume remaining in the lungs is 1.5 L in males (Schmidt & Prommer, 2005). In addition to this, to quantify the CO volume exhaled after disconnecting, end-tidal CO concentration was

measured 2 min after the end of the rebreathing period and multiplied by the alveolar ventilation (estimated at 5 $L \cdot min^{-1}$; Schmidt and Prommer, 2005). 200 µL arterialised fingertip capillary blood samples were collected before, and 7 min after the rebreathing procedure began, and a minimum of quintuplicate measurements of the fraction of carboxyhaemoglobin (%COHb) were determined using a spectrophotometer (ABL 90 FLEX, Radiometer, Brønshøj, Denmark).



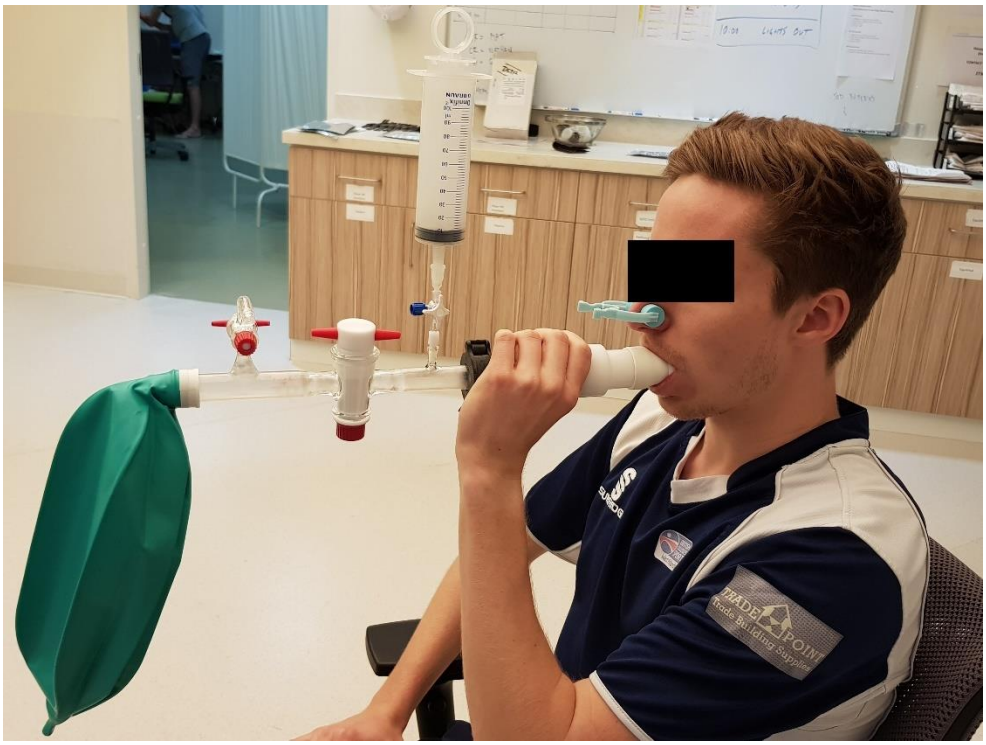


Figure 3.12: Top: Spirometer used for CO rebreathing. (A): O_2 tube, (B): O_2 port (closed during test), (C): valve of O_2 reservoir (open during test), (D): CO syringe, (E): adapter for mouthpiece, (F): sleeve, (G): mouthpiece and, (H): anaesthetic bag for O_2. Redrawn from Schmidt and Prommer (2005). Bottom: example re-breathing procedure.

Total Hb_{mass} was then calculated at each point of venous blood sampling in Chapters 4 through 6 using the following equations of Schmidt and Prommer (2005):

$$Total\ Hb\ mass = K\ \times MCO\ \times 100\ \times (\Delta HbCO\%\ \times 1.39)^{-1}$$

Where K = current barometric pressure x 760^{-1} X [1 + (0.003661 x current temperature)]

$MCO = CO_{adm} - (CO_{system + lung\ (after\ disconnection)} + CO_{exhaled\ (after\ disconnection)})$

CO_{adm} = CO volume administered into the system

$CO_{system + lung\ (after\ disconnection)}$ = CO concentration in spirometer x (spirometer volume + lung residual volume)

$CO_{exhaled\ (after\ disconnection)}$ = end-tidal CO concentration x alveolar ventilation x time

$\Delta HbCO\%$ = difference between basal HbCO and HbCO in the blood after administration and;

1.39 = Hüfners number (ml CO x g Hb^{-1}).

3.8.2 – Blood and plasma volumes

BV, RCV and PV were determined at the time of each venous blood sample using two methods; changes in Hb concentration relative to the total amount of Hb in grams to determine absolute blood volume and relative changes, to identify percent changes in the plasma compartment to acute exercise with or without dehydration. The absolute BV, RCV and PV were calculated using total Hb mass, Hb concentration and haematocrit (Hct) using the following equations:

$$BV = (Hb\ mass\ \div Hb\ concentration) \times 100$$

$$RCV = BV\ \times (Hct\ \div 100)$$

$$PV = BV - RCV$$

Where BV, RCV and PV are in ml, Hb_{mass} is in g and Hb concentration is $g \cdot dl^{-1}$.

Relative changes in BV and PV were also determined using the equations of Dill and Costill (1976):

$$BV_A = BV_B\ \times \frac{Hb_B}{Hb_A}$$

$$RCV_A = BV_A\ \times\ Hct_A$$

$$PV_A = BV_A - RCV_A$$

Where the subscripts B and A represent samples that are obtained before and after exercise, respectively. Hb and Hct were obtained from venous blood samples. Initial blood volume is assumed to be 100 ml. Using the calculated changes in Hb and Hct, percent changes in BV and PV can therefore be calculated as:

$$\Delta BV = \frac{100(BV_A - BV_B)}{BV_B}$$

$$\Delta PV = \frac{100(PV_A - PV_B)}{PV_B}$$

Analyses of whole blood were determined using two techniques. Throughout days of HA, blood was collected into 4 ml EDTA tubes. Tubes were inverted several times and placed on a tilting table at an ambient room temperature until later analysis. Parameters were measured in the main hospital laboratory at Aspetar using a Coulter counter (UniCel DxH 800 Coulter Analysis System, Beckman Coulter, CA, USA). Typical error between duplicate measurements of whole blood in this analyser were 0.1 $g \cdot dl^{-1}$ and 0.34% for haemoglobin concentration and haematocrit, respectively. In the experimental trials of Chapters 5 and 6, a ~2 ml discard was collected into an untreated syringe. Then a 2 ml sample was collected into a lithium heparinised syringe (PICO 50, Radiometer, Brønshøj, Denmark) and the cannula was flushed with sterile saline. Syringes were immediately inverted several times and expressed to remove trapped air before a cap was placed on the tip. Whole blood was analysed in duplicate for oximetry, metabolite and electrolyte concentrations (ABL90 FLEX, Radiometer, Brønshøj, Denmark) and corrected for T_C.

3.9 – Statistical analyses

All data were analysed using a commercially available statistical software package (SPSS V.20, Chicago, IL, USA). Analyses used are described in detail in the relevant study sections. All results are expressed as mean ± standard deviation and the level of significance be set at an alpha level of $P < 0.05$. In general, the statistical analyses carried out on data collected in this study consisted of analysis of variance with repeated measures (RM-ANOVA). Mauchley's test was performed to determine any violations to sphericity. In cases where this occurred a Greenhous-Geisser correction factor was applied to the degrees of freedom. Effect sizes were determined using partial eta squared with values for small, medium and large effects defined in each experimental chapter. Typically, within and between group and interaction

effects may be reported along with a ratio of the within-between group variances (i.e. the F statistic) and 95% confidence intervals and may be used to contextualise two means where differences may or may not have been observed. However, these have been omitted for the sake of clarity to the reader.

Sample size was calculated *a priori* using G*Power with effect sizes derived from parameters relevant to the thesis. These were sourced from previously observed effect sizes for relative changes in PV with dehydrated compared to euhydrated HA (Garrett et al., 2014) and effects of HA on SV and $\dot{Q}$ (Tyler et al., 2016). For repeated measures ANOVA with a power ($\beta \geq 0.80$) and an alpha level of 0.05, a total of 8-10 participants were required to achieve a balanced cross-over designed study. Therefore, every attempt was made to recruit a minimum of 10 participants to complete the experiments within this thesis. However, due to logistical constraints this was not possible. Therefore, relevant findings of the thesis are addressed in context of sample size and statistical power where appropriate.

The reliability of data collected (the co-efficient of variation; i.e. the ratio of the standard deviation (σ): the mean value (μ)) during preliminary trials designed to test the sensitivity of main outcome measures are described in full detail below.

3.10 – Co-efficient of variation

Several of the variables collected throughout this thesis are both subjective in nature and highly dependent on competency of the primary investigator. This section describes the various within-participant co-efficient of variation for several dependent variables at independent time-points.

3.10.1 – Echocardiographic measurements co-efficient of variation

Despite echocardiography being a useful non-invasive clinical tool, the imaging modalities are susceptible to several sources of measurement error. For instance, inter-individual differences in anatomy results in variation of the

possible image quality and thus, participants were screened prior to experimentation to determine whether suitable windows and images were possible during exercise. However, perhaps the biggest sources of error introduced to image acquisition are the subjectivity in assessment of wall motion and sonographer skill. As such, it is key to ensure the sonographer is appropriately trained (Oxborough, 2008) and emphasis is placed on image reproducibility (Gottdiener, 2001).

The sonographer has undergone training from a qualified cardiologist and followed a systematic procedure according to the guidelines of echocardiographic image acquisition and analysis (Lang et al., 2015; Lang et al., 2006). To determine the measurement variability at rest and during exercise, a within-participant, between-day reliability trial was conducted. Ten healthy, active males were asked to attend the laboratory on 3 occasions. The average age, height and body mass of the participants was 33.4 ± 6.3 yr, 181 ± 9 cm and 79 ± 9 kg, respectively. During an initial visit, participants were familiarised to the stress echocardiography procedures and screened for image quality. All participants had no visible wall motion abnormalities, a preserved resting ejection fraction (i.e. >55%) and normal diastolic function (i.e. E'/A' >1.5).

In each of the reliability visits echocardiographic assessment of LV volumes were assessed at rest and while cycling at two submaximal power outputs. On arrival participants were instrumented for a 3-lead electrocardiogram and rested on the ergometer in a semi-recumbent position for a period of 5 min. After this, the ergometer was tilted, and a resting echocardiographic examination was completed.

Once all images were acquired, participants were returned to a semi-recumbent position. Participants then pedalled at a low cadence (55-65 rpm) with a resistance of 60 W for 5 min. After this period, the ergometer was tilted while participants continued exercising and images were recorded for a further 6-8 min. All images were taken at end expiration. Following image recording,

participants continued to exercise, and the process was repeated at 120 W. Total image acquisition time lasted ~30 min.

A systematic order of image acquisition was followed during each phase of the trial. LV volumes were first estimated using the method of Teichholz et al. (1976) from M-Mode measurement from the parasternal long axis view. Finally, apical four and two chamber views were then assessed for comparative measures of left ventricular volumes using the Simpsons bi-plane method of disk summation.

The co-efficient of variation for LV volumes are presented in Table 3.1 along with previously observed reliability data of some variables (Stöhr, 2010). The agreement between the two methods is displayed in Figure 3.13.

Table 3.1: Co-efficient of variation of echocardiographic variables at rest and two sub-maximal intensities of semi-recumbent cycling

Intensity	Method	LV index	Mean of trial 1 & 2	SD of trial 1 & 2	C of V (%)	Previously reported C of V (Stöhr, 2011)	Absolute change required
Rest	Teicholz	EDV (ml)	126	7	5.6	3.1	7
		ESV (ml)	47	3	6.7	12.6	3
		SV (ml)	80	5	7	4.4	5
	Simpsons	EDV (ml)	140	6	4.4	-	6
		ESV (ml)	58	3	5.7	-	3
		SV (ml)	82	5	6.3	-	5
	Tissue Doppler	IVRT (ms)	81	11	13	7.9	9
60 W	Teicholz	EDV (ml)	134	5	3.6	-	5
		ESV (ml)	39	6	16.4	-	6
		SV (ml)	94	7	8	-	7
	Simpsons	EDV (ml)	146	8	5.2	-	8
		ESV (ml)	56	6	10.2	-	6
		SV (ml)	90	5	6.1	-	5
120 W	Teicholz	EDV (ml)	137	11	8	-	11
		ESV (ml)	41	8	20	-	8
		SV (ml)	96	10	10	-	10
	Simpsons	EDV (ml)	148	9	6.2	-	9
		ESV (ml)	53	6	11.1	-	6
		SV (ml)	95	6	5.8	-	6

C of V; co-efficient of variation. IVRT; iso-volumetric relaxation time.

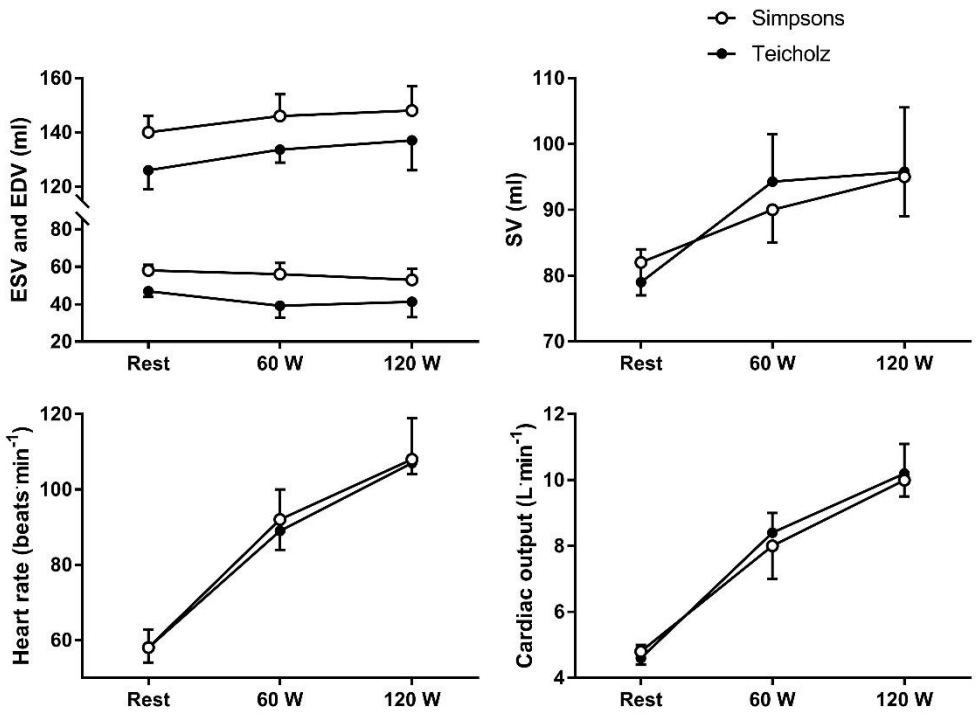


Figure 3.13: Cardiovascular responses at rest and during two step incremental exercise intensities. Volumes measured via either motion-mode imaging of the parasternal long-axis (Teicholz; filled circles) or the bi-plane method of disk summation (Simpsons; closed circles) from apical four and two chamber views. Values are means ± SD.

The co-efficient of variation of this sonographer for LV volumes ranged from 4-13% at rest. This is in accordance with previously reported intra-observer reliability for LV volumes of 3-13 % from Brunel University London (Stöhr, 2010) and others (George et al., 2004). During submaximal exercise the reliability of measurements made by the sonographer decreased for some motion-mode measures of LV volume. In addition, the volumes differed substantially between methods. Therefore, methods used within a participant were standardised between trials and times. Using the Simpsons method of disk-summation the variation remained between 6-11%. Moreover, the absolute change to detect a meaningful difference during exercise is relatively small (6 – 11 ml in central haemodynamics). Given the typical expected changes in cardiovascular function with heat stress and dehydration, the sensitivity of this measurement technique is acceptable.

3.10.2 – Haemoglobin mass co-efficient of variation

The optimised CO rebreathing technique has been shown to be a simpler valid means of assessing total Hb_{mass} when compared to rebreathing of a gas mixture (Schmidt & Prommer, 2005). Furthermore, the inhalation of a single CO bolus and capillary blood sampling is less invasive than the gold-standard technique of radioactive labelling. Despite its simplicity, the technique also has several possible sources of error. Timing of measurements are key due to the initially rapid and transient diffusion of CO out of the circulatory system (Bruce & Bruce, 2003) and the reproducibility and reliability of results can be affected by the spectrophotometer used (Ulrich, Strunz, Frese, Bartsch, & Friedmann-Bette, 2012). Therefore, the primary investigator underwent supervised training for this technique and care was taken to ensure the spectrophotometer was kept constant for all trials, thus minimising between-trial measurement error.

The binding of CO to Hb can lower $\dot{V}O_{2max}$ due to the oxygen carrying capacity of the blood and the leftward shifting of the oxygen dissociation curve that impairs muscle $\dot{V}O_2$ (Hogan et al., 1990; Richardson, Noyszewski, Saltin, & González-Alonso, 2002). Washout of CO is reported to vary depending on whether exercise is performed between measurements. Washout is expected to be complete within 12 h of dose administration (Heinicke et al., 2001) but previous research has highlighted that subsequent dosages can be administered as little as 2 h apart without affecting CO-Hb exceeding 10% or impairing the accuracy of the rebreathing method (Naef, Steiner, & Wehrlin, 2015). However, to ensure maximal performance is unaffected a minimum of 24 h separated the rebreathing and any subsequent measures of $\dot{V}O_{2max}$ in experimental chapters.

A within-participant, between-day study for intra-observer reliability was conducted prior to commencing the experiments in this thesis. Nineteen participants were recruited to attend the laboratory on two occasions a minimum of 24 h apart. All participants were healthy males. Their characteristics are displayed in Table 3.2. Participants were asked to refrain

from alcohol intake and strenuous exercise for 24 h and smoking for 12 h prior to attending the laboratory. Experimental procedures were identical to those described above for CO re-breathing. The results of each trial along with the between-day within-subject co-efficient of variation are displayed below (Table 3.2).

The average Hb_{mass} (μ) measured across all participants in both trials was 878 g. The standard deviation of the differences between trials 1 and 2 was ±14 g (σ). Co-efficient of variation was 0.84% (7.4 g) and was calculated as σ /μ X100. A meta-analysis of the data published in the scientific literature using this technique (Gore, Hopkins, & Burge, 2005) pooled a total of 69 estimates of measurement error and observed a co-efficient of variation of 2.2% (90% confidence limits 1.4-3.5%). The results of the above trial are well below this value and are comparable to the lowest recorded between-measurement error for determining Hb_{mass} using CO-rebreathing reported in the literature (0.9%; Burge & Skinner 1995). It is also possible to calculate the typical error of the measurement by dividing the average standard deviation of the difference scores by $\sqrt{2}$ (Hopkins, 2000). This indicates a typical error of 10 g between measurements. Therefore, the smallest absolute detectible difference between trials was 7.4 g, and a minimum of 10 g change in Hb_{mass} may be considered meaningful pre- to post-HA.

Despite the relatively small variation in values obtained by the primary investigator, there are still a number of potential sources of error when using CO-rebreathing to determine absolute Hb_{mass} (Gore et al., 2005). These include the subject not exhaling to residual volume prior to connecting to the closed circuit, spirometer leakage and measurement error of the CO bolus. Therefore, a cut-off between relative differences of the duplicate baseline in Hb_{mass} was set. If pre-acclimation values differed by >2% within individuals at baseline, they were asked to report to the laboratory for an additional measurement before undergoing experimentation.

Table 3.2: Characteristics and results of 19 participants who underwent two measurements of Hb_{mass}.

Participant	Age (yr)	Height (cm)	BM (kg)	CO dose (ml)	Hb_{mass} – trial 1 (g)	Hb_{mass} – trial 2 (g)	Trial 1 - 2 (g)	Average (g)	SD (g)	%difference	C of V (%)
1	27	175	82.0	100	943	943	0	943	0	0	0
2	26	192	74.5	90	1055	1038	17	1047	12	1.6	1.15
3	33	175	78.5	94	898	902	-4	900	3	-0.4	0.31
4	32	185	73.0	88	898	903	-5	901	4	-0.6	0.39
5	37	175	72.1	88	754	766	-12	760	8	-1.6	1.12
6	40	173	70.0	84	880	870	10	875	7	1.1	0.81
7	37	172	72.0	86	939	953	-14	946	10	-1.5	1.05
8	35	172	81.9	98	850	848	2	849	1	0.2	0.17
9	25	182	77.0	92	849	851	-2	850	1	-0.2	0.17
10	25	175	66.4	80	962	938	24	950	17	2.5	1.79
11	26	178	65.8	80	884	859	25	872	18	2.9	2.03
12	26	176	65.7	80	824	836	-12	830	8	-1.4	1.02
13	24	180	67.3	80	688	692	-4	690	3	-0.6	0.41
14	32	179	68.7	82	918	903	15	911	11	1.6	1.16
15	18	187	73.7	88	1000	1009	-9	1005	6	-0.9	0.63
16	33	173	65.7	80	783	783	0	783	0	0	0
17	27	184	68.8	84	929	926	3	928	2	0.3	0.23
18	22	172	65.3	78	951	918	33	935	23	3.5	2.50
19	32	169	56.7	68	706	699	7	703	5	1.0	0.70
Average	29.3	177.6	70.8	85.3	880	876	4	878	7	0.4	0.8
SD	5.8	6.1	6.3	7.6	96	92	14	94	7	1.5	0.7

Hb_{mass} haemoglobin mass (in g). C of V co-efficient of variation.

CHAPTER 4

Study 1: Heat acclimation with controlled heart rate: the influence of hydration status on induction of adaptations, maximal aerobic capacity and self-paced exercise performance

4.0 – Abstract

Heat acclimation (HA) may be optimised by exercise but can be difficult to safely implement outside of a controlled laboratory environment. This study sought to characterise adaptive responses to acclimation with a controlled heart rate protocol and determine whether hydration strategy altered the adaptation process. A secondary aim was to determine the influence such interventions had on maximal aerobic capacity in a cool environment and self-paced exercise performance in the heat. Eight males performed a graded exercise test in cool conditions followed by a 30 min cycling time trial in the heat (35°C and 60% relative humidity) before and after two exercising HA interventions conducted in a counterbalanced, crossover experiment. Both euhydrated and dehydrated HA interventions consisted of cycling for 90 min in 40°C and 40% relative humidity for 10 consecutive days. Workload was altered over the final 75 min of each exposure to maintain a target heart rate equivalent to 65% $\dot{V}O_{2max}$ (146 ± 7 beats·min^{-1}). Fluid was prescribed to replace either 90% (euhydration) or 10% (dehydration) of expected sweat losses to exercise. Core temperature was 38.4 ± 0.2°C over the last 75 min of each exposure, regardless of hydration status (both $P > 0.05$). Resting core temperature, heart rate and plasma volume were unaltered by either HA intervention (all $P > 0.05$). HA tended to increase sweat rate ($P = 0.06$). Skin temperature decreased in euhydrated (0.6 ± 0.5°C, $P = 0.03$) but not dehydrated acclimation. Acclimation led to slight 0.16 ± 0.12 L·min^{-1} (~3 ml·kg^{-1}·min^{-1}) increases in $\dot{V}O_{2max}$ ($P = 0.02$) while 30 min time trial performance was improved following euhydrated (19 ± 16 W, $P = 0.02$), but not dehydrated acclimation (13 ± 26 W, $P = 0.21$). These findings suggest exercise with controlled heart rate induces several adaptive responses typical of heat acclimation, which may be optimised by maintaining euhydration. These adaptations result in significant improvements in self-paced exercise performance in the heat. However, HA does not appear to significantly increase $\dot{V}O_{2max}$ in cool conditions.

4.1 – Introduction

The level of heat strain experienced by an individual to a given stress improves with the repeated exposure to the combined stressors of internal metabolic heat production and high ambient temperatures. The adaptations that lead to this improvement can occur in naturally hot environments (acclimatisation) or with repeated exposure to artificial climates; termed heat acclimation (HA; Armstrong & Maresh, 1991). HA increases sweating and skin blood flow, improves fluid balance and reduces heart rate (HR) and thermal strain during exercise heat stress (Periard et al., 2015; Taylor, 2014; Tyler et al., 2016). For athletes, exercise HA is considered the most effective strategy to alleviate physiological strain and optimise performance in the heat (Racinais et al., 2015a) as it mimics conditions experienced during competition.

The most frequent HA intervention in research is the use of repeated exposures of fixed intensity exercise that are of a pre-determined duration (Tyler et al., 2016). This experimental approach has the advantage that metabolic heat production is constant. However, this may result in a transient withdrawal of the endogenous thermal stimuli to adapt, as increases in core temperature (T_C) are lowered across exposures (Taylor, 2014). Instead, controlled hyperthermia or isothermal HA, whereby T_C is elevated and clamped, has been suggested to induce more complete adaptation (Taylor, 2014). For the elite athlete however, it is difficult to incorporate HA into training and travel schedules while budgetary and practicality considerations must also be made. Furthermore, monitoring T_C accurately is expensive and often requires invasive measurements that involve the support of practitioners. Other methods have recently been explored, such as post-exercise hot water immersion (Zurawlew et al., 2016) and overdressing during exercise (Ely et al., 2018), that may offer a practical alternative. However, these techniques do not fully replicate responses to exercise in hot environments.

Another important consideration when exercising in the heat is that of dehydration. Acutely, dehydration increases the risk of heat exhaustion (Carter et al., 2005) and heat stroke (Sawka et al., 1992), particularly in those

who are unacclimated. It is therefore recommended to maintain euhydration during HA (Bergeron et al., 2012). Recently however, HA with controlled hyperthermia and fluid restriction has been used to investigate the thermally-independent effects of dehydration on adaptation. Patterson et al., (2004a) demonstrated continual thermal and cardiovascular adaptations over a 28-day intervention, with plasma volume (PV) remaining elevated from day 8 (Patterson et al., 2004b). However, the effect of hydration status on the development of adaptations remains unclear as previous studies have found both slight (Garrett et al., 2014) or no additional beneficial effect of dehydration (Neal et al., 2016b) on the time course or magnitude of acclimation compared to euhydration. Interestingly, these studies and others also observed a similar daily exercising HR, independent of hydration status, while exercise intensity was either increased or maintained to elicit a T_c ~38.5°C as adaptations developed (Garrett et al., 2012; Garrett et al., 2014; Neal et al., 2016b).

Together, these findings suggest that altering exercise intensity during heat exposure to maintain a target HR throughout acclimation would lead to the development of adaptations typical of HA. This approach could provide favourable real-world application and relevance to those undergoing exercise HA (Periard et al., 2015). Such an intervention may also allow for the maintenance of T_c despite exercise induced dehydration and therefore potentially offers a safe, practical method of HA for athletes. However, no study to date has fully described the adaptive responses to HA with controlled HR, or whether hydration status alters responses observed. This was therefore the primary aim of this investigation.

HA has also been reported to augment maximal aerobic capacity ($\dot{V}O_{2max}$), the power output at lactate threshold and improve mechanical efficiency and exercise economy by 5-7% in cool ambient conditions (Lorenzo et al., 2010; Neal et al., 2016a; Sawka et al., 1983b). Many of the adaptations to HA have been proposed to cause these increases in aerobic exercise performances in cool conditions (Corbett et al., 2014). For instance, the pronounced hypervolemia observed following dehydrated HA (Garrett et al., 2014) may enhance maximal cardiac output. However, whether HA *per se* increases

$\dot{V}O_{2max}$ in temperate conditions is unclear, regardless of hydration strategy. Few studies have employed appropriate control arms and it has been proposed that HA does not improve temperate exercise performance beyond typical training since other investigations using counterbalanced cross-over designs have shown no improvements in temperate exercise performance (Karlsen et al., 2015; Keiser et al., 2015). Keiser et al. (2015) reported that euhydrated HA with controlled HR improved $\dot{V}O_{2max}$ and self-paced exercise performance in the heat, but not cool conditions. This suggests HA specifically benefits oxygen uptake, delivery and thermoregulation during exercise heat stress. However, the effects of hydration status throughout HA with controlled HR on $\dot{V}O_{2max}$ in a temperate environment and self-paced exercise performance in the heat remains unknown and was a secondary aim of the present investigation. It was hypothesised that both maintained euhydration via fluid ingestion and dehydration via fluid restriction throughout HA with controlled HR would i) lead to similar adaptations typical of the heat acclimated phenotype and, ii) while these interventions would not increase $\dot{V}O_{2max}$ in cool conditions, iii) self-paced exercise performance in the heat would be improved following HA, regardless of hydration strategy.

4.2 – Methodology

4.2.1 – Participants

Eight healthy males provided written informed consent to take part in this study following a health questionnaire. Participants were cyclists and triathletes training a minimum of 5 h per week and had an average age, height, body mass and $\dot{V}O_{2max}$ of 33 ± 5 years, 176 ± 5 cm, 75.7 ± 4.5 kg and 4.01 ± 0.61 $L \cdot min^{-1}$, respectively. The study was approved by the ethics review board of the Anti-Doping Laboratory Qatar.

4.2.2 – Experimental design

A within participant, counterbalanced crossover design was employed, with participants completing two exercise HA interventions with controlled HR. An overview of the experimental protocol is displayed in Figure 4.1. Experimentation took place between December 2016 and February 2018 in the research department laboratories at Aspetar, Qatar. To minimise the potential confounding influence of natural heat acclimatisation, participants were invited to train indoors in the laboratories at Aspetar three weeks prior to commencing the study and encouraged to limit outdoor exercising exposures to once per week over this period. This time was also used to familiarise participants to laboratory equipment and procedures.

Interventions differed in the fluid intake strategy used during HA, with participants maintaining euhydration (HA-EUH) or reaching a similar level of dehydration (HA-DEH) each day. A graded exercise test was conducted before and after each acclimation period to determine the effects of HA on $\dot{V}O_{2max}$ in temperate conditions. In the same visit, a 30 min cycling time trial was also conducted in hot humid conditions to determine the effects of each intervention on self-paced exercise performance in the heat. Changes in red cell (RCV), blood (BV) and PV were determined via carbon monoxide rebreathing before and after each HA intervention. Each intervention was separated by a washout period of 10 ± 3 weeks. All experiments were

conducted at a similar time of day to minimise the effect of circadian variation. Participants were asked to refrain from smoking for 12 h and alcohol consumption for 24 h prior to each visit to the laboratory.

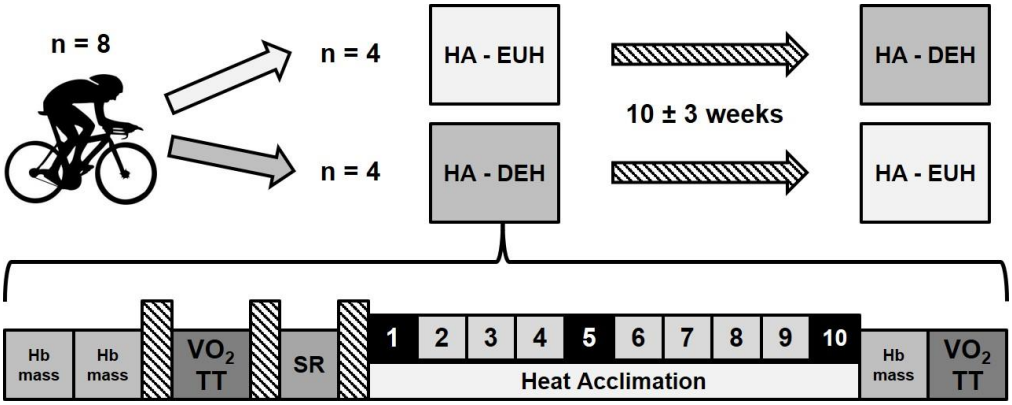


Figure 4.1: Outline of counterbalanced crossover study design. Each 10-day acclimation period was preceded by a dual baseline measurement of haemoglobin mass (Hb_{mass}), a $\dot{V}O_{2max}$ test, 30 min self-paced exercise performance trial ($\dot{V}O_2$ & TT) and sweat rate (SR) evaluation. Hb_{mass} and exercise performance was re-assessed following each intervention. Hatched bars represent a minimum of 24 h between visits to the laboratory.

4.2.3 – Graded exercise test and cycling time trial

On arrival to the laboratory, participants provided a urine sample for measurement of urine specific gravity (USG; PAL-10S, ATAGO, Tokyo, Japan) and measured their nude body mass before self-inserting a rectal thermistor, placing on a HR monitor and dressing in cycling shorts, socks and cycling shoes. Skin temperature (T_{sk}) thermistors were then applied (iButton^{TM}, Maxim Integrated Products, Sunnyvale, CA, USA) and after a 10 min supine resting period, T_C and HR (T31, Polar, Kempele, Finland) were recorded. Participants then completed a graded exercise test on an electronically braked cycle ergometer (Lode, Excalibur Sport, Groningen, The Netherlands) in cool conditions (19.2 ± 1.9°C and 63 ± 10% relative humidity). The test consisted of 5 submaximal stages, each lasting 4 min, that began at 90 W and increased in 30 W step-increments. After the final submaximal stage, resistance increased by 1 W every 2 s until volitional exhaustion. Participants were instructed to maintain a steady cadence throughout. Breath-by-breath pulmonary gas exchange (Oxycon Pro, Jaeger, CareFusion,

Hoechberg, Germany) and HR were recorded continuously throughout. Submaximal values were averaged over the final minute of each stage, while $\dot{V}O_{2max}$ was defined as the highest minute average. The HR and power output associated with 65% $\dot{V}O_{2max}$ were calculated via linear regression. T_C and T_{sk} were measured during the final 30 s of each stage and at exhaustion. Ratings of perceived exertion (RPE; Borg, 1982) and thermal comfort (Bedford, 1936) were recorded immediately after the test.

Following completion of the graded exercise test, participants rested in the main laboratory for 30 min. Participants were provided with 5 $ml \cdot kg^{-1}$ of water that they were instructed to consume by the end of this period. Participants then entered an environmental chamber (TEMI 1000, Sanwood Environmental Chambers Co., Taiwan) set to 35°C and 60% relative humidity and completed 5 min of light pedalling on a cycle ergometer (Schoberer Rad Meßtechnik; SRM, Jülich, Germany). Participants were then instructed to complete a 30 min self-paced cycling time trial at the highest sustainable power output. Feedback was limited to time-remaining in the form of a digital stopwatch. No interaction occurred between participants and investigator except for provision of additional water which was consumed *ad libitum* throughout the trial. Power output and HR were measured continuously while T_C and T_{sk} were measured at the start and every 6 min of the time trial. RPE and thermal comfort was recorded immediately after the trial.

4.2.4 – Heat acclimation with controlled heart rate

Heat acclimation consisted of 90 min of cycling exercise for 10 consecutive days in 40°C and 40% relative humidity. Euhydration was confirmed via USG on arrival each day. Nude body mass was recorded before instrumentation with T_C and T_{sk} thermistors and a HR monitor. Resting T_C and HR were measured at the end of a 10 min supine resting period in the main laboratory. Each HA session involved an initial 15 min period of fixed intensity cycling at 65% $\dot{V}O_{2max}$ before work rate was adjusted automatically via computer software (Lode ergometry manager 9.0, Lode, Groningen, The Netherlands) to maintain an exercising HR corresponding to that intensity. The initial fixed

intensity period was chosen to sufficiently raise HR and promote the onset of sweating while preventing the initial increases in T_C being dampened by cardiovascular strain. A fan in front of the ergometer provided convective airflow at a constant windspeed of 3 $m \cdot s^{-1}$. Participants were instructed to maintain a steady cadence at a minimum of 80 $rev \cdot min^{-1}$ to minimise the conscious manipulation of HR and exercising power output. HR was recorded continuously throughout each session while T_C, T_{sk} and environmental conditions were recorded every 5 min. RPE and thermal comfort were recorded every 10 min.

A minimum of 24 h prior to the first exposure participants attended the laboratory and completed 60 min of exercise at 65% $\dot{V}O_{2max}$ in the heat (33°C and 50% relative humidity). Nude body mass was recorded before and after the exposure to determine sweat rate. On day one of each intervention either 90% (HA-EUH) or 10% (HA-DEH) of expected hourly sweat lost was provided in the form of a 0.1% electrolyte drink (HIGH5 ZERO, H5 Ltd, Bardon, UK). Fluid was divided into 6 equal aliquots that were provided at the onset of exercise and then every 15 min thereafter. After each exposure, participants towel dried and measured their nude body mass to determine sweat lost. Fluid volumes were adjusted in the subsequent exposure to match sweat rate, ensuring a similar end-exercise hydration status. Following HA-EUH sessions participants were permitted to drink *ad libitum*. In HA-DEH participants were also permitted to drink *ad libitum* but were provided with water equalling ~150% of their body mass deficit and encouraged to consume this within ~2-3 h. No other instructions were given for the control of posture or general light physical activity.

Resting thermal and haematological responses to each intervention were assessed over short-term (day 5) and medium-term (day 10) HA and compared to values measured on day 1. Prior to day 1 of the first HA intervention, participants were provided with a 24 h food diary and asked to record all food and fluid intake. To ensure adequate hydration, participants were advised to consume 5-7 $ml \cdot kg^{-1}$ of water and a light standardised meal 3 h prior to attending the laboratory. Participants were then instructed to

replicate the diary as best as possible on these days so that fluid and macronutrient intake was similar between days and interventions.

4.2.5 – Haematological analyses

Before each HA intervention haemoglobin mass (Hb_{mass}) was determined in duplicate using a modified version of the optimised carbon monoxide rebreathing technique (Schmidt & Prommer, 2005), with calculations based on values obtained from arterialised fingertip capillary blood. Measurements were made in quintuplicate (ABL 90FLEX, Radiometer, Copenhagen, Denmark) before and after rebreathing a standardised bolus (1.2 $ml \cdot kg^{-1}$) of 99.5% medical grade carbon monoxide. If pre-acclimation measurements of Hb_{mass} differed by >2%, i.e. the typical error reported in the literature (Gore et al., 2005), the test was repeated. RCV, BV and PV were calculated from subsequent venous blood samples using the equations outlined in Chapter 3. HA was not expected to alter Hb_{mass}. This assumption was tested by conducting a single measurement within 24 h of day 10 of each acclimation intervention.

Resting and exercise haematological responses to HA were measured via venepuncture of an antecubital vein (BD Eclipse, 21 g, BD, Utah, USA). Care was taken to ensure <60 s between application of a tourniquet and collection of 4 ml of blood into an EDTA coated vacutainer (BD Vacutainer, BD Diagnostics, Franklin Lakes, USA). Resting samples were collected after a 10 min supine resting period, while end-exercise samples were collected from a bed adjacent to the ergometer immediately after exercise. Samples were analysed in a Coulter counter (UniCel DxH 800 Coulter Analysis System, Beckman Coulter, CA, USA).

4.2.6 – Data analyses

Two-way ANOVA with repeated measures analyses were used to determine differences in resting and exercise responses to each HA intervention. Separate two-way ANOVA tests were conducted to analyse effects of HA interventions on exercise capacity in temperate conditions and exercise performance in the heat. Mauchly's test was used to test the assumption of Sphericity. In cases where this assumption was violated a Greenhouse-Geisser correction factor was applied. Bonferroni *post-hoc* testing was employed to determine where pairwise differences occurred. Wilcoxon signed rank test was used to analyse ordinal (RPE and thermal comfort) data. Separate t-tests were conducted between interventions for Tc and HR at rest and after 15 min of exercise on days 2 and 3 to determine if an order effect of HA interventions induced a rapid re-induction of these adaptations. All statistical analyses were conducted using SPSS (Version 21, IBM, Armonk, US). Results are reported as mean ± SD unless otherwise stated. Significance was set at $P < 0.05$ with a $P \leq 0.1$ considered a statistical trend. In such cases, effect sizes are presented using partial eta squared values for analysis of variance ($\eta_p^2 \leq 0.02$: small; 0.02-0.13: medium; 0.13-0.26: large; Cohen 1988).

4.3 – Results

4.3.1 – Acclimation intervention summary

A brief overview of each HA intervention is outlined in Table 4.1. The average ambient temperature (40.0 ± 0.3°C) and relative humidity (40.1 ± 1.6%) within the climatic chamber did not differ between days of acclimation (all $P > 0.05$). Power and HR targets were similar between HA interventions (both $P > 0.05$, Table 4.1). Prescribed fluid increased significantly throughout HA ($P = 0.03$), with 90 min intake increasing by 334 ± 316 ml and 39 ± 23 ml between day 1 and 10 in HA-EUH and HA-DEH, respectively. Fluid restriction resulted in significantly greater body mass changes in HA-DEH compared to HA-EUH ($P < 0.001$, Table 4.1). Body mass deficits within each HA intervention did not differ between days ($P = 0.22$).

Table 4.1: Summary of euhydrated and dehydrated heat acclimation interventions.

	HA-EUH	HA-DEH
Average temperature (°C)	40.0 ± 0.5	40.0 ± 0.4
Average relative humidity (%)	40.0 ± 1.9	40.2 ± 1.2
Target power output (W)	171 ± 20	173 ± 22
Target HR (beats·min^{-1})	146 ± 7	145 ± 7
Average fluid prescribed (L)	2.05 ± 0.33	0.23 ± 0.04*
Average body mass deficit (%)	-0.60 ± 0.26	-2.85 ± 0.48*

*Significantly different from HA-EUH

4.3.2 – Resting thermal, cardiovascular and haematological responses to HA with controlled HR

USG measurements indicated participants arrived at the laboratory in a euhydrated state each day and resting body masses were similar between days within each intervention (all $P > 0.05$, Table 4.2). Neither intervention altered resting T_C or HR (both $P > 0.05$). Additional comparisons between the first and second intervention completed indicated successful wash-out and no prior effect of HA on these parameters (all $P > 0.05$). One participant had a 59 g (-7%) decrease in Hb_{mass} in HA-EUH, that did not occur (0 g) in HA-DEH.

Therefore, this value was excluded, and their pre-acclimation value was used in the analysis of HA effects on Hb_{mass}. Hb_{mass} tended to decrease with HA (P = 0.07, η_p^2 = 0.39) and a significant interaction between hydration and HA day was also observed (P = 0.03). Pairwise analyses identified a slight 11 g (1.3 ± 1.4%) decrease in Hb_{mass} with HA-EUH (P = 0.04). Although minimal, this decrease was more than twice as a large as the coefficient of variation observed between the two intra-individual pre-acclimation measurements (0.6%). Therefore, calculated RCV, PV and BV for day 10 of HA-EUH were made using the post-acclimation Hb_{mass}. Calculations for all other days were made using pre-acclimation measurements. Resting thermal, cardiovascular and haematological responses to acclimation are presented in Table 4.2. There was a significant effect of HA day on PV and BV (both P < 0.05). However, pairwise analyses did not identify any significant differences between days (all P > 0.05). Similarly, no differences were observed in relative changes in resting PV or BV compared to day 1 of HA (Table 4.2). RCV did not change with HA (P = 0.47).

Table 4.2: Resting responses to heat acclimation with controlled HR

	Day 1		Day 5		Day 10	
	EUH	**DEH**	**EUH**	**DEH**	**EUH**	**DEH**
USG	1.016 ± 0.01	1.016 ± 0.01	1.013 ± 0.01	1.019 ± 0.00	1.016 ± 0.01	1.016 ± 0.00
BM (kg)	75.3 ± 4.9	76.1 ± 4.8*	75.1 ± 5.0	75.8 ± 4.5	74.8 ± 4.9	75.4 ± 4.4
T_C (°C)	37.0 ± 0.2	37.0 ± 0.3	37.0 ± 0.3	36.9 ± 0.3	36.9 ± 0.2	36.8 ± 0.4
HR (beats·min^{-1})	57 ± 8	57 ± 7	54 ± 5	56 ± 8	55 ± 6	53 ± 7
RCV (ml)	2585 ± 216	2583 ± 201	2608 ± 225	2579 ± 204	2561 ± 208	2590 ± 211
PV (ml)	3582 ± 290	3518 ± 306	3655 ± 271	3737 ± 221	3679 ± 181	3570 ± 222
BV (ml)	6168 ± 396	6100 ± 474	6263 ± 403	6315 ± 327	6240 ± 301	6160 ± 334
ΔPV (ml vs. Day 1)	-	-	73 ± 270	220 ± 299	97 ± 248	53 ± 304
ΔBV (ml vs. Day 1)	-	-	96 ± 262	215 ± 313	73 ± 250	60 ± 302

*Significantly different from HA-EUH

4.3.3 – Exercising HA responses

Similar HR and T_C responses on days two and three were observed between the first and second intervention completed (all $P > 0.05$), suggesting sufficient wash-out between interventions and there was no order effect on re-induction of these parameters. The relative decreases in PV and BV with exercise and fluid restriction in HA-DEH did not differ between days 1, 5 or 10 of HA (both $P > 0.05$), averaging 14.0 ± 3.2% and 8.1 ± 1.9%, respectively. In contrast and as expected, significantly smaller reductions occurred within HA-EUH sessions, averaging 7.7 ± 3.8% and 4.4 ± 2.3%, respectively (both $P < 0.05$). The power output, T_C and T_{sk} responses to exercise with controlled HR on days 1, 5 and 10 of each HA intervention are displayed in Figure 4.2. Neither acclimation intervention lowered the initial T_C response to 15 min fixed intensity cycling (all $P > 0.05$). In contrast, HR after 15 min of exercise was significantly lowered by acclimation ($P < 0.001$). In HA-EUH, 15 min HR was similar between day 1 and 5 ($P = 0.13$) before becoming 11 ± 8 beats·min^{-1} lower on day 10 compared to day 1 ($P = 0.02$, Figure 4.2). In HA-DEH, a significant 7 ± 6 beats·min^{-1} decrease was observed by day 5 ($P = 0.04$) and did not decrease further throughout the intervention ($P = 0.33$). For the proceeding 75 min, HR was successfully controlled via changes in power output. Exercising HR over this period did not differ between days of acclimation ($P = 0.72$, Figure 4.2), averaging 147 ± 6 and 146 ± 7 in HA-EUH and HA-DEH, respectively. T_C averaged 38.4 ± 0.2°C over the final 75 min of exercise ($P = 0.49$) and did not differ between days or interventions ($P = 0.90$, Figure 4.2). T_{sk} decreased by 0.63 ± 0.50°C between days 1 and 10 in HA-EUH ($P = 0.027$) and was accompanied by tendency for increased sweating rate (0.19 ± 0.18 L·h^{-1}, $P = 0.06$, $\eta_p^2 = 0.46$). Neither T_{sk} (0.35 ± 0.54°C, $P = 0.3$) nor sweating rate (0.15 ± 0.16 L·h^{-1}, $P = 0.12$) changed significantly with HA-DEH.

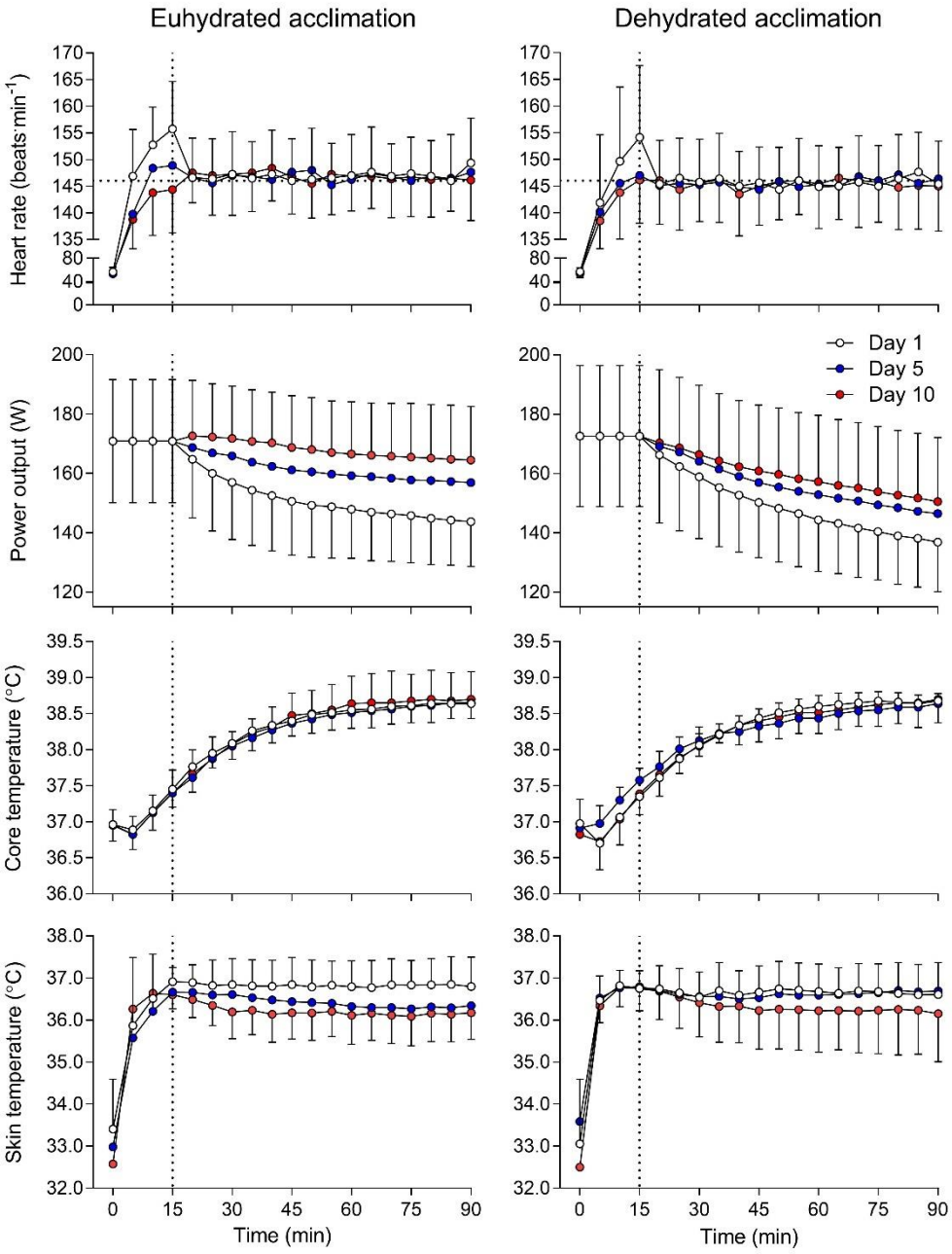


Figure 4.2: T_C and T_{sk} responses to euhydrated and dehydrated HA with controlled HR via alterations in power output. Vertical dotted lines show point HR control began. Horizontal dotted lines show target HR of each respective intervention. Between day differences omitted for clarity. See text for details.

By design, HR was maintained by progressive decreases in power output. The reductions in power output necessary to maintain HR were greater in HA-DEH than HA-EUH throughout HA ($P < 0.05$, Figure 4.3). Average power during HR controlled exercise was greater during HA-EUH than HA-DEH and was 139 ± 15 and 130 ± 16 W on day 1, respectively (both $P < 0.05$). With acclimation, the +25 ± 10 W increase in average power for the same HR between days 1

and 10 of HA-EUH was greater than the +16 ± 18 W increase in HA-DEH (P = 0.007). Perceptual responses to exercise remained unchanged between days of acclimation and did not differ between interventions (both P > 0.05), with RPE and thermal comfort during controlled HR exercise averaging 12.3 ± 1.4 and 5.3 ± 0.7 units, respectively, indicating the exercise and environmental conditions were not too hard or warm.

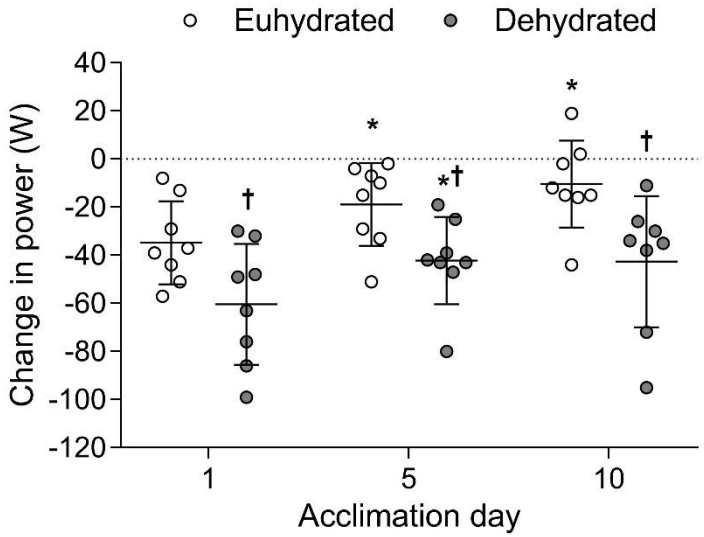


Figure 4.3: Difference between initial 15 min power output and average power during the final 5 min of HR controlled exercise on days 1, 5 and 10 of HA. Open circles are individual euhydrated responses. Closed circles are dehydrated responses. Lines are mean ± SD * Significantly different from day 1. † Significantly greater than HA-EUH.

4.3.4 – Maximal aerobic capacity and self-paced exercise performance with acclimation

Thermal, HR, ventilatory and metabolic responses to graded submaximal exercise in cool ambient conditions were similar prior to each HA intervention and were unaltered by either protocol (all P > 0.05). There was a main effect of acclimation on $\dot{V}O_{2max}$ (P = 0.017, η_p^2 = 0.58), however pairwise analyses only identified a tendency for slight ~0.16 L·min^{-1} increases with HA-EUH and HA-DEH (both P = 0.08, Figure 4.4).

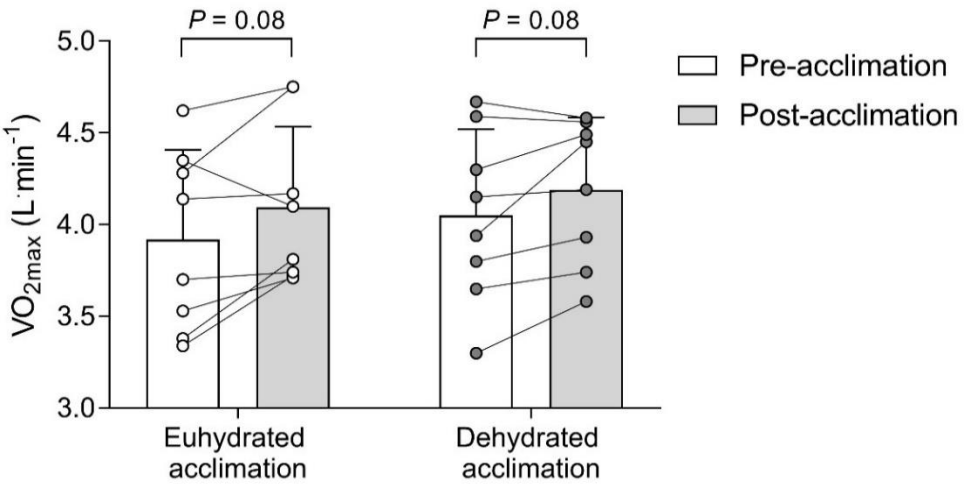


Figure 4.4: $\dot{V}O_{2max}$ in cool ambient conditions before and after euhydrated and dehydrated HA with controlled HR. Circles and lines represent individual responses, clear and shaded bars are pre- and post-HA means, respectively.

Time trial power output in the heat was similar prior to each HA intervention, averaging 231 ± 33 and 235 ± 32 W before HA-EUH and HA-DEH, respectively (P = 0.62). Average power output significantly increased by 19 ± 16 W following HA-EUH (P = 0.012) but was not significantly improved following HA-DEH (13 ± 26 W, P = 0.21, Figure 4.5). This performance improvement was due to a consistently greater power output over the 30 min period as no differences were observed in pacing strategy and was associated with similar HR, T_C and T_{sk} responses pre- and post-acclimation (all P > 0.05, Figure 4.6).

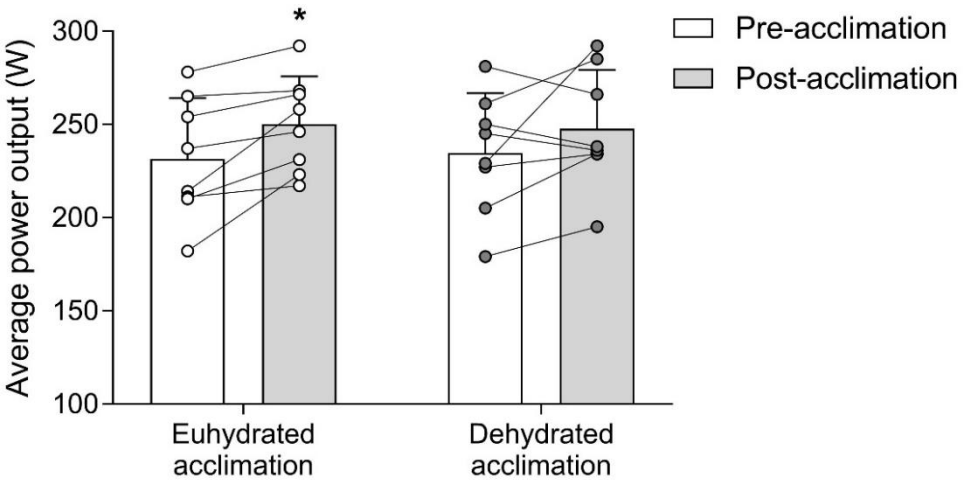


Figure 4.5: Individual average 30 min power outputs during self-paced cycling time trial exercise with euhydrated and dehydrated HA. White and shaded bars are pre- and post-HA means. * Significantly different from pre-acclimation.

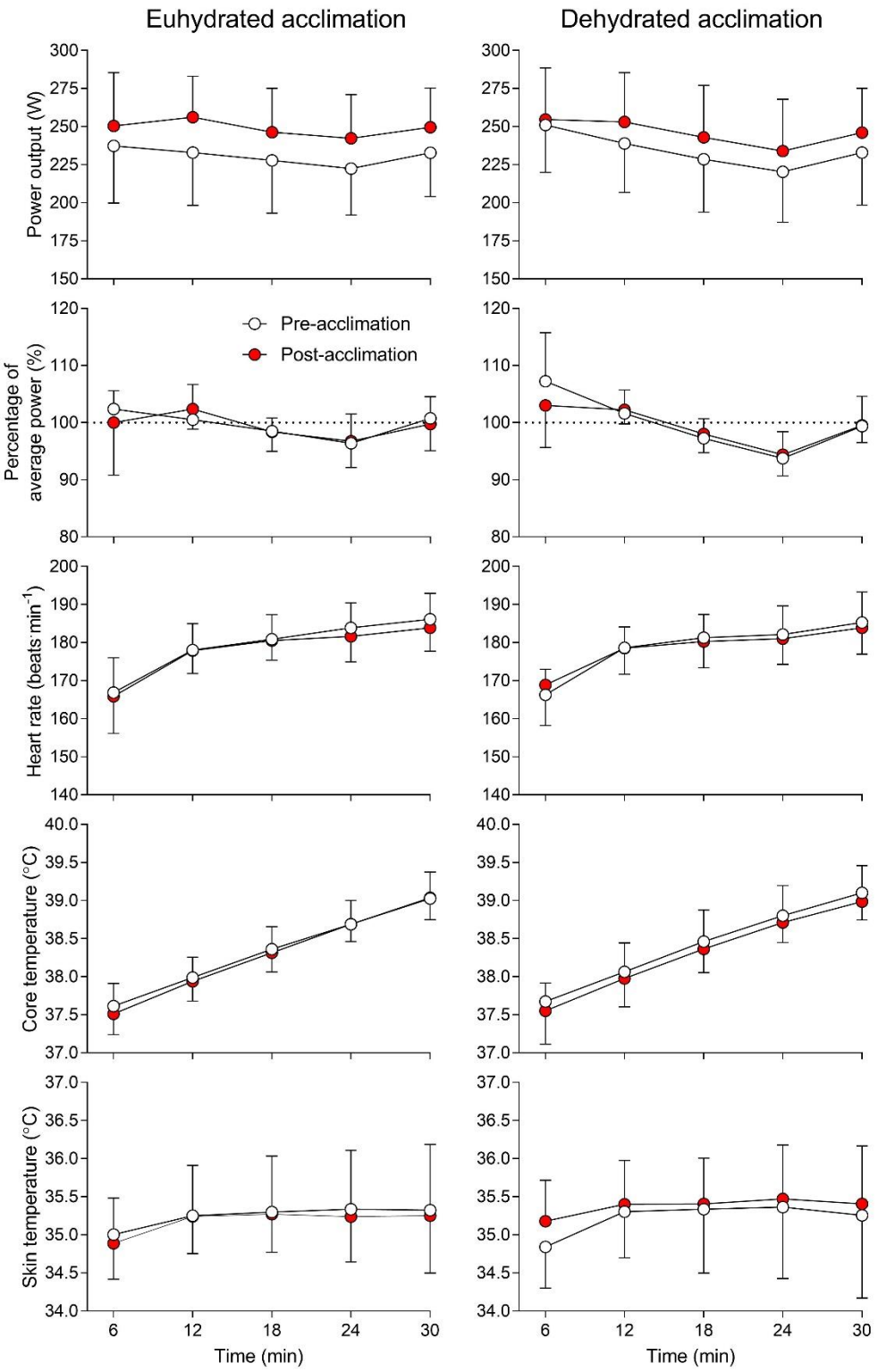


Figure 4.6: Power output, HR, T_C and T_{sk} responses during a 30 min cycling time trial before (open) and after (red) controlled HR heat acclimation with and without dehydration.

4.4 – Discussion

This study sought to characterise the adaptive responses to exercise HA with controlled HR and whether hydration status altered the time course and magnitude of responses observed. Secondly, this study determined the potential ergogenic effects of HA with controlled HR on maximal aerobic capacity in cool ambient conditions and self-paced exercise in humid heat. The main findings of the present investigation are that 1) across 10 days of exercise HA with controlled HR both the HR response to initial fixed-intensity exercise and average exercise T_{sk} were significantly lowered, while sweat rate and power output for the same HR were increased. However, 2) adaptations were more pronounced when euhydration was maintained throughout each exposure, as dehydrated HA did not significantly increase sweat rate, reduce exercising T_{sk} or alter 30 min self-paced exercise performance in the heat. Furthermore, 3) neither HA intervention had a significant effect on resting T_C, HR, PV or BV. Finally, 4) whilst there was a trend for an increase in $\dot{V}O_{2max}$ with HA, neither euhydrated or dehydrated heat training with controlled HR independently resulted in notable effects on maximal aerobic capacity in cool conditions and were not associated with altered thermoregulatory, ventilatory or metabolic responses to maximal incremental exercise.

Considering these main findings, the hypotheses that both interventions elicited similar responses to HA and led to significant improvements in self-paced exercise performance are rejected. However, as anticipated, neither intervention led to significant increases in $\dot{V}O_{2max}$. and this hypothesis is therefore accepted.

4.4.1 – Adaptations to controlled heart rate exercise heat acclimation

The possible efficacy of controlled HR acclimation is a recent suggestion (Periard et al., 2015; Periard et al., 2016) and the known responses to this method of acclimation are limited (Keiser et al., 2015; Pethick et al., 2018; Philp et al., 2017). The present study observed several adaptive responses that would be considered typical of HA (Periard et al., 2015; Tyler et al., 2016).

The average overall responses throughout both HA interventions were a lowering of fixed-intensity exercise HR (~10 beats·min^{-1}) and 90 min average exercise T_{sk} (~0.5°C), while there were increases in sweating rate (~0.17 L·h^{-1}) and the power output required to maintain 75 min exercising HR (~21 W). It is reasonable to assume these responses are adaptations to repeated exercise heat stress despite participants not completing a third control training intervention. Prior to each intervention self-paced exercise performances in the heat were similar and the cardiovascular and thermoregulatory responses to day 1 of acclimation did not differ between interventions. Therefore, it is likely that the observed intra-individual responses to daily HR controlled exercise in the heat are the result of the development of HA.

Previous studies investigating HA with controlled HR have reported conflicting responses. Most recently Philp et al. (2017) conducted 5-days of HA consisting of 50 min exposures at 70% of HR reserve in both hot-humid heat and cool ambient conditions. They observed both interventions resulted in similar reductions in exercising HR and intermittent running performance (Philp et al., 2017). The lack of a clear adaptation is perhaps not surprising considering exposure durations were below the recommended 60 min minimum (Tyler et al., 2016), resulted in low average internal heat loads (~37.6°C) and intervention responses were determined in cool conditions (Philp et al., 2017). Keiser et al. (2015) however, found several significant adaptive responses to euhydrated HA with controlled HR compared to the same training conducted in control conditions that are somewhat at odds with the present findings. The authors reported a significant and consistent 6% (~200 ml) expansion of resting PV, a 26% increase in sweat output and reduction of sweat sodium concentration, as well as an increase in exercise performance in the heat (Keiser et al., 2015). In the present investigation, there was no effect of either intervention on resting PV or BV, and a smaller (~200 ml·h^{-1}, 13%) change in sweat rate. It is unlikely that these differences are due to HA protocols considering the similarities in intervention period (10 days), durations of exposure (90 min) and environmental conditions used (hot-dry heat). Furthermore, it is also unclear whether differences in fitness status between the volunteers in the study of Keiser et al. (2015) and those in the

present study (~63 *vs.* 53 $ml \cdot kg^{-1} \cdot min^{-1}$) may explain the discrepancies between the increases in sweating rate and lack of PV expansion. For example, while $\dot{V}O_{2max}$ is positively associated with the lowering of exercising T_C and HR (Pandolf, Burse, & Goldman, 1977), trained, untrained and unfit individuals all exhibit similar increases in sweat rate with HA (Shvartz et al., 1977). Conversely, fitter individuals exhibit larger whole body and local sweat rates than those who are less fit during exercise at the same relative intensity (Jay, Bain, Deren, Sacheli, & Cramer, 2011). It is possible volunteers in the study of Keiser et al. (2015) could have had larger increases in absolute power output for the same HR as HA progressed. However, the relative intensity prescribed was lower (50% $\dot{V}O_{2max}$ HR) and the absolute workloads and increases in T_{sk} and body temperature throughout HA were not reported (Keiser et al., 2015). Regardless, a clear effect of HA was observed since the same training conducted in control conditions did not alter peak power, maximal aerobic capacity or self-paced exercise in a cool environment. Furthermore, the time trial performance improvement in the heat following acclimation (~10%; Keiser et al., 2015) was similar to that observed in the present euhydrated intervention (~9%). Therefore, the reasons for these differences in adaptive responses are not entirely clear and cannot be fully elucidated with the limited data available regarding this type of HA intervention. In the light of similar between-day resting BV and PV and T_C and T_{sk} responses, a possible mechanism determining the lowering of fixed-intensity exercise HR across HA was not directly determined. A reduction in deep body tissue temperatures, alterations in venous tone and blood flow distribution, increased sympathetic nervous activity and altered cardiac filling have all been suggested to be altered by HA (Periard et al., 2016) and may explain the reductions in exercising HR seen in the current study.

The present study did not compare the effect of controlled HR acclimation against another intervention designed to induce heat adaptation. Therefore, the reported responses herein remain relatively limited to the intra-individual changes throughout each 10-day period. However, the present data suggest some comparisons of the adaptive responses may be made to those reported following controlled hyperthermia HA due to similarities in daily cardiovascular

and endogenous thermal loads observed here and in other investigations, independently of hydration status (Garrett et al., 2012; Garrett et al., 2014; Neal et al., 2016b; Patterson et al., 2004a).

4.4.2 – Influence of hydration status on adaptation

Dehydration has previously been proposed to facilitate adaptation (Taylor & Cotter, 2006; Taylor, 2014) as it results in an increased fluid regulatory response to exercise (Kenefick et al., 2007). Previously, controlled hyperthermia with permissive dehydration has been shown to enhance electrolyte retention and expand PV (Patterson et al., 2004b). However, few investigations have directly assessed the influence of hydration status on the development of HA (Garrett et al., 2014; Neal et al., 2016b) and the proposed benefit is unclear (Akerman, Tipton, Minson, & Cotter, 2016). Garrett et al. (2014) reported significantly lower exercising HR and a greater relative increase in PV with dehydration compared to euhydrated HA. A more recent study, however, observed similar time courses and magnitudes of adaptations regardless of hydration strategy (Neal et al., 2016b). In the present investigation fixed-intensity exercising HR was decreased by both interventions but the increase in sweat rate and significant decrease in average T_{sk} that occurred with euhydrated HA was not observed during dehydrated HA. Furthermore, neither intervention independently altered resting BV or PV. The differences observed between the current study and findings of others using dehydrated HA (Garrett et al., 2014; Neal et al., 2016a; Neal et al., 2016b; Patterson et al., 2004a, 2004b) may partly be methodological. The studies mentioned above reported improvements during a standardised heat response test, which was not conducted in the present investigation. Instead, the thermoregulatory responses reported here are from between-day measurements with different levels of metabolic heat production and progressive dehydration. Acutely, dehydration results in the development of hyperosmotic hypovolemia (Sawka et al., 1992) that may lower skin blood flow and sweat output and increase fluid regulatory, thermal and cardiovascular strain (Kenefick et al., 2007; Sawka et al., 1992). In this regard a clear effect of acute dehydration was still evident on day 10 of HA given the

significantly lower sweat rate and metabolic heat production for the same T_C and HR compared to euhydrated HA. Furthermore, a lower sweat rate also likely contributed to the observation of similar T_{sk} between days with dehydration, but a significant decrease with euhydrated HA. Changes in sweat rate may also take longer to adapt as increases appear to be larger the more exposures are repeated (Tyler et al., 2016). However, although short-term HA has a small (~5%) effect on sweat rate (Tyler et al., 2016), medium-term HA interventions using a similar number and durations of exposures have reported between similar and larger changes than those observed in the current euhydrated HA intervention (~10-30%; Regan et al., 1996; Magalhães et al., 2006; Kirby & Convertino, 1986; Patterson et al., 2004a; Neal et al., 2016). Whether greater differences would be observed during a standardised heat response test or following a longer intervention is currently unknown. There was a main effect of HA day on BV and PV but no absolute or relative differences between days within either intervention was identified (Table 4.2). The reason for this lack of an effect is unclear. PV is typically expanded ~4% by HA (Tyler et al., 2016) and dehydrated controlled hyperthermia HA interventions have reported large and sustained increases between 5 and 10% (Garrett et al., 2014; Neal et al., 2016b; Patterson et al., 2004a, 2004b), although this is not always observed (Garrett, Goosens, Rehrer, Patterson, & Cotter, 2009; Neal et al., 2016a). By design, dehydrated HA routinely resulted in similar daily body mass deficits ~2.8%, surpassing the 2% change expected to stimulate a fluid regulatory response (Cheuvront & Kenefick, 2014; Cheuvront, Kenefick, Montain, & Sawka, 2010). Other investigators have fixed fluid intakes during exposures, resulting in progressively greater daily levels of body water deficit as sweat losses increase with acclimation (Garrett et al., 2014; Neal et al., 2016b; Patterson et al., 2004a). Progressively greater body mass deficits may be necessary throughout HA to maintain physiological strain (Patterson et al., 2014). An additional consideration is that participants began each day of HA in a euhydrated state. Therefore, given the average sweat rate throughout HA, participants would have only been expected to have a body mass deficit beyond 2% of baseline during the latter ~15 minutes of exercise. It is unclear whether this level of dehydrated stimulus is sufficient at eliciting an enhanced response compared to exercise with maintained

euhdyration. In line with this, Neal et al. (2016b) induced body mass deficits between 2.3 to 3.1% over 10 days of acclimation. Although they observed slight increases in resting plasma osmolality with dehydrated acclimation, this was not greater than the 2% that may be necessary to stimulate renal water conservation (Cheuvront & Kenefick, 2014). Plasma osmolality was not directly measured in the current study, but this may explain in part the lack of a change in PV in the present study and the similar hypervolemic responses between euhydrated and dehydrated HA in the study of Neal and colleagues (2016b). Future studies may wish to investigate whether starting daily exercising heat exposures hypohydrated would alter the responses observed here and elsewhere.

It is also possible that a change in RCV with acclimation (Garrett et al., 2014) may potentially mask reported relative changes in BV and PV that are traditionally calculated from haematological parameters (Dill & Costill, 1974). In the current study however, Hb_{mass} and RCV were measured directly and were similar across both HA interventions. Despite this, large differences in inter-individual responses between days were still evident, with PV changes ranging from -13 to +13% between participants. It is possible that an increased duration of tourniquet application might artificially concentrate venous blood (Lippi, Salvagno, Solero, & Guidi, 2006). However, a standardised approach was followed to the control of resting posture and sampling technique. Moreover, considering the small repeated measure variabilities of the current methods (0.6%, 0.1 $g \cdot dL^{-1}$ and 0.31% for Hb_{mass}, haemoglobin concentration and haematocrit, respectively) it does not seem likely that measurement error has masked a potential hypervolemic response to the present HA interventions. Acute short-term PV expansion following exercise is also affected by posture (Nagashima, Mack, Haskell, Nishiyashu, & Nadel, 1999) and the timing of fluid intake and beverage composition (Kamijo et al., 2012; Okazaki et al., 2009). Post-exercise general light activity, posture and *ad libitum* fluid intake were not directly controlled in this study. However, it is unlikely that participants did not adequately replace post-exercise deficits in body water as USG measures and stable body masses indicated participants returned the laboratory euhydrated each day. Therefore, based on the findings

of the current investigation neither HA intervention significantly increased BV nor PV of participants and the reasons for this are presently unclear.

4.4.3 – Effect of acclimation on maximal aerobic capacity in cool conditions

In the present study there was a small main effect of HA on $\dot{V}O_{2max}$ (~0.16 $L \cdot min^{-1}$). However, as hypothesised, neither intervention individually resulted in a significant effect on $\dot{V}O_{2max}$ in a temperate environment. The premise that HA is ergogenic for exercise performance in cool conditions has recently been debated (Minson & Cotter, 2016; Nybo & Lundby, 2016). Many of the typical thermal and non-thermal adaptations to HA might translate to improved exercise performance in cool conditions (Corbett et al., 2014), although there is very little direct experimental evidence. In the case of this study however, the lack of a change in $\dot{V}O_{2max}$ is supported by similarities in resting and exercising cardiovascular, thermoregulatory, ventilatory and metabolic responses in cool conditions. For instance, an increase in RCV and/or PV with HA have been proposed to increase oxygen delivery and/or maximal cardiac output (Lorenzo et al., 2010; Scoon et al., 2007). There were no observed differences in resting RCV or PV (Table 4.2), while HR, $\dot{V}O_2$, T_C and T_{sk} responses to incremental exercise at ~20°C were similar in all trials. Given these similarities, it is does not seem likely either intervention altered oxygen carrying capacity or delivery to the locomotor muscles. Furthermore, the significant overall main effect of HA on $\dot{V}O_{2max}$ (4%, ~0.16 $L \cdot min^{-1}$) was similar to the differences between pre-acclimation values (3.6%, ~0.13 $L \cdot min^{-1}$; Figure 4.4). Further post hoc analysis also identified that given the number of participants, observations and main effect size of HA on $\dot{V}O_{2max}$, the experiment was sufficiently powered (actual power observed = 0.92). Together, this suggests that this effect is not meaningfully larger than the intra-individual between-day responses to maximal incremental exercise in these individuals.

Sub maximally, there were also similar minute ventilation and respiratory exchange ratio values between trials. Despite the possibility for HA to

decrease substrate metabolism and increase lactate threshold and exercise economy (Corbett et al., 2014; Lorenzo et al., 2010; Neal et al., 2016a), it appears neither intervention altered these parameters either, at least at the submaximal workloads assessed. Instead, our findings agree with others (Houmard et al., 1990; Karlsen et al., 2015; Keiser et al., 2015), that HA does not alter maximal incremental exercise performance in cool conditions.

4.4.4 – Self-paced exercise performance following acclimation

Euhydrated HA with controlled HR significantly improved 30 min self-paced cycling performance in hot humid conditions. Average power produced for 30 min was 19 W greater (9%) following euhydrated HA, with all participants observing an increase in performance. These findings are typical of the performance effects of HA on a closed-loop self-regulated task, with others observing improvements in cycling time-trials between 8 and 15% (Garrett et al., 2012; Karlsen et al., 2015; Keiser et al., 2015; Lorenzo et al., 2010). However, dehydrated HA did not reliably alter performance in the heat (~6%, 13 W, P = 0.21) with 3 of the 8 participants having performance decreases between 9 and 15 W (Figure 4.5). These findings support the notion for limited adaptive responses to dehydrated HA with controlled HR that were observed between days 1 and 10. Conversely, euhydrated HA resulted in adaptations that specifically facilitated improved performance in hot-humid conditions. There are several potential reasons for these conclusions. Firstly, pre-acclimation $\dot{V}O_{2max}$ and time-trial performances were similar between interventions. Secondly, $\dot{V}O_{2max}$ was not increased significantly with either acclimation intervention. Finally, the pacing profiles, thermoregulatory and cardiovascular responses to exercise did not differ between trials. Self-paced exercise in the heat tends to be conducted at a stable relative exercise intensity (Periard & Racinais, 2015), and decrements in power output occur over time with the development of cardiovascular strain and hyperthermia (Periard et al., 2011). Although oxygen uptake was not measured in this study, given the similar thermal and cardiovascular responses to an increased average power production with HA, it is likely euhydrated HA facilitated improved heat exchange between the body and environment. Conversely,

since the same participants did not show increases in sweat rate and decreases in T_{sk} throughout dehydrated HA, it is not reasonable to expect a significant increase in metabolic power production for similar thermoregulatory responses. It is possible that the differences between interventions are due to greater work done in euhydrated compared to dehydrated HA periods (i.e. a training effect). The HA interventions were intended to match the relative cardiovascular stimulus during exercise heat stress (Periard et al., 2015) with euhydration and daily progressive dehydration. Keiser et al. (2015) did not show an effect of HR controlled exercise in cool conditions on temperate or hot exercise performance. Despite neither intervention altering $\dot{V}O_{2max}$, due to the lack of a control training arm and large differences in average power output between interventions in the present study it is not possible to completely dismiss the possibility altered training stimuli for differences in exercise performance in hot-humid environments. Future studies should seek to determine the effectiveness of interventions against appropriate work-matched controls (Corbett et al., 2014).

4.4.4 – Limitations

Neither of the HA interventions carried out in the present study resulted in an alteration of resting T_C or HR. Both adaptations might be considered typical hallmarks of the heat acclimated phenotype. Medium-term interventions tend to decrease resting T_C by 0.17°C, regardless of measurement site (Tyler et al., 2016) and although small, this change in resting T_C is likely physiologically meaningful as it may reflect a shift in the thermal control of effector responses that occur at a lower absolute T_C (Gisolfi & Wenger, 1984). The reason T_C was not reduced in the current interventions is unclear. The average change we observed might be typical of the expected decrease in T_C, however there was significant variation between participants (Table 2). The similarities in resting HR is less surprising considering the stable resting BV and T_C throughout acclimation. Care was taken to ensure each participant had designated re-usable rectal thermistors and measurements were conducted at a similar time of day after a 10 min stabilisation period to minimise any confounding effects of circadian rhythm and between-thermistor and -day variability. There is

however the possibility that some subjects may have been partially heat acclimatised. All participants were residents of Qatar, where monthly average highest temperatures range from 22-42°C (Climate & Weather Averages in Doha, 2018) and three participants underwent experimentation in the summer months. Residents to such climates exhibit seasonal fluctuations in resting T_C (Buguet et al., 1988). While participants limited weekly outdoor daylight exercise exposures to once per week for a minimum of 3 weeks prior to each HA intervention, the time-course of decay in resting T_C from acclimation/acclimatisation is not currently clear (Daanen, Racinais, & Periard, 2018). However, given the pre-acclimation determination of sweat rate, self-paced performance trial and additional experimentation (see Chapters 5 and 6), day-1 of each intervention was not a true 'first' heat exposure for un-acclimated participants. It is possible some acclimation could have occurred prior to HA periods being undertaken, particularly as it seems medium-term acclimation does not appear to additively alter resting T_C beyond short-term HA (Tyler et al., 2016). Despite this, as discussed above, given the similar baseline exercise performances as well as the similarities in thermal and cardiovascular responses to the first HA exposures it seems likely that daily exposures induced some heat adaptation beyond seasonal climate changes.

It is acknowledged that long term residence to a hot climate may have affected the magnitude of responses seen here. Whether the present HA interventions would illicit larger adaptive responses in long-term residents of cooler climates is unknown, however much of the data within the HA literature has been gathered from Caucasian residents of temperate western climates. Limited data suggests that, aside from a possible tendency for morphological differences, there does not appear to be a genetic basis for altered thermoregulatory responses between tropical and non-tropical natives (Taylor, 2006). Instead, it is likely that these differences are due to the level of phenotypic adaptation induced by unique environmental stressors across climates (Taylor, 2014; Taylor, 2006). In addition, tropical natives have been shown to exhibit enhancements in sweat rate to medium-term HA (Magalhães et al., 2010). Together, this suggests that given sufficient thermal stimulus (i.e.

exercise under conditions of elevated ambient and skin temperatures) some adaptations still occur in these populations (Saat et al., 2005; Taylor, 2014). However, given no control arm was conducted, it remains unclear if basal acclimatisation status altered the present results and is an accepted limitation.

4.4.5 – Conclusions

Both euhydrated and dehydrated exercise at a controlled HR in the heat results in elevated and sustained T_C and facilitates several adaptive responses that are typical of HA. Therefore, such an intervention may be easily implemented by athletes as a means of safely regulating exercising intensity during heat exposures. The adaptive potential of this type of intervention compared to another method, remains to be determined. Furthermore, hydration status appears to slightly alter the time-course and magnitude of responses to these interventions. Maintaining euhydration during exposure enhanced reductions in exercising HR and T_{sk} throughout HA and improved 30-min self-paced exercise performance in a hot-humid environment. Conversely, dehydration consistently impairs the exercising component of HR controlled HA and limited adaptation beyond 5 days of acclimation. Whether similar differences would be observed in a standardised heat response test is presently unknown. Finally, neither euhydrated nor dehydrated HA with controlled HR significantly altered the thermal, cardiovascular, ventilatory or metabolic responses to submaximal and maximal exercise in cool conditions.

CHAPTER 5

Study 2: Effects of heat acclimation with controlled heart rate on thermal, haematological and haemodynamic responses at rest and during prolonged exercise in the heat with altered hydration

5.0 – Abstract

A common adaptation to heat acclimation (HA) is a lowered core temperature and heart rate during euhydrated exercise heat stress at submaximal workloads. However, the acute thermal, haematological and haemodynamic responses to exercise with and without progressive dehydration in acclimated humans is not fully characterised or understood. In this study, 8 males performed two trials of prolonged submaximal exercise in the heat (33°C and 50% relative humidity) while maintaining euhydration or becoming progressively dehydrated. Each trial consisted of three 20 min bouts of semi-recumbent cycling (50% $\dot{V}O_{2max}$) interspersed by 60 min periods of upright cycling (65% $\dot{V}O_{2max}$). Core and skin temperature, blood volume, mean arterial pressure and left ventricular volumes were measured during each bout of semi-recumbent cycling. Trials were repeated following 10 days euhydrated exercise HA with controlled heart rate in a 40°C and 40% relative humidity environment. Fluid intake was adjusted to match hydration status between pre- and post-HA experiments. HA did not alter resting core temperature, heart rate, blood, red cell or plasma volume (all $P > 0.05$), but slightly increased stroke volume ~5 ml ($P = 0.005$). Cardiac output was maintained with euhydration pre-HA ($P = 0.06$) and increased between bouts following HA ($P < 0.001$). Progressive dehydration resulted in similar body mass deficits at 80 min (1.8 ± 0.4%) and 160 min (3.6 ± 0.7%) in both trials ($P > 0.05$). Dehydration pre-HA resulted in significantly greater core temperature (0.6°C) and heart rate (11 beats·min^{-1}) compared to euhydration ($P < 0.05$), while blood volume (5.4%), cardiac output (1.14 L·min^{-1}) and mean arterial pressure (9 mmHg) declined over time ($P < 0.05$). HA did not alter these responses ($P > 0.05$). These findings suggest euhydrated HA slightly improves the haemodynamic responses to prolonged euhydrated exercise. However, HA does not prevent the deleterious effects of dehydration on cardiovascular function and core temperature regulation.

5.1 – Introduction

Heat acclimation (i.e. the repeated exposure to artificially hot environments; HA) is considered the most effective strategy to minimise impairments in exercise performance in the heat (Cohen & Gisolfi, 1982; Racinais et al., 2015a). HA lowers resting core temperature (T_C) and heart rate (HR) with similar responses observed during constant workload submaximal exercise in the heat (Periard et al., 2015; Taylor, 2014). However, the reported central haemodynamic responses to exercise heat stress following HA vary (Tyler et al., 2016). Cardiac output ($\dot{Q}$) has been shown to decrease (Wyndham, 1951), be maintained (Nielsen et al., 1997; Rowell et al., 1967) or increase (Nielsen et al., 1993) in acclimated individuals compared to pre-acclimation responses. The reasons for these differences are unclear and are suggested to be due to variations in exercise modality, environmental conditions, subject population and method of HA (Periard et al., 2015; Taylor, 2014). Previous studies using daily constant workload exercise to exhaustion in the heat have lowered exercising T_C and HR, increasing $\dot{Q}$ in dry (Nielsen et al., 1993; Rowell et al., 1967) but not humid heat (Nielsen et al., 1997). The responses to other methods of HA are less well characterised. For example, euhydrated HA with controlled HR has been shown to improve self-paced exercise performance in the heat (Chapter 4). However, the thermoregulatory and haemodynamic responses to prolonged submaximal exercise following such a HA intervention are unknown. Therefore, the primary aim of the present investigation was to assess the effects of exercising HA with controlled HR on thermoregulatory and cardiovascular function.

HA also results in an increase in the sensitivity and rate of sweating, lowering skin temperature (T_{sk}) and blood flow during exercise in environments that allow evaporative heat exchange (Fox et al., 1964; Tyler et al., 2016; Wyndham, 1967). Together these adaptations are associated with improved cardiovascular stability during exercise in the heat. However, profuse sweating may result in progressive dehydration during prolonged exercise if fluid replacement is inadequate. The development of dehydration during exercise under heat stress is associated with concomitant hypovolemia and

hyperthermia. Together, reductions in blood volume (BV) and elevated body temperature contribute to significant reductions in mean arterial pressure (MAP), stroke volume (SV) and $\dot{Q}$ compared to euhydrated exercise with parallel increases in peripheral vascular resistance (González-Alonso et al., 1995). These changes occur in proportion to the deficit of body water (Montain & Coyle, 1992b) and may be related to impaired LV filling (González-Alonso et al., 1997). Despite these acute impairments to thermoregulatory and cardiovascular function the adjustments to endurance exercise in the heat with progressive dehydration following HA are unknown. To date, studies have used overnight heat stress (Buskirk et al., 1958), fluid restriction (Sawka et al., 1983c) and diuretics (Ikegawa et al., 2011) to manipulate the hydration status of acclimated subjects prior to heat exposure. Therefore, a secondary aim of this investigation was to characterise the acute acclimated thermoregulatory and cardiovascular responses to progressive exercise-induced dehydration and heat stress.

The following hypotheses were tested to determine whether HA with controlled HR would 1) lead to lowered HR and increased SV during constant workload exercise in the heat and, 2) improve thermoregulation while euhydration was maintained whereas, 3) acute dehydration would blunt otherwise improved thermoregulatory and cardiovascular responses following HA, associated in part with a reduced BV and ventricular filling.

5.2 – Methods

5.2.1 – Participants and study design

Eight males completed this study. Participants were recreational cyclists and triathletes training ≥5 h per week and had an average (± SD) age, height, body mass and $\dot{V}O_{2max}$ of 33.8 ± 5.1 years, 176 ± 5 cm, 75.4 ± 4.7 kg and 3.97 ± 0.42 $L \cdot min^{-1}$, respectively. All participants provided written informed consent prior to experimentation. The study was approved by the review board of Anti-Doping Laboratory Qatar and conformed to the declaration of Helsinki.

All participants underwent the overall experimental procedures outlined in Figure 5.1. Participants were asked to refrain from caffeine intake for 12 h and exercise for 24 h prior to all visits to the laboratory. All experiments were conducted at the same time of day to minimise the effects of circadian variation.

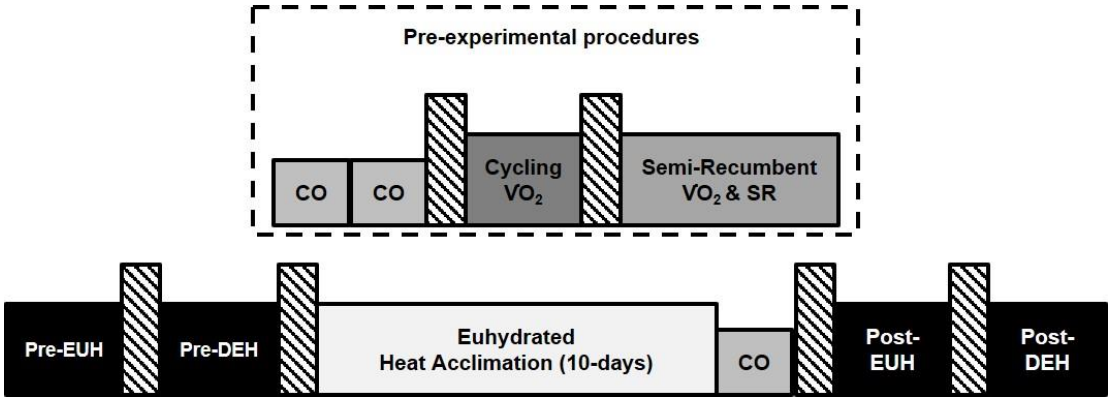


Figure 5.1: Schematic representation of study design. Pre-experimental procedures (top) were used to determine haemoglobin mass (Hb_{mass}), exercise workloads and sweat rate before the full intervention was completed (bottom). Experimental trials were completed with maintained euhydration (EUH) and progressive dehydration (DEH) via altered fluid intake before and after 10-days of euhydrated HA. Hatched bars represent a minimum of 24 h between visits to the laboratory. CO Carbon monoxide rebreathing. SR Sweat rate determination.

5.2.2 – Pre-experimental procedures

Pre-experimental procedures were conducted to determine Hb_{mass}, exercising workloads and sweat rate. Hb_{mass} was measured in duplicate using the optimised carbon monoxide rebreathing method (Schmidt & Prommer, 2005). Red cell (RCV), blood (BV) and plasma volumes (PV) were calculated from all subsequent venous blood samples using the equations outlined in Chapter 3, assuming a stable Hb_{mass} throughout all trials. Relative changes in BV and PV were also calculated using the method of Dill and Costill (1974).

On a separate occasion a step-incremental upright cycling test to exhaustion (Lode, Excalibur Sport, Groningen, The Netherlands) was conducted to determine $\dot{V}O_{2max}$ and prescribe exercise intensity and HR targets during subsequent experimental and HA trials. The test was conducted in cool ambient conditions (19.5 ± 1.7°C and 62 ± 12% relative humidity) and consisted of 5 submaximal step-incremental stages, each lasting 4 min. Following the final stage (210 W), resistance increased by 1 W every 2 s until volitional exhaustion. Participants were instructed to maintain a steady cadence throughout. $\dot{V}O_2$ (Oxycon Pro, Jaeger, CareFusion, Hoechberg, Germany) and HR were averaged over the final minute of every stage and $\dot{V}O_{2max}$ was defined as the highest minute average. The power output and HR associated with 65% $\dot{V}O_{2max}$ were determined via linear regression and used to set the initial workload and HR target throughout the heat acclimation period.

Following a minimum of 24 h rest, participants re-attended the laboratory to complete a step-incremental semi-recumbent cycling test to exhaustion. The purpose of the test was to determine study eligibility via echocardiographic assessment, semi-recumbent exercise workloads, and sweat rate of participants. The incremental test was conducted in the main laboratory (~20°C) with fan cooling (3 $m \cdot s^{-1}$) throughout. Briefly, participants mounted an ergometer (Ergoselct, Ergoline GMbH, Germany) wearing cycling shorts, socks and running shoes before a resting echocardiographic assessment was performed. Study eligibility was confirmed by the presence of a preserved

ejection fraction (i.e. >55%) and visual confirmation of symmetrical ventricular contraction. An incremental test was then conducted, beginning at 60 W, with power output increasing in 30 W step-increments every 3 min until volitional exhaustion despite strong verbal encouragement. HR (T31, Polar, Kempele, Finland) and $\dot{V}O_2$ were recorded throughout and averaged over the last 30 s of each stage. $\dot{V}O_{2max}$ was defined as the highest average 30 s value. The workload at 50% $\dot{V}O_{2max}$ was calculated via linear regression. Following the test participants rested in the main laboratory for a period of 60 min to allow sweating to cease and T_C to return to a resting value. Participants then entered an environmental chamber and completed 60 min of upright cycling exercise at 65% $\dot{V}O_{2max}$ in 33°C, 50% relative humidity. During this period the volume of *ad libitum* fluid intake was recorded and sweat rate was determined as the corrected change in nude body mass. This value was used to prescribe fluid intake in the two-subsequent control experimental trials.

5.2.3 – Experimental trials

Two experimental trials were completed in a randomised, counterbalanced order. Trials were designed to determine the thermoregulatory, haematological and cardiovascular responses to prolonged exercise heat stress with progressive dehydration and maintaining euhydration via alterations in fluid intake (Figure 5.2). Trials were separated by a minimum of 1 day of complete rest. Environmental conditions were identical to those used in the pre-experimental sweat rate estimation trial. Participants were instructed to attend the laboratory in a well-hydrated state and encouraged to consume approximately 5-7 ml of water per kg of body mass 2 h prior to attending the laboratory. Participants were also instructed to record their food and fluid intakes in the 24 h prior to experimentation and then replicate this as best as possible prior to all other experimental trials. Upon arrival to the laboratory, participants provided a urine sample for the measurement of urine specific gravity (USG), their nude body mass was determined and they then self-inserted a rectal thermistor. Participants then lay supine on a bed and a cannula was inserted into a right antecubital vein and flushed with 10 ml of sterile saline. During a 10-min rest period they were instrumented with T_{sk}

thermistors (iButton^{TM}, Maxim Integrated Products, Sunnyvale, CA, USA). Measurements of resting HR, T_C and blood pressure were then obtained, followed by a resting blood sample.

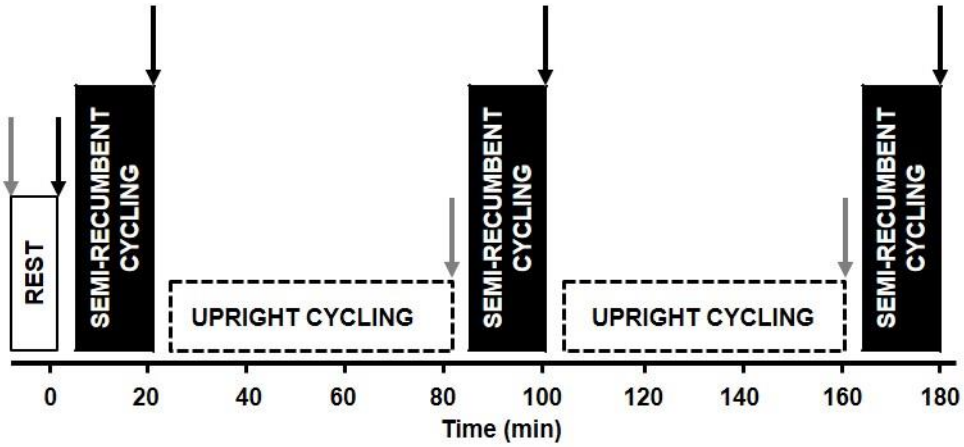


Figure 5.2: Schematic outline of experimental trials and example of echocardiographic assessment during semi-recumbent cycling. Nude body mass (grey arrows) was measured before 3 bouts of semi-recumbent cycling. Black arrows show measurement of T_C, T_{sk}, MAP, LV volumes and blood sampling. Bouts of semi-recumbent cycling were interspersed with 60 min periods of upright cycling during which fluid intake was altered depending on the target hydration status.

Participants then entered the environmental chamber and mounted a semi-recumbent cycle ergometer. The ergometer was tilted and resting echocardiographic images were taken in the left lateral decubitus position as outlined in Chapter 3. Once resting images were obtained, participants cycled at 50% $\dot{V}O_{2max}$ (135 ± 18 W) for 6 min before MAP was recorded in duplicate. The ergometer was again tilted while HR and left ventricular (LV) volumes were measured during exercise. Semi-recumbent exercise was terminated when images were collected, and a blood sample was taken (~20 min). Bouts of semi-recumbent exercise were repeated from 80-100 and 160-180 min. Between these periods, participants cycled on an upright cycle ergometer at 65% $\dot{V}O_{2max}$ (171 ± 21 W) for 60 min. A fan placed in front of the ergometer provided a constant windspeed of 3 $m \cdot s^{-1}$. Fluid was provided in 4 equal aliquots every 15 min during upright cycling in the form of a 0.1% electrolyte drink (HIGH5 ZERO, H5 Ltd, Bardon, UK). Fluid volumes were prescribed to the nearest 1 ml to match either 90% (maintained euhydration) or 10% (progressive dehydration) of estimated hourly sweat losses. At 80 and 160 min, participants dismounted and sweat losses were determined by changes in nude body mass from within the chamber (Figure 5.2). Blood samples were obtained following all exercising echocardiographic measurement periods and were analysed in duplicate (ABL 90FLEX, Radiometer, Copenhagen, Denmark).

5.2.4 – Euhydrated heat acclimation protocol

Following completion of pre-HA experimental trials, participants underwent 10 days of euhydrated exercising HA with controlled HR on a cycle ergometer. Each session lasted 90 min in environmental conditions of 40°C and 40% relative humidity. Prior to HR controlled exercise, a 15 min period was conducted at a fixed workload corresponding to 65% $\dot{V}O_{2max}$. This period was designed to sufficiently raise HR and promote the onset of sweating whilst avoiding initial increases in T_C being dampened by reductions in metabolic workload. Automatic adjustments in power output were then made via software on a computer terminal (LODE ergometry manager, LODE, Groningen, The Netherlands) to maintain an exercising HR associated with

65% $\dot{V}O_{2max}$ for the final 75 min (average target: 146 ± 7 beats·min^{-1}). Total work performed over this period increased by 112 ± 46 kJ throughout acclimation and resulted in an average HR of 147 ± 6 beats·min^{-1} and T_C of 38.4 ± 0.2°C. An average exercising body mass change of 0.6 ± 0.2% was achieved each day by providing fluid matching 90% of predicted sweat losses. Six equal boluses were consumed throughout each exposure from the beginning of exercise and every 15 min thereafter. To match the 0.19 ± 0.18 L·h^{-1} increase in sweat rate with HA, fluid intake was adjusted for each exposure to the recorded sweat losses of the previous day, ensuring body mass deficits were similar. Sweating rate on the final day of HA was used to determine the volume of fluid prescribed in the post-HA experimental trials. A single CO measurement was conducted 24 h following the HA intervention to determine if acclimation resulted in a stable or altered in Hb_{mass}. All post-HA trials were completed within 5 days of the final day of HA.

5.2.5 – Echocardiography

All echocardiographic images were taken in the same order as outlined in Chapter 3. Image depth was standardised for each participant and phase of the trial (i.e. rest and exercise). Frame rate was set to 60 Hz for 2D image acquisition (S5-1 5 MHz sector array probe; CX50 POC, Philips Healthcare, The Netherlands). All images were taken in the left lateral decubitus position at the end of expiration. A minimum of 6 images from each view were recorded.

Images were analysed offline using dedicated computer software (Q-Station, Version 3.8.5, Phillips Healthcare, The Netherlands). All trials were de-identified and analysed at the end of the data collection period. A minimum of three cardiac cycles were analysed for all measurements over consecutive cardiac cycles where possible. LV mass was measured at end diastole during the resting period of each trial using the area-length method (Schiller et al., 1989). LV volumes were calculated from apical 4- and 2-chamber views using the Simpson's bi-plane method of disk summation. $\dot{Q}$ was calculated as HR

multiplied by SV. Systemic vascular resistance (SVR) was calculated as MAP divided by Q̇.

5.2.6 – Data analysis

A 2-way (hydration x acclimation) ANOVA with repeated measures analysis was used to test for differences in thermoregulatory and cardiovascular responses at rest. A separate trial (4) x time (3) ANOVA with repeated measures analysis was conducted to test differences between thermoregulatory, thermal, haemodynamic and haematological responses to bouts of semi-recumbent cycling at 20, 100 and 180 min of exercise before and after HA. Mauchly's test was used to test the assumption of Sphericity. In cases where this assumption was violated a Greenhouse-Geisser correction factor was applied to the degrees of freedom. Bonferroni *post-hoc* testing was employed to determine where pairwise differences occurred. All statistical analyses were conducted using SPSS (Version 21, IBM, Armonk, US). Results are reported as mean ± SD. Significance was set at $P < 0.05$ with a $P \leq 0.1$ considered a statistical trend. Effect sizes are presented using partial eta squared values for analysis of variance ($\eta_p^2 \leq 0.02$: small; 0.02-0.13: medium; 0.13-0.26: large; Cohen 1988).

5.3 – Results

5.3.1 – Resting thermal, haemodynamic and haematological responses to euhydrated heat acclimation

Participants attended all experimental trials in a well-hydrated state as indicated by similar USG measurements (1.013 ± 0.008, *P* = 0.24) and nude body masses (75.2 ± 4.7 kg, *P* = 0.21) between trials. Resting T_C and T_{sk} were unaltered by HA (both *P* > 0.05, Table 5.1, Figure 5.3). There was a significant interaction between trial and HA status on resting HR. Pairwise analyses showed resting HR was significantly lower following HA prior to the euhydration (*P* = 0.03) but not the progressive dehydration trial (*P* = 0.39; Table 5.1). EDV tended to be ~5 ml higher at rest following HA (*P* = 0.06) whilst ESV remained unaltered. This explained a small, but significant increase in resting SV following HA (*P* = 0.04, Table 5.1, Figure 5.5). However resting $\dot{Q}$ did not differ from pre-HA values (*P* = 0.21). There was no effect of HA on LV mass with calculated values being similar between all experimental trials (174 ± 11 and 174 ± 9 g in pre-HA trials, and 174 ± 9 and 175 ± 10 g post-HA, respectively, P = 0.80).

There was a slight but significant 2.1% decrease in Hb_{mass} from 882 ± 69 g pre-HA to 863 ± 78 g 24 h following day 10 of HA (*P* < 0.05). All acclimated haematological responses were therefore calculated using this value. The lowered Hb_{mass} resulted in a tendency for RCV to decrease with HA, but this was not significant (*P* = 0.06, Table 5.1). There was no absolute or relative change in resting BV or PV with HA (all *P* > 0.05, Table 5.1).

Table 5.1: Baseline thermal, haemodynamic and haematological parameters prior to each experimental trial.

	Maintained Euhydration		Progressive Dehydration		Hydration	Acclimation	Interaction
	Pre-HA	**Post-HA**	**Pre-HA**	**Post-HA**			
Thermal							
T_C (°C)	37.1 ± 0.3	37.0 ± 0.3	36.8 ± 0.4	36.9 ± 0.3	$P = 0.01^{\#}$ $\eta_p^2 = 0.72$	$P = 1.00$ $\eta_p^2 = 0.00$	$P = 0.18$ $\eta_p^2 = 0.24$
T_{sk} (°C)	33.7 ± 0.7	33.5 ± 0.9	33.5 ± 0.7	33.1 ± 1.1	$P = 0.27$ $\eta_p^2 = 0.17$	$P = 0.26$ $\eta_p^2 = 0.18$	$P = 0.43$ $\eta_p^2 = 0.09$
Haemodynamic							
HR (beats·min^{-1})	61 ± 7	55 ± 4*	58 ± 8	57 ± 5	$P = 0.56$ $\eta_p^2 = 0.01$	$P = 0.41$ $\eta_p^2 = 0.30$	$P = 0.02^{\#}$ $\eta_p^2 = 0.55$
EDV (ml)	140 ± 19	145 ± 19	139 ± 12	145 ± 19	$P = 0.81$ $\eta_p^2 = 0.01$	$P = 0.06$ $\eta_p^2 = 0.42$	$P = 0.83$ $\eta_p^2 = 0.00$
ESV (ml)	50 ± 10	50 ± 10	50 ± 7	49 ± 10	$P = 0.89$ $\eta_p^2 = 0.00$	$P = 0.99$ $\eta_p^2 = 0.00$	$P = 0.76$ $\eta_p^2 = 0.01$
SV (ml)	90 ± 11	95 ± 12*	89 ± 6	96 ± 9*	$P = 0.86$ $\eta_p^2 = 0.01$	$P < 0.01^{\#}$ $\eta_p^2 = 0.71$	$P = 0.36$ $\eta_p^2 = 0.12$
Haematological							
RCV (ml)	2701 ± 213	2671 ± 223	2701 ± 212	2668 ± 222	$P = 0.24$ $\eta_p^2 = 0.20$	$P = 0.06$ $\eta_p^2 = 0.44$	$P = 0.33$ $\eta_p^2 = 0.14$
BV (ml)	6024 ± 457	6049 ± 417	6042 ± 480	6095 ± 376	$P = 0.62$ $\eta_p^2 = 0.04$	$P = 0.59$ $\eta_p^2 = 0.05$	$P = 0.62$ $\eta_p^2 = 0.04$
PV (ml)	3323 ± 294	3378 ± 295	3341 ± 347	3427 ± 254	$P = 0.60$ $\eta_p^2 = 0.04$	$P = 0.29$ $\eta_p^2 = 0.16$	$P = 0.59$ $\eta_p^2 = 0.05$

* Significantly different from pre-HA. # Significant main effect.

5.3.2 – Prolonged exercise with maintained euhydration

By design, fluid intake was increased from 2.53 ± 0.42 to 3.03 ± 0.29 L, (P < 0.001) to match 90% of expected hourly sweat losses following HA in the maintained euhydration experiments. This resulted in slight relative decreases in body mass from rest to immediately prior to the final bout of euhydrated semi-recumbent exercise of 0.74 ± 0.40% before HA and 0.36 ± 0.26% following HA, that did not differ between trials (P = 0.11).

Following an initial reduction in PV from the onset of exercise, adequate fluid intake in the maintained euhydration trial before HA resulted in a stable RCV, PV and BV throughout exercise (all P > 0.05). Similar to resting conditions, no effect of HA was observed on RCV or PV throughout exercise (all P > 0.05). Therefore, BV at 180 min did not differ between euhydration trials, averaging 5679 ± 404 before HA and 5764 ± 408 ml following HA, respectively (P = 0.80, Table 5.2).

T_C increased by 1.0 ± 0.2°C throughout exercise before HA (P < 0.001), reaching 38.4 ± 0.2°C at 180 min. T_{sk} remained similar throughout this period, averaging 34.0 ± 0.1°C (P > 0.05). HA did not lower the exercise T_C and T_{sk} responses during semi-recumbent cycling with maintained euhydration (all P > 0.05, Figure 5.3), which reached 38.3 ± 0.1°C and 34.0 ± 0.1°C at 180 min following HA, respectively.

Exercising HR with maintained euhydration was not significantly lowered by HA, reaching 139 ± 7 and 135 ± 8 beats·min^{-1} at 180 min before and after HA, respectively (P = 0.70, Figure 5.4). Over the same period, SV tended to be 9 ± 10 ml higher following HA (P = 0.07, Figure 5.4). This was primarily due to differences in responses over time within each euhydrated trial. Before HA, EDV and SV significantly declined between 20 and 180 min by 7 ± 5 and 9 ± 5 ml, reaching 146 ± 19 and 103 ± 9 ml at the end of exercise, respectively (both P < 0.05). ESV remained unchanged during exercise (P = 0.71) and $\dot{Q}$ was largely maintained between 20 and 180 min before HA in parallel to the increases in HR (P = 0.07, Figure 5.5). EDV was not significantly increased

following HA (P = 0.75), but did not decline throughout exercise (P > 0.05), instead averaging 152 ± 21 ml at 180 min. This resulted in exercising $\dot{Q}$ increasing significantly throughout exercise, reaching 15.1 ± 1.63 $L \cdot min^{-1}$ at 180 min (P < 0.001). However, $\dot{Q}$ before and after HA at 180 min did not differ significantly (0.7 ± 0.7 $L \cdot min^{-1}$, P = 0.18, Figure 5.5).

MAP was not altered throughout euhydrated exercise and there was no effect of HA on responses observed, averaging 91 ± 10 and 89 ± 6 mmHg at 180 min before and after HA, respectively (P = 1.00). SVR significantly decreased by 10 ± 5% (0.7 ± 0.4 $mmHg \cdot L \cdot min^{-1}$) throughout euhydrated exercise before HA (P = 0.02). Following HA, SVR was significantly lower than before HA at 100 min (0.5 ± 0.4 $mmHg \cdot L \cdot min^{-1}$, 8%, P = 0.03) but not 180 min (0.4 ± 0.4 $mmHg \cdot L \cdot min^{-1}$, 7%, P = 0.34).

Table 5.2: BV, PV and RCV during each bout of semi-recumbent cycling

		Maintained Euhydration		Progressive dehydration		Trial	Time	Interaction
		Pre-HA	Post-HA	Pre-HA	Post-HA			
BV (ml)	20 min	5656 ± 447	5743 ± 432	5671 ± 465	5736 ± 370	$P = 0.01^{\#}$ $\eta_p^2 = 0.44$	$P < 0.001^{\#}$ $\eta_p^2 = 0.66$	$P < 0.001^{\#}$ $\eta_p^2 = 0.83$
	100 min	5674 ± 432	5766 ± 442	5532 ± 407*	5599 ± 312*			
	180 min	5679 ± 404	5764 ± 408	5365 ± 392*†‡	5383 ± 289*†‡			
PV (ml)	20 min	2952 ± 278	3076 ± 294	2970 ± 326	3068 ± 219	$P = 0.01^{\#}$ $\eta_p^2 = 0.50$	$P < 0.001^{\#}$ $\eta_p^2 = 0.66$	$P < 0.001^{\#}$ $\eta_p^2 = 0.83$
	100 min	2971 ± 270	3099 ± 307	2827 ± 276*	2930 ± 189*			
	180 min	2977 ± 247	3097 ± 269	2663 ± 249*†‡	2716 ± 163*†‡			
RCV (ml)	20 min	2704 ± 213	2667 ± 222	2701 ± 213	2668 ± 222	$P = 0.04^{\#}$ $\eta_p^2 = 0.51$	$P = 0.25$ $\eta_p^2 = 0.18$	$P = 0.82$ $\eta_p^2 = 0.06$
	100 min	2703 ± 212	2668 ± 221	2705 ± 212	2668 ± 220			
	180 min	2701 ± 210	2667 ± 222	2702 ± 212	2667 ± 222			
Hb ($g \cdot dl^{-1}$)	20 min	15.60 ± 0.61	15.17 ± 0.92	15.57 ± 0.79	15.17 ± 0.74	$P = 0.001^{\#}$ $\eta_p^2 = 0.56$	$P = 0.01^{\#}$ $\eta_p^2 = 0.67$	$P < 0.001^{\#}$ $\eta_p^2 = 0.84$
	100 min	15.55 ± 0.62	15.11 ± 0.92	15.95 ± 0.78*	15.54 ± 0.82*			
	180 min	15.53 ± 0.62	15.11 ± 0.88	16.44 ± 0.72*†‡	16.16 ± 0.78*†‡			
Hct (%)	20 min	47.8 ± 1.9	46.5 ± 2.8	47.7 ± 2.5	46.5 ± 2.3	$P < 0.001^{\#}$ $\eta_p^2 = 0.57$	$P = 0.01^{\#}$ $\eta_p^2 = 0.67$	$P < 0.001^{\#}$ $\eta_p^2 = 0.85$
	100 min	47.7 ± 1.9	46.3 ± 2.8	48.9 ± 2.4*	47.6 ± 2.5*			
	180 min	47.6 ± 1.9	46.3 ± 2.7	50.4 ± 2.2*†‡	49.5 ± 2.4*†‡			

* Significantly different from 20 min. † Significant difference from 100 min. ‡ Significantly different from euhydration trial. # Significant main effect.

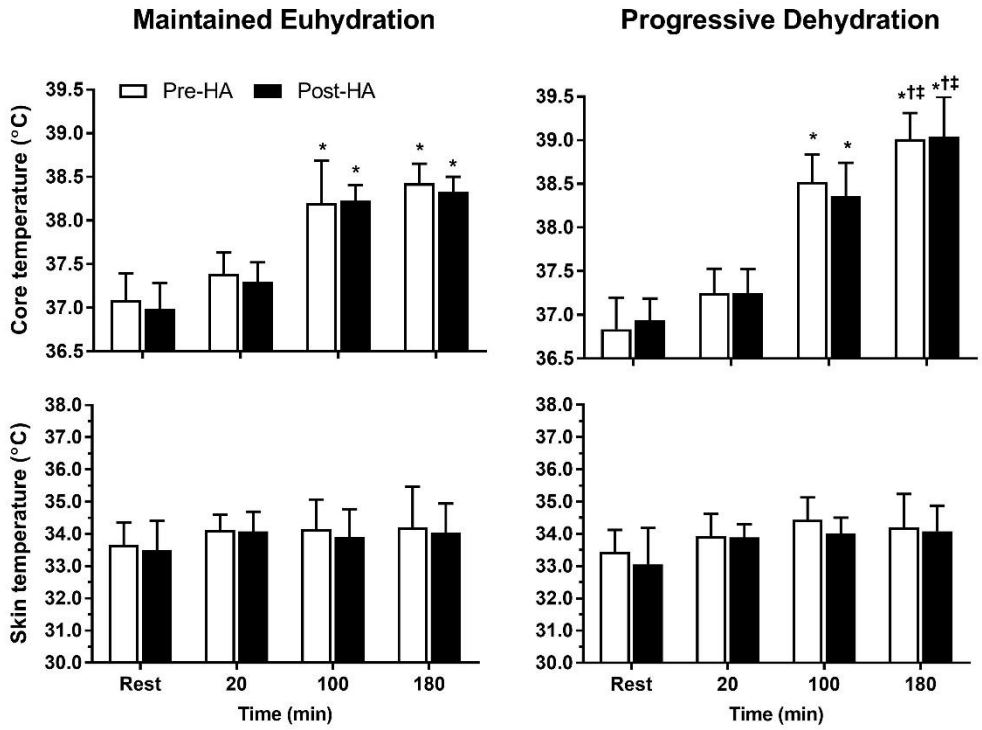


Figure 5.3: T_C and T_{sk} responses at rest and during repeated bouts of semi-recumbent cycling in the heat while maintaining euhydration (left) or progressive dehydration (right) via altered fluid ingestion. White and black bars represent respective pre- and post-HA trials. * Significantly greater than 20 min. † Significantly greater than 100 min. ‡ Significantly different from maintained euhydration.

5.3.3 – Prolonged exercise with progressive dehydration

Average fluid intake in the progressive dehydration experimental trials was similar before and after HA, averaging 0.3 ± 0.6 and 0.4 ± 0.1 L (P = 0.22). This resulted in 3.53 ± 0.70% and 3.75 ± 0.72% decreases from resting body mass, respectively that did not differ between dehydration trials (P = 0.50). In parallel to whole-body progressive dehydration PV and BV declined significantly throughout exercise (both P < 0.0001). However, these decreases were not altered by HA. RCV was not affected by dehydration or HA. Therefore, both PV and BV at 180 min following HA did not differ from values measured before HA (both P > 0.05, Table 5.2).

HA did not lower T_C or T_{sk} during exercise with progressive dehydration compared to pre-HA responses (both P > 0.05, Figure 5.3). HR responses to semi-recumbent exercise were also not affected by HA, reaching 150 ± 10

and 151 ± 10 beats·min^{-1} at 180 min before and after HA, respectively (P = 1.00, Figure 5.4). SV declined significantly to a similar extent between 20 and 180 min with progressive dehydration before and after HA (both P < 0.05), averaging 85 ± 8 and 86 ± 8 ml at the end of exercise, respectively (P = 0.41). $\dot{Q}$ was not altered at the end of dehydrating exercise by HA, averaging 12.7 ± 1.7 and 13.0 ± 1.5 L·min^{-1} at 180 min before and after HA, respectively (P = 1.00).

MAP significantly declined during exercise by 8 ± 5 mmHg with progressive dehydration before HA (P = 0.01). HA did not alter this response and a similar decline occurred between 20 and 180 min (9 ± 5 mmHg, P = 0.003). Therefore, MAP was similar at the end of exercise before and after HA, averaging 86 ± 15 and 83 ± 13 mmHg, respectively (P = 0.41, Figure 5.5). SVR remained unaltered between 20 and 180 min of exercise in both dehydration trials and responses were not altered by HA (all P > 0.05).

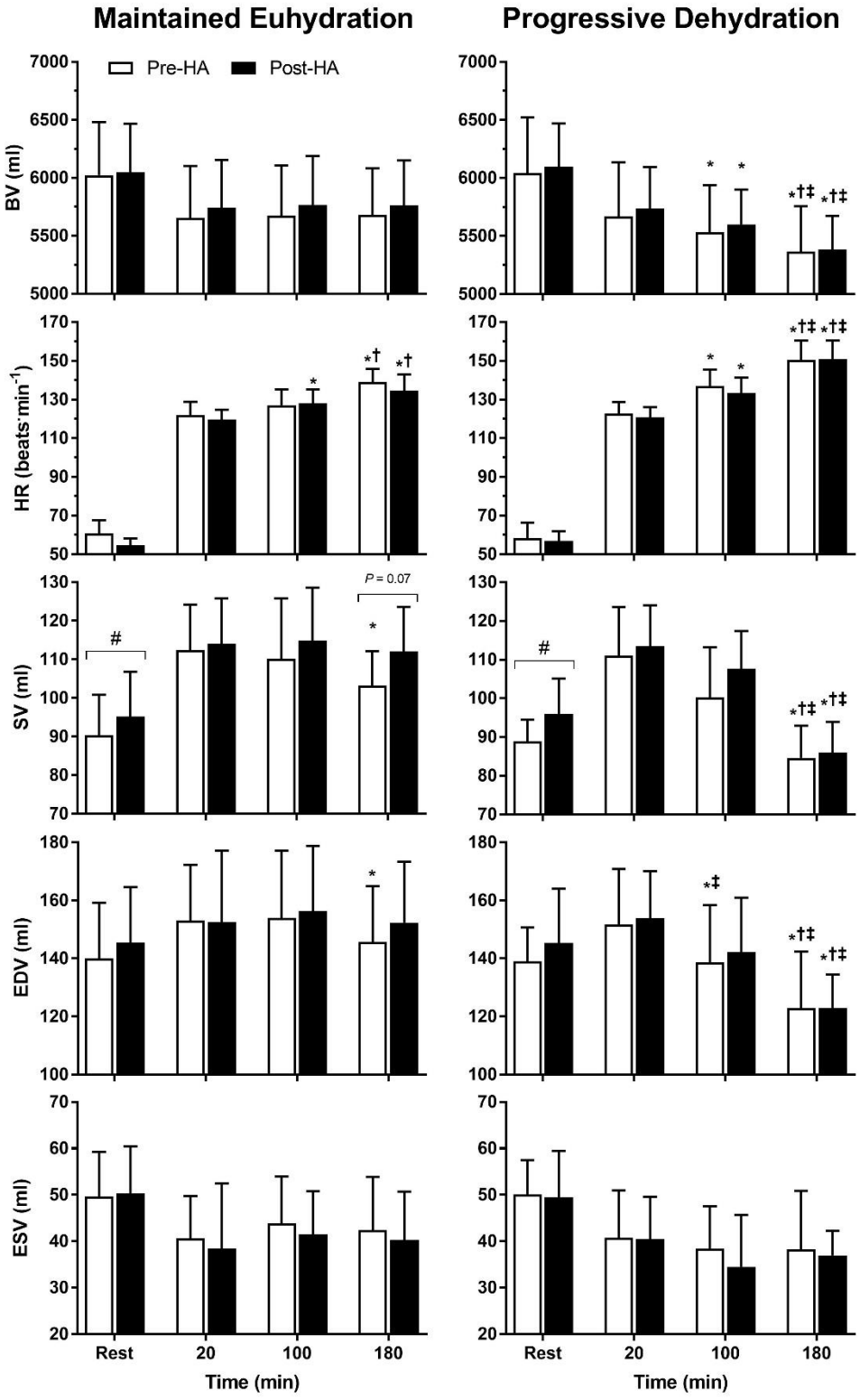


Figure 5.4: Pre- (white) and Post-HA (black) BV, HR and LV volumes at rest and during semi-recumbent cycling while maintaining euhydration (left) and becoming progressively dehydrated (right) via altered fluid ingestion. # Significantly different to Pre-HA. * Significantly different from 20 min in same condition. † Significantly different from 100 min in same condition. ‡ Significantly different from euhydration trial.

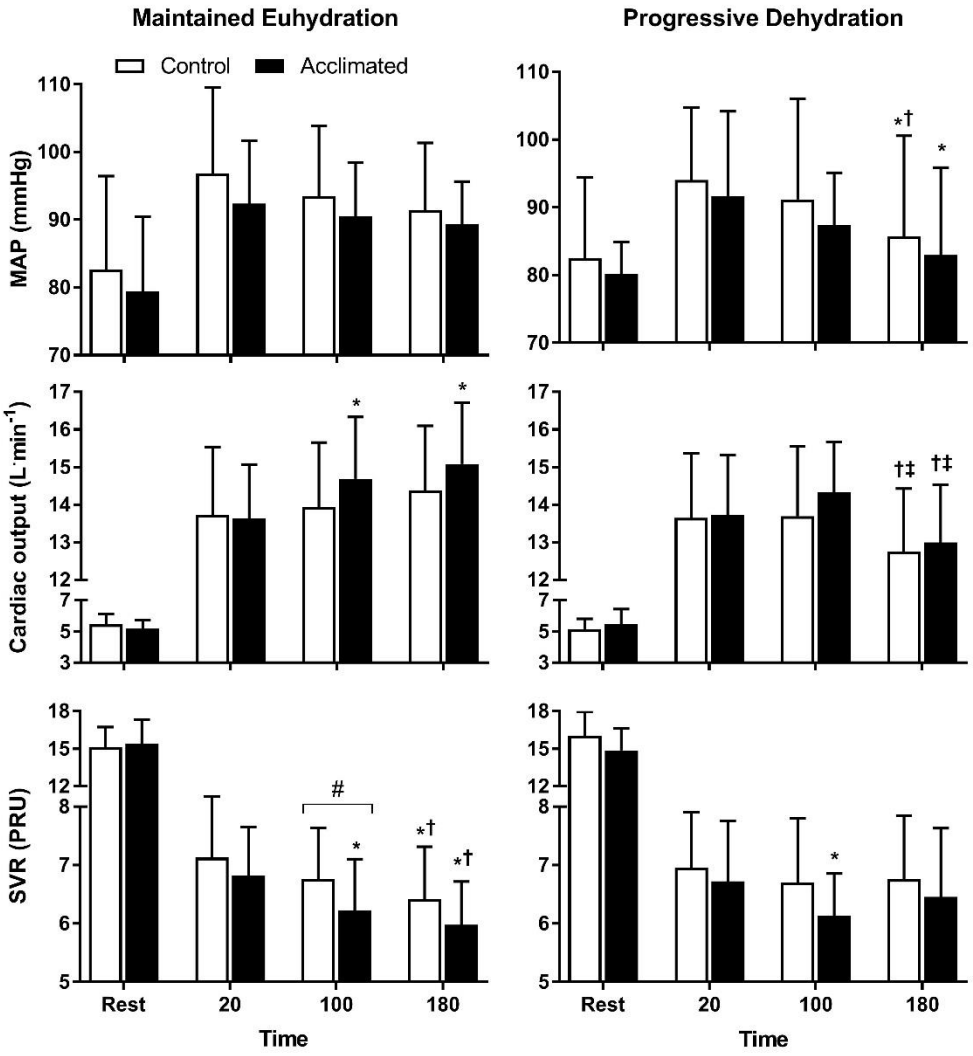


Figure 5.5: Pre- (white bars) and Post-HA (black bars) MAP, Q̇ and SVR during repeated bouts of semi-recumbent cycling in the heat with maintained euhydration (left) and progressive dehydration (right) via altered fluid ingestion. # Significantly different from Pre-HA. * Significantly different from 20 min. † Significant difference from 100 min. ‡ Significantly different from euhydration trial.

5.3.4 – Effect of hydration status following heat acclimation

Progressive dehydration resulted in significant haemoconcentration, hyperthermia and cardiovascular strain compared to maintained euhydration via altered fluid intake following HA. The significant haemoconcentration that occurred with progressive dehydration meant that both PV and BV were 12 ± 6 and 6 ± 3% lower, respectively than maintained euhydration at 180 min following HA ($P < 0.05$, Table 5.2).

T_{sk} was similar throughout exercise between euhydration and dehydration trials following HA (all $P > 0.05$). However, progressive dehydration resulted in a 0.8 ± 0.4°C greater increase in T_C throughout exercise compared to maintained euhydration, reaching 39.0 ± 0.5°C at 180 min ($P = 0.001$, Figure 5.3). This was accompanied by significantly greater increases in HR over the same period and resulted in HR being 16 ± 7 beats·min^{-1} higher with dehydration compared to euhydration at the end of exercise ($P = 0.003$).

Despite significant declines throughout exercise, MAP with progressive dehydration was similar to maintained euhydration at 180 min following HA (-6 ± 8 mmHg, $P = 0.41$). Similarly, there were no differences in SVR during exercise between trials (all $P > 0.05$). However, $\dot{Q}$ was 2.1 ± 0.8 L·min^{-1} lower than euhydration with progressive dehydration following HA after 180 min ($P = 0.001$, Figure 5.5). This was second to a significantly diminished SV and EDV, which were 26 ± 9 and 29 ± 16 ml lower than euhydration at 180 min, respectively (both $P < 0.05$). ESV was similar between dehydration and euhydration following HA, averaging 37 ± 5 and 40 ± 10 ml at 180 min, respectively ($P = 1.00$).

5.4 – Discussion

This study sought to determine the thermal, haematological and haemodynamic responses at rest and during repeated bouts of exercise in the heat following euhydrated HA with controlled HR. In doing so, this study provided non-invasive measurements of LV volumes during dynamic exercise with maintained euhydration and progressive dehydration. Several novel observations were made. Firstly, when euhydration was maintained in heat acclimated individuals, the decline in SV secondary to a fall in EDV observed during prolonged exercise heat stress prior to HA was not observed. As euhydrated exercise progressed MAP was maintained and $\dot{Q}$ increased. However, HA did not result in a reduction in T_C and HR or an increase in PV and BV during semi-recumbent exercise with maintained euhydration. Secondly, the responses to progressive exercise and heat stress induced dehydration observed before HA (i.e. significant whole-body hyperthermia, haemoconcentration and cardiovascular strain) were remarkably similar following HA when a similar ~3.6% deficit in body mass occurred. These results expand our understanding of the effects of HA and highlight the influence of hydration status on thermoregulatory and central haemodynamics to exercise as thirdly, progressive dehydration following HA resulted in a significantly elevated T_C and HR and reduced SV and $\dot{Q}$ compared to when euhydration was maintained.

Given these findings, the hypotheses that an increase in SV during euhydrated exercise would be related to a decrease in HR and improved thermal responses following HA is only partly accepted. However, the hypothesis that thermoregulatory and cardiovascular responses to acute dehydration before and after HA would be similar is accepted.

5.4.1 – Effect of acclimation on cardiovascular stability

The central circulatory responses to endurance exercise and heat stress following HA are relatively unclear. It is widely reported that HA results in improvements in cardiovascular stability, specifically via a decrease in HR and

increase in SV. Therefore, it is considered that Q̇ may be better defended during exercise following HA (Periard et al., 2015; Sawka et al., 2011; Taylor, 2014; Tyler et al., 2016). Together with the decrease in HR, an increased PV and therefore BV is proposed to enhance LV filling, permitting this increase in SV (Rowell et al., 1967; Senay, 1986). However, there is little direct mechanistic evidence for changes in LV volumes following HA, with indirect measurements in the literature reporting conflicting responses (Nielsen et al., 1993; Nielsen et al., 1997; Rowell et al., 1967; Wyndham, 1951; Wyndham et al., 1968a). Here non-invasive diastolic and systolic LV volumes were determined via echocardiography during repeated bouts of semi-recumbent cycling in 33°C and 50% relative humidity. Absolute PV and BV were also determined. Therefore, this study provides novel insight into the haemodynamic and haematological responses to exercise with and without progressive dehydration in humans who have undergone 10-day euhydrated HA with controlled HR.

In contrast to the responses seen before HA, when euhydration was maintained following HA EDV and SV did not decline during exercise. Instead, both remained elevated following the onset of exercise around 151 and 113 ml, respectively. Therefore, SV tended to be ~9 ml higher at 180 min following HA, while HR responses remained similar. However, despite these differences between euhydrated unacclimated and acclimated responses, HA did not significantly increase Q̇ at 180 min (~0.7 $L \cdot min^{-1}$). These acclimated haemodynamic responses differ somewhat to previous observations (Nielsen et al., 1993; Nielsen et al., 1997; Rowell et al., 1967; Wyndham, 1951; Wyndham et al., 1968a). For example, Rowell et al. (1967) observed similar Q̇ responses following HA in hot-dry heat. The authors concluded this was due to an increase in SV and decreased HR, rather than an increase in central BV (Rowell et al., 1967). In addition, Nielsen et al. (1993) previously reported greater skin and forearm blood flows following HA in hot-dry heat. This was accompanied by 1.8 $L \cdot min^{-1}$, 21 ml and 13% increases in Q̇, SV and PV, respectively (Nielsen et al., 1993). Considering HR reached ~137 $beats \cdot min^{-1}$ before and after HA and PV and BV were not expanded by the current intervention it is perhaps not surprising that Q̇ was not significantly increased.

Instead the maintenance of EDV and SV with prolonged exercise heat stress following HA is unclear and points to another mechanism. In the rodent model cardiac muscle exhibits a transient increase in autonomic excitability, enhanced LV compliance and contractility with HA (Horowitz, 2002; Levi et al., 1993). While such responses to HA have never been directly observed in humans and may be unlikely given the relatively smaller adaptive stimuli, alterations in myocardial function have been observed to occur with (Levy, Cerquiera, Abrass, Schwartz, & Stratton, 1993) and independently of changes in LV wall thickness with exercise training (Stöhr et al., 2012). Furthermore, cross-sectional studies show aerobic fitness is closely related to LV compliance, with fitter individuals exhibiting a larger EDV at lower filling pressures (Stickland et al., 2006). Considering the similarities in cardiovascular responses and absence of a structural change, a functional change is possible. However, no direct measurements of LV function were made in the present study and this possibility is merely speculative. Despite this, the present data does suggest that HA leads to an improvement in the ability to sustain $\dot{Q}$ during prolonged exercise heat stress while euhydration is maintained.

The ~9 ml decrease in SV with maintained euhydration prior to HA is similar to previous observations (González-Alonso et al., 1997), whereby exercising HR and T_C increased by 16 beats·min^{-1} and 1°C over the same period, respectively. Several reasons for this acute decline in SV during euhydrated exercise have been proposed. Firstly, central BV and venous pressure may fall as blood is distributed peripherally to the cutaneous circulation (Rowell, 1986; Shaffrath & Adams, 1984). In addition, several others have observed plateaus in forearm and cutaneous blood flow during exercise in temperate (Fritzsche et al., 1999) and hot conditions (González-Alonso et al., 2000a; Trinity et al., 2010). Therefore, the significant fall in EDV and SV with euhydration prior to HA may be related to paradoxical increases in HR and reductions in LV filling time. In support of this increasing HR via right atrial pacing has been shown to lower SV at a given exercise intensity (Munch et al., 2014). Furthermore, β1-adrengeric blockade prevents increases in HR and reductions in SV during prolonged exercise in temperate and hot

conditions (Fritzsche et al., 1999; Trinity et al., 2010). In the present study, T_C, $\dot{Q}$ and MAP remained similar between 100 and 180 min. Over the same period, HR increased ~12 beats·min^{-1}. Although skin blood flow was not determined in the present study, it seems this increase in HR may be behind the lowering of SV and EDV over this period, rather than increases in cutaneous blood flow. SVR did slightly decrease between bouts of exercise. This however may be due to initial transient vasoconstrictor adjustments in the cutaneous vascular bed (Johnson & Park, 1982) and the delayed vasodilatory response to a higher T_C at the onset of exercise (Kellogg, Johnson, & Kosiba, 1991). Several investigations of moderate dynamic exercise heat stress have also shown that muscle blood flow is maintained (González-Alonso et al., 1998; Savard et al., 1988) while cutaneous blood flow plateaus as T_C exceeds ~38°C (Brengelmann, Johnson, Hermansen, & Rowell, 1977; González-Alonso et al., 2000a; González-Alonso et al., 1999b; Kenney, Tankersley, Newswanger, & Puhl, 1991; Savard et al., 1988). This, along with the similarities in T_C and Tsk between 100 and 180 min suggest skin blood flow requirements remained unchanged (Sawka et al., 2011). It is therefore likely the fall in SV accompanying the lowering of EDV in the pre-HA euhydrated trial was primarily due to decreases in filling time, secondary to increases in HR.

5.4.2 – Effect of acclimation on responses to exercise and progressive dehydration

To date very few have investigated the effect HA has upon haematological, thermal and haemodynamic responses to acute dehydration. In this study fluid intake was manipulated to match 10% of expected hourly sweat losses. This resulted in similar ~3.6% relative reductions body mass during exercise before and after HA, permitting the effects of HA on a standardised deficit of total body water via exercise-induced heat stress to be determined. T_C and HR were not lowered by the 10-day euhydrated HA with controlled HR intervention of the present study and instead reached ~39°C and ~150 beats·min^{-1} at the end of semi-recumbent cycling in both pre- and post-HA dehydration trials, respectively. These responses were accompanied by similar reductions in BV,

MAP and Q̇ before and after HA. The loss of more dilute sweat following HA may create an osmotic gradient between the intracellular and extracellular space (Nose, Mack, Shi, & Nadel, 1988b), expand the extracellular compartment and buffer against reductions in PV associated with exercise induced dehydration (Maw, Mackenzie, & Taylor, 1998). However, no study to date has characterised the responses to matched levels of progressive dehydration that occur during exercise following HA. In a recent investigation it was shown that a 22-day dehydrated-HA intervention was associated with T_C and HR being 0.6°C and 14 beats·min^{-1} lower, respectively after 120 min of exercise and fluid restriction. However, the dehydration challenges were not standardised and instead reflected end-exercise deficits in total body water of ~1.8 L pre- and ~2.7 L post-HA, respectively (Patterson et al., 2014). The findings of the present study therefore extend these observations and suggest that euhydrated HA does not alter thermal, haematological or haemodynamic responses to a matched level of progressive dehydration >3%. Whether these responses would be similar following a dehydrated HA intervention is currently unknown.

Several previous investigations have explored the influence of HA on exercise heat stress with prior hypohydration. These have involved overnight heat stress and fluid restriction to induce significant body water deficits (i.e. >5% body mass loss; Buskirk et al. 1958; Sawka et al. 1983c) or iso-osmotic hypohydration via diuretics (Ikegawa et al., 2011), neither of which are typically experienced by individuals undertaking endurance exercise. Sawka et al. (1983c) observed similar thermoregulatory responses with dehydration pre- to post-HA, whereas HR was ~20 beats·min^{-1} lower. This was ascribed to smaller reductions in PV that may have permitted improved cardiac filling (Sawka et al., 1983c). In contrast, the present investigation observed very similar reductions in BV and PV, while there were similar HR and T_C responses. Instead, these findings more closely reflect those of Ikegawa et al. (2011). The average body mass deficit of the present study was similar to that of Ikegawa et al. (2011; ~3%) but was associated with slightly different responses, primarily due to the nature of exercise heat stress with fluid restriction compared with pre-exercise body water loss via diuretics. Q̇ and SV

responses to exercise post acclimation were similar. However, HR and T_C responses were slightly dampened post-acclimation during a 30 min exercise bout (Ikegawa et al., 2011). Conversely, both dehydration trials in the present study resulted in significantly greater increases in T_C compared to Ikegawa et al. (2011; ~39.0°C *vs.* ~38.4°C). These greater temperature elevations may also explain the similar HR increases between 20 and 180 min (~28 beats·min^{-1}) in both pre- and post-HA trials of the present study.

Inadequate fluid replacement resulted in similar significant degrees of haemoconcentration, as similar reductions in PV occurred pre- and post-HA. This finding provides further indication that PV is not preferentially maintained during acute exercise induced dehydration following HA (Patterson et al., 2014). Therefore, similar to pre-HA responses, significant dehydration during exercise leads to marked elevations in body temperature and HR along with reductions in PV and BV. Together, it appears that dehydration may result in a consistent impairment in LV filling following HA as the 31 and 27 ml reductions in EDV and SV were remarkably similar to pre-HA responses. Data from the present study indicates that the sequence of events associated with progressive declines and $\dot{Q}$ and MAP during exercise with progressive dehydration (González-Alonso, 1998) is not altered following 10 days of euhydrated HA. This influence is further highlighted when acclimated responses to maintained euhydration and progressive dehydration are compared.

5.4.3 – Acute influence of fluid intake following acclimation

Since responses to exercise with progressive dehydration were broadly similar before and after HA, this study therefore highlights the importance of maintained euhydration in the cardiovascular and thermoregulatory responses to exercise and heat stress following HA. For instance, the ~0.7°C and ~16 beats·min^{-1} greater increases in T_C and HR that occurred after 180 min of exercise with progressive dehydration compared to euhydration post-HA are typical of those seen with acute dehydration beyond ~2% of body mass (Montain & Coyle, 1992b). In addition, dehydration before and after HA in the

current study was coupled with similar ~11 and ~7% lower PV and BV at the end of exercise compared to euhydration. However, the present study shows when similar whole-body dehydration beyond ~3% occurs, there are somewhat larger relative differences in SV and $\dot{Q}$ following HA. For example, with dehydration, $\dot{Q}$ was 1.6 $L \cdot min^{-1}$ lower at the end of exercise compared to euhydration before HA. However, this difference was 2.2 $L \cdot min^{-1}$ following HA and is predominantly due to EDV and SV being relatively lower with dehydration compared to euhydration.

The interplay between temperature, hydration and SV is well established with varying contributions of reduced BV and increased body temperature observed following manipulations of posture and heat stress (González-Alonso et al., 1995, 1997; González-Alonso et al., 1999a, 2000a). Following HA, SV was ~29 ml lower at 180 min with dehydration compared to euhydration. This was second to a ~26 ml lower EDV whilst ESV was similar. Dehydration during dynamic upright exercise and heat stress typically results in a ~5% lowering of MAP with concomitant ~10% increase in SVR compared to maintained euhydration (González-Alonso et al., 1998; González-Alonso et al., 1995, 1997). However, in the current study MAP at the end of exercise with dehydration was not significantly lower than euhydration, despite a ~10% decrease between 20 and 180 min within the dehydration trial. This is perhaps due to the semi-recumbent body position during exercise as SVR was not significantly greater with dehydration, suggesting there was not a significant increase in systemic vasoconstrictor tone throughout dehydrating exercise. Conversely, the lower SV with dehydration was not likely due to increased cutaneous blood flow despite the greater increases in body temperature compared to euhydration (González-Alonso et al., 1998; González-Alonso et al., 1995, 1997). Instead, it appears that the significantly greater increases in T_C and HR together with the reduced BV with dehydration compared to maintained euhydration may contribute to an impaired filling of the LV following HA as previously discussed.

5.4.4 – Resting thermal, haematological and haemodynamic responses to heat acclimation

HA did not result in a decrease in resting T_C (Table 5.1). A recent meta-analysis reported that 5-days of HA typically results in a ~0.17°C decrease in resting T_C, with minimal additional reductions induced by longer protocols (Tyler et al., 2016). The pre-HA experimental trials in the current study would have been the participants' third and fourth heat exposures prior to undergoing HA. However, it does not seem likely this parameter was altered by heat exposures over this period. Pre-HA trials were randomised and counterbalanced for hydration status amongst participants. It is also unlikely differences were masked by circadian variation or sampling error. This is because of intra-individual standardisation of the time of day of experimentation, thermistors used, and resting period observed within this study. In addition, both post-HA experimental trials were conducted within ≤10 days of the final HA exposure to ensure adequate recovery and additional experimentation conducted in Chapter 4. According to the limited data in the literature, a reduction in T_C persists for at least 8 days without heat exposure (Neal et al., 2016b) and exhibits rapid re-induction (Weller, Linnane, Jonkman, & Daanen, 2007). Therefore, these recordings are likely reflective of a stable resting T_C throughout HA and it is unclear why T_C was not reduced by the present HA intervention.

PV was not increased with the present euhydrated HA intervention. The slight ~2% decrease in Hb_{mass} is unlikely to have masked an expansion of BV or PV. The reason for this slight decrease is not entirely clear. Regardless, calculating RCV, BV and PV with uncorrected values also yielded similar results. Relative changes in BV and PV with HA from venous blood constituents (Dill & Costill, 1974) also did not alter the responses observed (data not shown). The reason PV may not have increased with euhydrated HA may have been due to an insufficient stimulus for renal water conservation (Cheuvront & Kenefick, 2014). The present data therefore suggest BV or PV were not expanded following HA. Since T_C and BV were unaltered by HA in the present study, the similarities in resting HR are perhaps not surprising.

5.4.5 – Limitations

The present thermoregulatory and HR data suggest HA may not have been induced in the current subjects. However, this seemingly absent effect is more likely due to a combination of the experimental procedures, exercise modality and environmental conditions. Firstly, clear adaptive responses were evident in subjects throughout HA, with increases in sweat rate and power output along with reductions in exercising HR that were similar to those observed in Chapter 4. Secondly, the apparent similarities during bouts of semi-recumbent cycling exercise in the pre- and post-HA trials may be in part due to the periods used to determine hydration status (i.e. non-exercising periods) during which T_C declined, as well as the relatively short periods of semi-recumbent cycling used to determine cardiovascular function. In support of this, during euhydrated periods of upright cycling both average and end-exercise T_C and HR were lowered by 0.3°C and 6 beats·min^{-1} respectively following acclimation (data not shown). Furthermore, given the level of experimental control, reproducibility of the non-invasive LV measurements and the clear differences observed via manipulations of hydration status, it is likely that differences in responses observed between pre-HA and post-HA trials may be related to an adaptive response brought about by HA. Future investigations are however required to determine the responses to work-matched HR control exercise in cool conditions to conclusively determine the specificity of these responses.

HA appears to have only resulted in small adjustments in sweating rate. A ~0.2 L·min^{-1} increase in sweat rate did occur with HA, but the magnitude of this improvement was likely dampened by the differences in heat stress between experimental trials and HA. As such the prescribed fluid intake in the acclimated euhydrated trial was >200 ml greater than sweat lost. However, by design fluid prescription intentionally matched 90% of expected losses to prevent net increases in total body water throughout the course of the trial. Therefore, given the similar slight reductions in body mass as well as the maintained BV and PV observed in pre-HA and post-HA trials, the additional fluid consumed had no discernible impact on haematological or cardiovascular responses of the present study.

5.4.6 – Conclusions

This study provides evidence that the central haemodynamic responses during prolonged euhydrated exercise in the heat are slightly altered following exercise HA with controlled HR. The acute reduction in SV characteristic of cardiovascular strain when T_C and HR are elevated (González-Alonso et al., 1998; González-Alonso et al., 2000a; Trinity et al., 2010) did not occur following HA via the maintenance of EDV. Since HA did not lower T_C or HR or result in a greater BV during euhydrated exercise, this response may instead be due to altered cardiovascular function. However, diastolic or systolic LV function was not determined in the present study. In addition, strong evidence is provided that euhydrated HA with controlled HR does not alter haematological or haemodynamic responses to matched levels of progressive dehydration beyond 3% of body mass. These findings support the notion that HA improves cardiovascular stability during exercise heat stress but indicates $\dot{Q}$ decreases with progressive dehydration, possibly via impaired venous return and LV filling.

CHAPTER 6

Study 3: Effect of controlled heart rate heat acclimation with dehydration on thermoregulatory and cardiovascular function during prolonged exercise in the heat

6.0 – Abstract

Dehydration via fluid restriction may enhance the fluid regulatory response to HA, resulting in a sustained expansion of plasma and blood volume. However, greater losses of plasma volume may occur during acute exercise induced dehydration and heat stress, potentially leading to significant hyperthermia and diminished cardiac output. This study investigated the thermoregulatory, haematological and haemodynamic responses to matched levels of dehydration before and after dehydrated HA. Nine males underwent 10 days of HA with controlled heart rate and fluid restriction. Average daily body mass deficits were 2.83 ± 0.46%. Two trials of prolonged exercise heat stress (33°C and 50% relative humidity) with or without progressive dehydration were performed pre- and post-HA. Core temperature, blood volume, mean arterial pressure and left ventricular volumes were measured at rest and during bouts of submaximal semi-recumbent cycling at 20, 100 and 180 min. Resting core temperature, heart rate and blood volumes were similar post-HA ($P > 0.05$). Similar 1.0 ± 0.5 $L \cdot min^{-1}$ increases in cardiac output were observed between exercise bouts when euhydration was maintained pre- and post-HA ($P > 0.05$). Over the same period mean arterial pressure was maintained (89 ± 12 mmHg) whilst systemic vascular resistance decreased slightly ($P < 0.05$). Progressive dehydration resulted in matched 3.63 ± 0.48% body mass deficits pre- and post-HA ($P > 0.05$) and was accompanied by greater 0.4 ± 0.2°C and 11 ± 10 $beats \cdot min^{-1}$ elevations in core temperature and heart rate compared to euhydration ($P < 0.05$). Between bouts of exercise, similar significant 11 ± 3%, 1.3 ± 1.0 $L \cdot min^{-1}$ and 6 ± 7 mmHg reductions in plasma volume, cardiac output and mean arterial pressure occurred with dehydration pre- and post-HA ($P < 0.05$). Parallel reductions in end diastolic and stroke volume also occurred over time, both averaging -27 ± 7 ml ($P > 0.05$). These findings indicate that following dehydrated exercise HA, acute dehydration beyond ~3% of body mass results in marked hyperthermia and reductions in blood volume, cardiac output and mean arterial pressure that are similar to pre-HA levels.

6.1 – Introduction

Heat acclimation (HA) is suggested to improve cardiovascular stability during submaximal exercise in the heat (Periard et al., 2015; Sawka et al., 2011; Taylor, 2014; Tyler et al., 2016). Various adaptive responses that occur with acclimation support these improvements. These include reductions in exercising core temperature (T_C) and heart rate (HR; Rowell et al., 1967; Nielsen et al., 1993; Patterson et al., 2004b; Neal et al., 2016) along with increases in the sensitivity and rate of sweating and skin blood flow (Lorenzo & Minson, 2010; Patterson et al., 2004b). HA also typically results in an expansion of plasma volume (PV; Senay et al., 1976; Wyndham et al., 1976; Patterson et al., 2004a) and hence a greater blood volume (BV), which potentially enhances ventricular filling (Senay, 1986). Together these responses may contribute to an increase in stroke volume (SV), while cardiac output ($\dot{Q}$) may be maintained (Nielsen et al., 1997; Rowell et al., 1967) or increased (Nielsen et al., 1993) following acclimation. However, PV responses to HA may also be transient in nature (Wyndham et al., 1968a) and reductions in PV throughout HA previously observed is possibly related to sub-optimal thermal and fluid regulatory stressors within acclimation protocols (Taylor, 2014). HA with controlled hyperthermia and permissive dehydration has been shown to result in a sustained expansion in PV (Patterson et al., 2004a). However, if progressive dehydration develops throughout a subsequent acute bout of submaximal exercise, a relatively greater reduction in PV may also occur (Harrison et al., 1981; Patterson et al., 2014). To date, direct measurements of left ventricular (LV) volumes during prolonged exercise heat stress following HA with dehydration have not been determined. In the previous chapter it was demonstrated that the transient increases in $\dot{Q}$ as euhydrated exercise progresses following HA was related to a maintenance of SV as HR and T_C progressively rose (Chapter 5). This occurred in the absence of an expansion in PV or BV. Moreover, during prolonged exercise with progressive dehydration, the increases in T_C and HR were greater than euhydrated exercise, while reductions in $\dot{Q}$ and mean arterial pressure (MAP) were similar to those observed in unacclimated individuals (González-Alonso

et al., 1998; Montain & Coyle, 1992b). The purpose of the current investigation was to determine the thermal, haematological and haemodynamic responses to prolonged exercise with maintained euhydration or progressive dehydration following dehydrated HA.

Acclimation was previously believed to result in better maintenance of PV during exercise induced dehydration (Senay et al., 1976). The greater loss of more dilute sweat that occurs with HA (Patterson et al., 2004a) increases extracellular osmolality, creating a gradient that promotes fluid shifts into this compartment, potentially buffering otherwise sweat-dependent reductions in PV in acclimated individuals (Sawka et al., 2007). Instead however, acute dehydration results in greater losses of PV in acclimated humans (Harrison et al., 1981; Patterson et al., 2014) and might be expected to result in impaired or similar thermoregulatory and cardiovascular responses to exercise induced dehydration and heat stress compared to euhydration.

Previously, individuals who have undergone dehydrated HA with controlled hyperthermia exhibited a lower T_C and HR during an acute bout of exercise heat stress and dehydration of ~2.7% (Patterson et al., 2004a). However, with dehydration of 3-5% of body mass induced by diuretics or overnight fluid restriction prior to exercise in the heat, no improvements in exercising T_C are observed, while SV and $\dot{Q}$ remain suppressed compared to euhydrated exercise (Buskirk et al., 1958; Ikegawa et al., 2011). Exercise in the heat may not be typically undertaken in a dehydrated state, but these findings suggest more pronounced dehydration might blunt the otherwise improved thermoregulatory and cardiovascular responses to dehydrated HA. Therefore, this study aimed to characterise the haematological, thermoregulatory and cardiovascular responses to matched levels of dehydration beyond 3% before and after dehydrated HA with controlled HR. A further aim was to determine the effect of acute dehydration on these responses compared to euhydrated exercise following HA.

It was hypothesised that dehydrated HA with controlled HR would result in i) improved thermoregulatory and cardiovascular responses during prolonged

euhydrated exercise in the heat. However, ii) progressive dehydration >3% would result in significant haemoconcentration and hyperthermia and would be accompanied by decreases in SV and $\dot{Q}$ compared to euhydration, that would be iii) similar to responses observed during pre-HA exercise in the heat.

6.2 – Methodology

6.2.1 – Participants

Following completion of a health questionnaire and provision of written informed consent, nine male recreational cyclists and triathletes (age = 32.4 ± 5.4 years, height = 178 ± 7 cm, body mass = 75.5 ± 4.3 kg and $\dot{V}O_{2max}$ = 4.12 ± 0.43 $L \cdot min^{-1}$) took part in the experiment. The procedures of the study were approved by Anti-Doping Lab Qatar and conformed with the declaration of Helsinki.

6.2.2 – Experimental design

All participants underwent a 10-day HR controlled HA intervention with fluid restriction. Pre- and post-HA experimental procedures were identical to those undertaken in Chapter 5, assessing the thermoregulatory and cardiovascular responses to prolonged exercise in the heat. The experimental procedures are outlined in Figure 6.1 and described briefly below for reference.

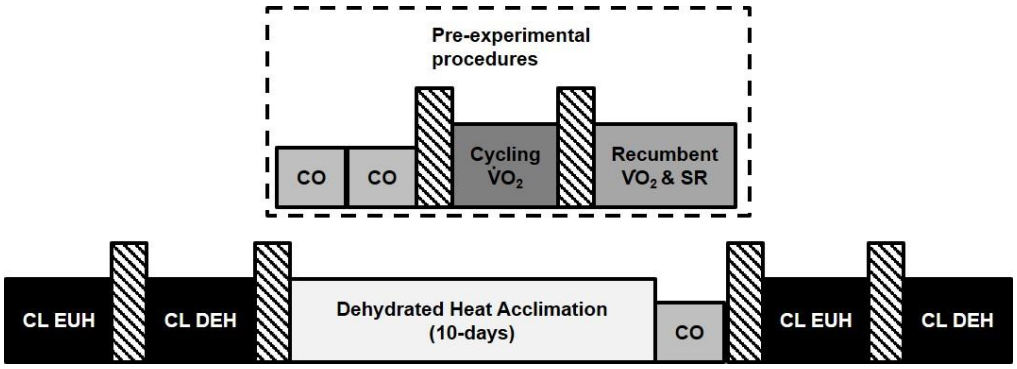


Figure 6.1: Outline of pre-experimental procedures (top) and main experimental intervention (bottom). Experimental trials were identical to those conducted in in Chapter 5. Heat acclimation exposures were conducted with fluid restriction to invoke similar daily body mass deficits.

6.2.3 – Pre-experimental procedures

Prior to the experimental interventions haemoglobin mass (Hb_{mass}), $\dot{V}O_{2max}$ and sweating rate were determined. Duplicate measures of Hb_{mass} (Schmidt & Prommer, 2005) were used to determine red cell (RCV), BV and PV from

subsequent venous blood samples. The measurement was repeated 24 h after the final HA exposure to test the assumption Hb_{mass} remained stable with dehydrated HA. An incremental cycling test to exhaustion was conducted on a cycle ergometer (Lode, Excalibur Sport, Groningen, The Netherlands) to determine $\dot{V}O_{2max}$ and prescribe exercise intensities for experimental trials and heat acclimation training. The test began at 90 W with four 30 W step-increments, each occurring every 4 min. Thereafter resistance increased by 1 W every 2 s until volitional fatigue. $\dot{V}O_2$ (Oxycon Pro, Jaeger, CareFusion, Hoechberg, Germany) and HR (T31, Polar, Kempele, Finland) were measured continuously throughout and averaged during the final minute of each stage. $\dot{V}O_{2max}$ was defined as the highest minute average prior to volitional fatigue. The power output and HR associated with 65% $\dot{V}O_{2max}$ were calculated for each participant using linear regression.

On a separate occasion, participants completed a maximal incremental semi-recumbent cycling test to exhaustion followed by 60 min of exercise in the heat. This was to determine exercise intensities during measurement of LV volumes, familiarise participants to the environment and protocol and to measure participants hourly sweat rate. Participants initially rested on a semi-recumbent cycle ergometer (Ergoselct, Ergoline GMbH, Germany) for 5 min in cool ambient conditions in the main laboratory (~20°C). The ergometer was tilted, and echocardiographic assessments were conducted to determine the clarity of the acoustic window and presence of a preserved ejection fraction (i.e. > 55%) whilst a visual assessment of LV wall motion symmetry was also used to determine study eligibility. The ergometer was returned to a semi-recumbent position and participants completed a step-incremental test to exhaustion at a self-selected cadence. $\dot{V}O_2$ and HR were recorded throughout. Power output at 50% $\dot{V}O_{2max}$ was then calculated via linear regression. Following the test, participants rested in the laboratory for 60 min before entering an environmental chamber with conditions of 33°C and 50% relative humidity. They then completed 60 min of exercise on a cycle ergometer at 65% $\dot{V}O_{2max}$ while *ad libitum* fluid intake was recorded. Hourly sweat rate was determined by the change in nude body mass before and after exercise.

6.2.4 – Experimental trials

Two experimental trials were conducted on separate occasions in a randomised order before HA (Figure 6.2). Trials consisted of prolonged exercise in the heat (33°C and 50% relative humidity) while maintaining euhydration or becoming progressively dehydrated via altered fluid intake. In euhydration trials, fluid intake matched 90% of predicted hourly sweat losses. This was to ensure; i) body mass did not increase pre- to post-exercise and ii) a body mass deficit >1% did not occur. Conversely, progressive dehydration was achieved via fluid intake that equalled 10% of expected hourly sweat losses. Following HA, the trials were repeated in the same order with adjustments made to prescribed fluid intake to account for changes in sweat rate and thus match hydration status between pre- and post-HA experimentation.

Participants were instructed to attend the laboratory in a well-hydrated state having consumed a similar diet over the preceding 24 h prior to experimentation. Participants provided a urine sample, measured their nude body mass and inserted a rectal thermistor. Once dressed in socks and cycling shorts, participants lay supine in the main laboratory. A cannula was inserted into a right antecubital vein and flushed with sterile saline and temperature thermistors were applied to the skin for the measurement of average T_{sk} (Ramanathan, 1964). After 10 min supine rest, a 2 min period was observed for the measurement of resting T_C and HR before a duplicate measurement of blood pressure was taken with a sphygmomanometer. A 2 ml resting blood sample was collected into a lithium heparin syringe (PICO 50, Radiometer, Brønshøj, Denmark) and analysed without stasis in triplicate in a blood gas analyser (ABL 90FLEX, Radiometer, Brønshøj, Denmark).

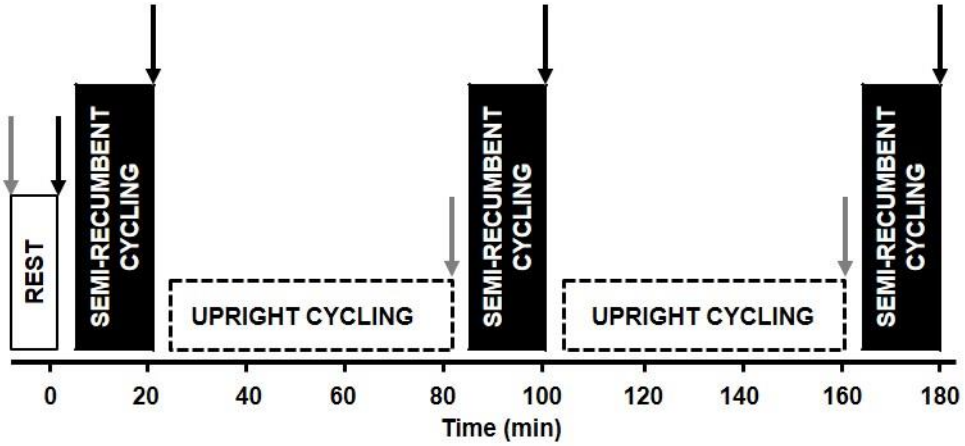


Figure 6.2: Schematic of experimental trials reproduced from Chapter 5. Nude body mass (grey arrows) was measured before three bouts of semi-recumbent cycling. T_C, T_{sk}, MAP and LV volumes were measured prior to the end of semi-recumbent exercise bouts (black arrows). Bouts were interspersed with 60 min periods of upright cycling, during which fluid intake differed depending on hydration strategy.

Participants then entered the environmental chamber and mounted the recumbent cycling ergometer. A resting echocardiographic assessment of LV volumes was conducted before participants began exercising at 50% $\dot{V}O_{2max}$ (140 ± 19 W) for a period of 6 min. Blood pressure was measured in duplicate and the ergometer was tilted for exercising measurement of LV volumes. Following image acquisition T_{sk} and T_C were recorded, and a blood sample was obtained (20 min). Bouts of semi-recumbent exercise were repeated at

approximately 100 and 180 min. In between these periods participants exercised upright on cycle ergometer for 60 min at 65% $\dot{V}O_{2max}$ (176 ± 24 W). A fan placed in front of the ergometer provided a constant windspeed of 3 $m \cdot s^{-1}$. After each 60 min period, participants towel dried non-evaporated sweat and measured their nude body mass within the chamber before each bout of semi-recumbent exercise (Figure 6.2). Fluid was provided in the form of 0.1% electrolyte solution drink (HIGH5 ZERO, H5 Ltd, Bardon, UK) at 15 min intervals throughout each bout of upright cycling in four equal boluses.

6.2.5 – Echocardiography

2D echocardiographic images were acquired in the same order using a cardiac ultrasound machine and S5-1 5 MHz sector array probe (CX50 POC, Philips Healthcare, The Netherlands). A short axis of the LV base, parasternal long-axis, apical 4- and apical 2-chamber views were recorded. All 2D images were acquired at a frame rate of 60 Hz at the end of expiration. Care was taken to ensure similar image depth between trials and time points.

Images were analysed offline using dedicated computer software (Q-Station, Version 3.8.5, Phillips Healthcare, The Netherlands). Trials were de-identified and analysed in a random order at the end of the data collection period to minimise the effect of confirmation bias. LV volumes were determined using the Simpson's method of bi-plane disc summation using a minimum of three cardiac cycles. Consecutive cycles were analysed where possible. LV mass was calculated using the method of Schiller et al. (1989). HR was measured from a 3-lead electrocardiogram. $\dot{Q}$ was calculated as HR multiplied by SV. Systemic vascular resistance (SVR) was calculated as MAP divided by $\dot{Q}$.

6.2.6 – Dehydrated heat acclimation

Following baseline experimentation, participants had a minimum of 24 h complete rest before undertaking a 10-day exercising HA intervention. The intervention design was identical to the dehydrated HA protocol of Chapter 4. Briefly, the intervention consisted of 10 daily 90 min exposures to 40°C and 40% relative humidity. Sessions were conducted at a similar time each day.

Exercise was governed during each session by computer software (LODE ergometry manager, LODE, Groningen, The Netherlands). The initial 15 min period of each session was the same intensity as the constant load trials (i.e. 65% $\dot{V}O_{2max}$). Workload was then automatically adjusted with the aim of maintaining exercising HR at a similar value associated with this intensity (142 ± 11 beats·min^{-1}). Equal boluses of fluid were provided every 15 min to replace ~10% of expected sweat losses. Fluid intake was adjusted daily to match participants sweat rate during the previous session. This was to induce a similar level of moderate dehydration with each exposure.

Hydration status and nude body mass were determined on arrival to the laboratory each day. Once dressed, participants observed a 10 min period of quiet supine rest in the laboratory while they were instrumented with skin thermistors. Resting T_C and HR were recorded during the final 2 min of this period. On days 1, 5 and 10 of HA a blood sample was collected into a vacutainer via venepuncture of an antecubital vein at the end of the resting period to minimise the effect of changes in posture. Blood samples were also collected immediately post exercise after participants transferred to a bed adjacent to the chamber. Blood samples were analysed using a complete blood count (UniCel DxH 800 Coulter Analysis System, Beckman Coulter, CA, USA). Changes in haemoglobin concentration, haematocrit and mean corpuscular haemoglobin content were used to determine absolute changes in RCV, PV and BV from the calculations outlined in Chapter 3.

6.2.7 – Data analysis

An ANOVA with repeated measures was used to determine any changes in resting measurements during the 10-day HA period. A 2-way (hydration x acclimation) ANOVA with repeated measures analysis was used to test for differences in thermoregulatory and cardiovascular responses at rest. A separate trial (4) x time (3) ANOVA with repeated measures analysis was conducted to test differences between thermoregulatory, thermal, haemodynamic and haematological responses to bouts of semi-recumbent cycling at 20, 100 and 180 min of exercise before and after HA. Mauchly's test

was used to test the assumption of Sphericity. In cases where this assumption was violated a Greenhouse-Geisser correction factor was applied. Bonferroni *post-hoc* testing was employed to determine where pairwise differences occurred. All statistical analyses were conducted using SPSS (Version 21, IBM, Armonk, US). Results are reported as mean ± SD unless otherwise stated. Significance was set at $P < 0.05$. A $P \leq 0.1$ was considered a statistical trend. In such cases, main effect sizes are also presented using partial eta squared values for analysis of variance ($\eta_p^2 \leq 0.02$: small; 0.02-0.13: medium; 0.13-0.26: large; Cohen 1988).

6.3 – Results

Nine participants completed pre-HA experiments and the HA intervention. However, one participant fell ill in between day 10 of HA and the post-HA experimental trials. Therefore, the data presented below are n = 9 for HA and n = 8 for pre- and post-HA experimental trials.

6.3.1 – Heat acclimation summary

The average environmental conditions during the HA intervention were 40.01 ± 0.36°C and 40.1 ± 1.2% relative humidity and did not differ between days (all $P > 0.05$). A summary of the responses throughout days 1, 5 and 10 of the HA period is presented in Table 6.1. Participants attended the laboratory in a well-hydrated state each day. Fluid prescribed on day 1 of HA was 217 ± 33 ml and was increased significantly, reaching 249 ± 39 ml on day 10 ($P = 0.024$) to match the increase in sweating rate ($P = 0.012$). Despite this increase in fluid intake there was a significant effect of HA day on body mass deficit ($P = 0.048$, Table 6.1). However, pairwise analyses did not identify a significant difference between daily levels of dehydration, averaging 2.83 ± 0.46% throughout the intervention (all $P > 0.05$). Dehydration was associated with similar significant levels of haemoconcentration during exercising exposures ($P = 0.30$, Table 6.1), with an average 7.9 ± 2.0% decrease in BV on days 1, 5 and 10 of HA. Average T_C during the final 75 min of each exposure (i.e. the HR-controlled period) averaged 38.3 ± 0.2°C and did not differ between days ($P = 0.42$, Table 6.1). T_{sk} was not significantly lowered by HA and averaged 36.5 ± 0.6°C throughout HA.

Table 6.1: Average resting and exercising responses to heat acclimation with controlled heart rate and dehydration.

	Day 1	Day 5	Day 10	Acclimation average	Main effect	
Rest						
Body mass (kg)	75.9 ± 4.5	75.7 ± 4.2	75.4 ± 4.0	75.8 ± 4.0	$P = 0.18$	$\eta_p^2 = 0.19$
USG	1.014 ± 0.007	1.016 ± 0.008	1.013 ± 0.006	1.016 ± 0.001	$P = 0.09$	$\eta_p^2 = 0.20$
HR (beats·min^{-1})	57 ± 7	56 ± 7	53 ± 7	56 ± 6	$P = 0.046^{\#}$	$\eta_p^2 = 0.32$
T_C (°C)	37.0	36.9	36.9	37.0 ± 0.3	$P = 0.07$	$\eta_p^2 = 0.28$
Exercise						
Work done (kJ)	727 ± 90	784 ± 78*	822 ± 112	795 ± 97	$P = 0.04^{\#}$	$\eta_p^2 = 0.50$
Body mass deficit (%)	-2.69 ± 0.46	-2.78 ± 0.47	-2.94 ± 0.52	-2.83 ± 0.46	$P = 0.048^{\#}$	$\eta_p^2 = 0.39$
15 min HR (beats·min^{-1})	154 ± 13	147 ± 9*	144 ± 8*	-	$P = 0.03^{\#}$	$\eta_p^2 = 0.52$
75 min HR (beats·min^{-1})	144 ± 9	144 ± 9	143 ± 9	144 ± 8	$P = 0.64$	$\eta_p^2 = 0.07$
75 min T_C (°C)	38.4 ± 0.1	38.3 ± 0.2	38.4 ± 0.2	38.3 ± 0.2	$P = 0.42$	$\eta_p^2 = 0.10$
Average T_{sk} (°C)	36.6 ± 0.73	36.6 ± 0.49	36.2 ± 1.08	36.5 ± 0.63	$P = 0.18$	$\eta_p^2 = 0.17$
Sweat lost (L)	2.25 ± 0.33	2.34 ± 0.33	2.46 ± 0.34	2.37 ± 0.32	$P = 0.021^{\#}$	$\eta_p^2 = 0.43$
ΔPV from rest (%)	-12.53 ± 3.27	-13.39 ± 3.44	-14.93 ± 3.42	-	$P = 0.11$	$\eta_p^2 = 0.17$

* significantly different from Day 1. # Significant main effect.

There was a small but significant effect of HA on resting HR (P = 0.046), while T_C was similar each day (P > 0.07, Table 6.1). HR was stable over the final 75 min of each exposure, and values were similar between days of HA (P = 0.65, Table 6.1). This was achieved by progressive increases in average power over the same period, increasing from 121 ± 17 to 148 ± 21 W between days 1 and 10 of HA (P = 0.006).

6.3.2 – Resting thermal, haemodynamic and haematological response to acclimation

All participants were euhydrated prior to each experimental trial as indicated by similar USG (1.014 ± 0.007) and nude body mass measurements (75.8 ± 4.4 kg, both P > 0.05). Resting thermal, haemodynamic and haematological parameters are displayed in Table 6.2. Resting T_C was similar between pre- and post-HA trials. Despite the significant reduction in resting HR throughout HA, this reduction was not evident at rest in post-HA experimental trials, as values were similar to pre-HA (P > 0.05, Table 6.2) Resting $\dot{Q}$ was also unaltered by HA and was associated with similar resting EDV, ESV and SV between pre- and post-HA experimental trials (all P > 0.05, Table 6.2). Total Hb_{mass} remained unchanged with acclimation (895 ± 85 and 883 ± 88 g pre- and post-HA, respectively) and there were also no changes in RCV, BV or PV (all P > 0.05, Table 6.2).

Table 6.2: Thermal, haemodynamic and haematological parameters at rest prior to each experimental trial.

	Maintained Euhydration		Progressive dehydration		Hydration	Acclimation	Interaction
	Pre-HA	Post-HA	Pre-HA	Post-HA			
Thermal							
T_C (°C)	37.0 ± 0.3	37.0 ± 0.2	36.9 ± 0.2	36.9 ± 0.3	$P = 0.04^{\#}$ $\eta_p^2 = 0.48$	$P = 0.38$ $\eta_p^2 = 0.11$	$P = 0.92$ $\eta_p^2 = 0.002$
T_{sk} (°C)	33.7 ± 0.5	33.6 ± 0.6	33.7 ± 0.7	33.3 ± 1.0	$P = 0.55$ $\eta_p^2 = 0.05$	$P = 0.14$ $\eta_p^2 = 0.29$	$P = 0.11$ $\eta_p^2 = 0.33$
Haemodynamic							
HR (beats·min^{-1})	57 ± 7	59 ± 10	57 ± 5	58 ± 9	$P = 0.77$ $\eta_p^2 = 0.01$	$P = 0.36$ $\eta_p^2 = 0.12$	$P = 0.46$ $\eta_p^2 = 0.06$
EDV (ml)	162 ± 25	166 ± 23	163 ± 22	165 ± 25	$P = 0.97$ $\eta_p^2 = 0.000$	$P = 0.32$ $\eta_p^2 = 0.14$	$P = 0.73$ $\eta_p^2 = 0.02$
ESV (ml)	67 ± 14	66 ± 15	68 ± 13	66 ± 13	$P = 0.73$ $\eta_p^2 = 0.09$	$P = 0.28$ $\eta_p^2 = 0.16$	$P = 0.91$ $\eta_p^2 = 0.002$
SV (ml)	95 ± 12	100 ± 9	96 ± 11	99 ± 12	$P = 0.68$ $\eta_p^2 = 0.19$	$P = 0.24$ $\eta_p^2 = 0.19$	$P = 0.48$ $\eta_p^2 = 0.07$
$\dot{Q}$ (L·min^{-1})	5.38 ± 0.78	5.88 ± 1.06	5.48 ± 0.84	5.71 ± 1.05	$P = 0.71$ $\eta_p^2 = 0.02$	$P = 0.22$ $\eta_p^2 = 0.21$	$P = 0.26$ $\eta_p^2 = 0.18$
Haematological							
RCV (ml)	2757 ± 270	2761 ± 272	2759 ± 275	2748 ± 275	$P = 0.65$ $\eta_p^2 = 0.03$	$P = 0.76$ $\eta_p^2 = 0.01$	$P = 0.09$ $\eta_p^2 = 0.35$
BV (ml)	6257 ± 643	6259 ± 641	6258 ± 680	6356 ± 670	$P = 0.46$ $\eta_p^2 = 0.08$	$P = 0.33$ $\eta_p^2 = 0.14$	$P = 0.32$ $\eta_p^2 = 0.14$
PV (ml)	3499 ± 421	3501 ± 410	3498 ± 429	3603 ± 433	$P = 0.44$ $\eta_p^2 = 0.09$	$P = 0.29$ $\eta_p^2 = 0.16$	$P = 0.29$ $\eta_p^2 = 0.16$

$^{\#}$ Significant main effect.

6.3.3 – Hydration status during exercise

Prescribed fluid intake was significantly greater post-HA compared to pre-HA in maintained euhydration trials, increasing from 2.62 ± 0.42 to 2.99 ± 0.47 L (P = 0.003). Conversely, fluid intake was not significantly altered following HA in progressive dehydration trials, averaging 0.30 ± 0.7 and 0.33 ± 0.5 L pre- and post-HA, respectively (P > 0.39). Sweat losses throughout the experimental trials were similar pre- to post-HA (all P > 0.05), as were changes in body mass (all P > 0.05). Body mass after 180 min of exercise with maintained euhydration was similar to resting values (-0.6 ± 0.3% pre-HA and -0.3 ± 0.4% post-HA, respectively, P = 0.21). By comparison, significantly greater body mass deficits occurred with progressive dehydration (both P < 0.0001), averaging 3.6 ± 0.5% pre-HA and 3.7 ± 0.5% post-HA, respectively (P = 0.47, Figure 6.3).

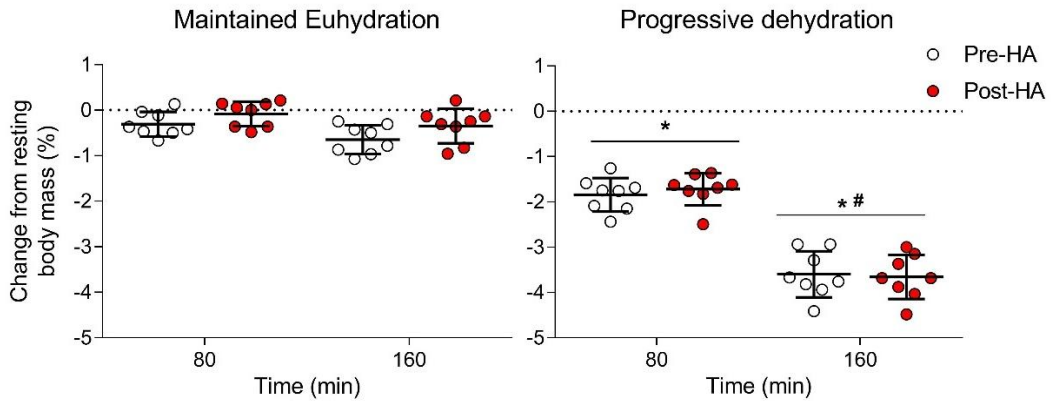


Figure 6.3: Changes from rest in nude body mass following prolonged exercise in the heat with maintained euhydration (left) and progressive dehydration (right). White and red circles are individual pre- and post-HA changes, respectively. N = 8. # Significantly different from 80 min. * Significantly greater than euhydration.

6.3.4 – Effect of acclimation on exercise with maintained euhydration

T_C reached 38.5 ± 0.4°C after 180 min (P < 0.001) whilst T_{sk} was maintained at 34.2 ± 0.6°C (P = 1.00) throughout exercise with maintained euhydration pre-HA. These responses were not significantly altered following HA, as T_C and T_{sk} averaged 38.2 ± 0.2°C and 34.1 ± 0.7°C, respectively (both P > 0.05,

Figure 6.4). Similar to the resting haematological responses, HA had no effect on RCV, PV or BV during exercise with maintained euhydration (all P >0.05, Table 6.3). There was also no effect of exercise or HA on plasma electrolyte concentrations, whilst glucose and lactate concentrations were also unaltered (all P > 0.05, Table 6.3).

HR responses to exercise with euhydration were unaltered by HA and averaged 135 ± 14 pre- and 134 ± 9 beats·min^{-1} post-HA at 180 min, respectively (P = 1.00, Figure 6.6). ESV and EDV remained unchanged throughout exercise and were unaffected by HA. SV was similar before and after HA after 180 min of exercise, averaging 112 ± 11 and 111 ± 12 ml pre- and post-HA, respectively (P = 1.00, Figure 6.6). $\dot{Q}$ increased between 20 and 180 min of exercise with euhydration (P < 0.05) but was also unaffected by HA, averaging 15.0 ± 2.3 and 15.1 ± 2.0 L·min^{-1} at the end of exercise pre- and post-HA, respectively (P = 1.00). MAP remained unchanged throughout exercise, averaging 87 ± 10 pre- and 88 ± 12 mmHg post-HA, respectively (P = 1.00). SVR progressively declined 9 ± 5% during exercise pre-HA (P = 0.004) and this response was unaltered post-HA (P = 1.00, Figure 6.5).

Table 6.3: BV, PV and RCV during each bout of semi-recumbent cycling

		Maintained Euhydration		Progressive dehydration		Trial	Time	Interaction
		Pre-HA	Post-HA	Pre-HA	Post- HA			
BV (ml)	20 min	5832 ± 604	5910 ± 605	5884 ± 635	5956 ± 679	$P = 0.07$ $\eta_p^2 = 0.27$	$P = 0.006^{\#}$ $\eta_p^2 = 0.66$	$P < 0.001^{\#}$ $\eta_p^2 = 0.82$
	100 min	5873 ± 526	5910 ± 554	5704 ± 575*	5813 ± 634*			
	180 min	5852 ± 476	5901 ± 549	5517 ± 543*†‡	5627 ± 615*†‡			
PV (ml)	20 min	3071 ± 386	3116 ± 387	3125 ± 380	3208 ± 445	$P = 0.07$ $\eta_p^2 = 0.28$	$P = 0.005^{\#}$ $\eta_p^2 = 0.67$	$P < 0.001^{\#}$ $\eta_p^2 = 0.82$
	100 min	3122 ± 335	3155 ± 350	2945 ± 324*	3059 ± 395*			
	180 min	3092 ± 295	3143 ± 348	2757 ± 289*†‡	2876 ± 374*†‡			
RCV (ml)	20 min	2761 ± 272	2760 ± 283	2759 ± 275	2748 ± 275	$P = 0.71$ $\eta_p^2 = 0.03$	$P = 0.48$ $\eta_p^2 = 0.08$	$P = 0.43$ $\eta_p^2 = 0.10$
	100 min	2750 ± 273	2755 ± 280	2759 ± 271	2754 ± 278			
	180 min	2759 ± 272	2758 ± 280	2760 ± 274	2751 ± 278			
Hb ($g \cdot dl^{-1}$)	20 min	15.46 ± 0.81	15.32 ± 0.88	15.32 ± 0.48	15.13 ± 0.73	$P = 0.05$ $\eta_p^2 = 0.30$	$P = 0.004^{\#}$ $\eta_p^2 = 0.69$	$P < 0.001^{\#}$ $\eta_p^2 = 0.83$
	100 min	15.34 ± 0.83	15.22 ± 0.88	15.79 ± 0.46*	15.49 ± 0.68*			
	180 min	15.39 ± 0.89	15.25 ± 0.90	16.32 ± 0.48*†‡	16.00 ± 0.69*†‡			
Hct (%)	20 min	47.4 ± 2.4	47.0 ± 2.7	46.9 ± 1.5	46.5 ± 2.3	$P = 0.07$ $\eta_p^2 = 0.28$	$P < 0.001^{\#}$ $\eta_p^2 = 0.69$	$P < 0.001^{\#}$ $\eta_p^2 = 0.81$
	100 min	46.9 ± 2.7	46.6 ± 2.7	48.4 ± 1.4*	47.6 ± 2.5*			
	180 min	47.2 ± 2.7	46.8 ± 2.8	50.0 ± 1.4*†‡	49.5 ± 2.4*†‡			

* Significantly different from 20 min. † Significant difference from 100 min. ‡ Significantly different from euhydration. # Significant main effect.

Table 6.4: Metabolic and electrolyte concentrations during each bout of semi-recumbent cycling

		Maintained Euhydration		Progressive dehydration		Trial	Time	Interaction
		Pre-HA	Post-HA	Pre-HA	Post-HA			
Glucose ($mmol \cdot L^{-1}$)	20 min	4.4 ± 0.7	4.4 ± 0.6	4.2 ± 0.7	4.4 ± 0.3	$P = 0.06$	$P = 0.04^{\#}$	$P = 0.26$
	100 min	4.6 ± 0.3	4.8 ± 0.4	4.8 ± 0.4	4.9 ± 0.2	$\eta_p^2 = 0.33$	$\eta_p^2 = 0.49$	$\eta_p^2 = 0.18$
	180 min	4.6 ± 0.4	4.6 ± 0.3†	5.0 ± 0.7*	5.1 ± 0.4*			
Lactate ($mmol \cdot L^{-1}$)	20 min	2.5 ± 1.0	2.1 ± 0.6	2.0 ± 0.5	2.0 ± 0.7	$P = 0.24$	$P = 0.33$	$P = 0.01^{\#}$
	100 min	2.1 ± 0.6	1.8 ± 0.5	2.4 ± 0.3	1.9 ± 0.4	$\eta_p^2 = 0.21$	$\eta_p^2 = 0.16$	$\eta_p^2 = 0.36$
	180 min	2.1 ± 0.6	1.9 ± 0.4	2.0 ± 0.7	2.0 ± 0.4			
Sodium ($mmol \cdot L^{-1}$)	20 min	141 ± 2	142 ± 1	141 ± 1	141 ± 1	$P < 0.001^{\#}$	$P < 0.001^{\#}$	$P < 0.001^{\#}$
	100 min	142 ± 2	142 ± 1	144 ± 1*‡	145 ± 1*‡	$\eta_p^2 = 0.71$	$\eta_p^2 = 0.82$	$\eta_p^2 = 0.83$
	180 min	141 ± 4	142 ± 1	147 ± 2*†‡	148 ± 1*†‡			
Potassium ($mmol \cdot L^{-1}$)	20 min	4.2 ± 0.1	4.3 ± 0.2	4.2 ± 0.1	4.3 ± 0.1	$P = 0.04^{\#}$	$P = 0.05$	$P < 0.001^{\#}$
	100 min	4.2 ± 0.1	4.3 ± 0.1	4.2 ± 0.1	4.3 ± 0.1	$\eta_p^2 = 0.42$	$\eta_p^2 = 0.55$	$\eta_p^2 = 0.60$
	180 min	4.2 ± 0.3	4.3 ± 0.2	4.5 ± 0.2*†	4.6 ± 0.2*†			

* Significantly different from 20 min. † Significant difference from 100 min. ‡ Significantly different from euhydration. $^{\#}$ Significant main effect.

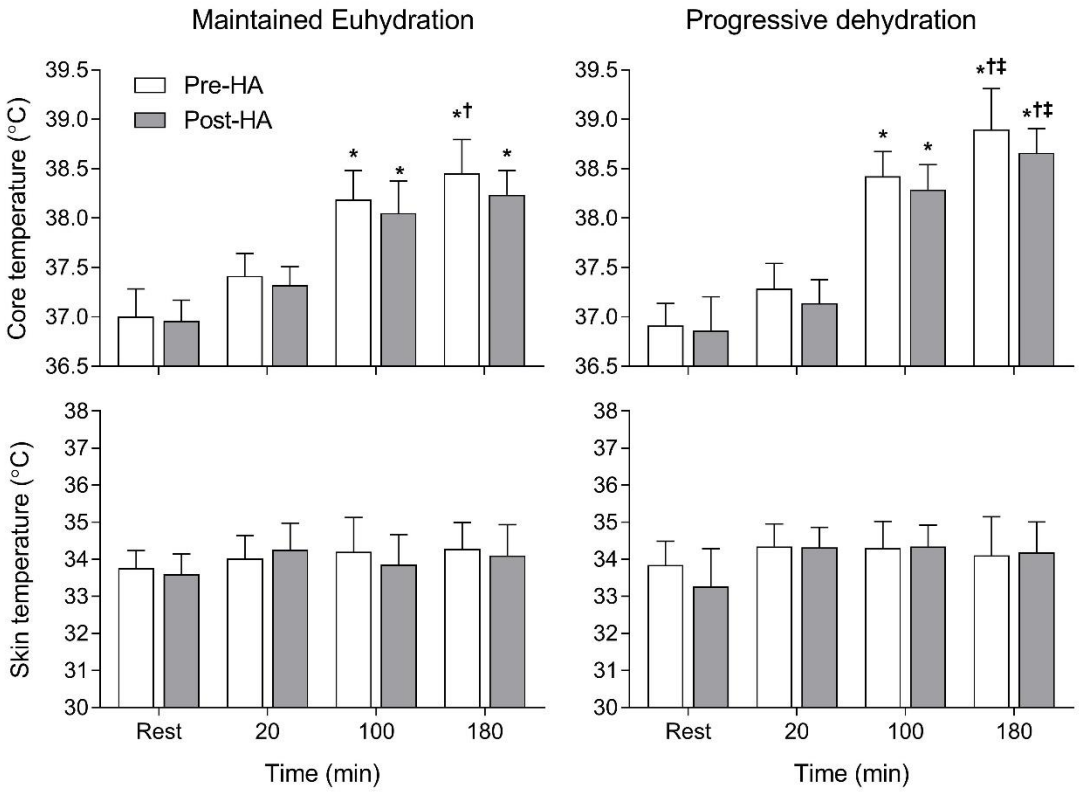


Figure 6.4: T_C and T_{sk} responses at rest and during repeated bouts of semi-recumbent cycling in the heat with maintained euhydration (left) or progressive dehydration (right) via altered fluid ingestion. Empty and filled bars are respective pre- and post-HA trials. * Significantly greater than 20 min. † Significantly greater than 100 min. ‡ Significantly different from euhydration.

6.3.5 – Effect of acclimation on exercise with progressive dehydration

T_C and T_{sk} responses to exercise with progressive dehydration were unaltered by HA, with T_C reaching 38.9 ± 0.4°C and 38.7 ± 0.2°C after 180 min (P = 0.10), and T_{sk} averaging 34.3 ± 0.8 and 34.3 ± 0.6°C during exercise (all P > 0.05) pre- and post-HA, respectively. Whole-body dehydration before and after HA were accompanied with similar degrees of haemoconcentration (Table 6.3 and Table 6.4). PV declined between 20 and 180 min by 368 ± 131ml pre-HA and 332 ± 136 ml post-HA, respectively (both P < 0.05), while RCV was unaltered (both P > 0.05). Therefore, BV after 180 min of exercise was similar before and after HA (P = 0.68, Table 6.3) and was associated with similar degrees of hypernatremia (P < 0.05, Table 6.4). Lactate responses to semi-recumbent cycling was unaltered by progressive dehydration before or after HA (all P > 0.05).

HR at 180 min with progressive dehydration was not altered by HA, averaging 145 ± 16 and 144 ± 10 beats·min^{-1} at the end of exercise pre- and post-HA, respectively (P = 1.00). ESV was maintained during exercise and was not affected by HA (all P > 0.05, Figure 6.6). EDV was also unchanged by HA and declined to similar extents during exercise by 28 ± 5 ml pre-HA and 26 ± 9 ml post-HA. Therefore, SV (89 ± 11 ml pre-HA and 92 ± 11 ml post-HA) and $\dot{Q}$ (13.1 ± 2.7 L·min^{-1} pre-HA and 13.2 ± 2.1 L·min^{-1} post-HA) were unaltered at 180 min by HA (all P > 0.05, Figure 6.6). MAP and SVR responses to exercise with progressive dehydration were not altered by HA (P > 0.05, Figure 6.5).

6.3.6 – Influence of hydration status following heat acclimation

Progressive dehydration post-HA was associated with PV (8 ± 6%) and BV (5 ± 3%) being significantly lower than maintained euhydration after 180 min (both P < 0.05). This was accompanied by significantly greater 0.5 ± 0.2°C increases in T_C with dehydration post-HA (P = 0.001). However, although seemingly large (+10 ± 10 beats·min^{-1}), HR at 180 min was not significantly greater with dehydration compared to euhydration post-HA (P = 0.18). With dehydration SV was 20 ± 8 ml lower than euhydration at 180 min post-HA (P = 0.001). This was predominantly due to a 21 ± 7 ml lower EDV with dehydration (P = 0.001), as ESV was similar between trials at the end of exercise (P = 1.00, Figure 6.6). Following HA, progressive dehydration resulted in a $\dot{Q}$ that was 1.8 ± 1.3 L·min^{-1} lower compared to euhydration at 180 min (P = 0.034). MAP and SVR were not significantly different between fluid intake strategies after 180 min of exercise following HA (both P > 0.05, Figure 6.5).

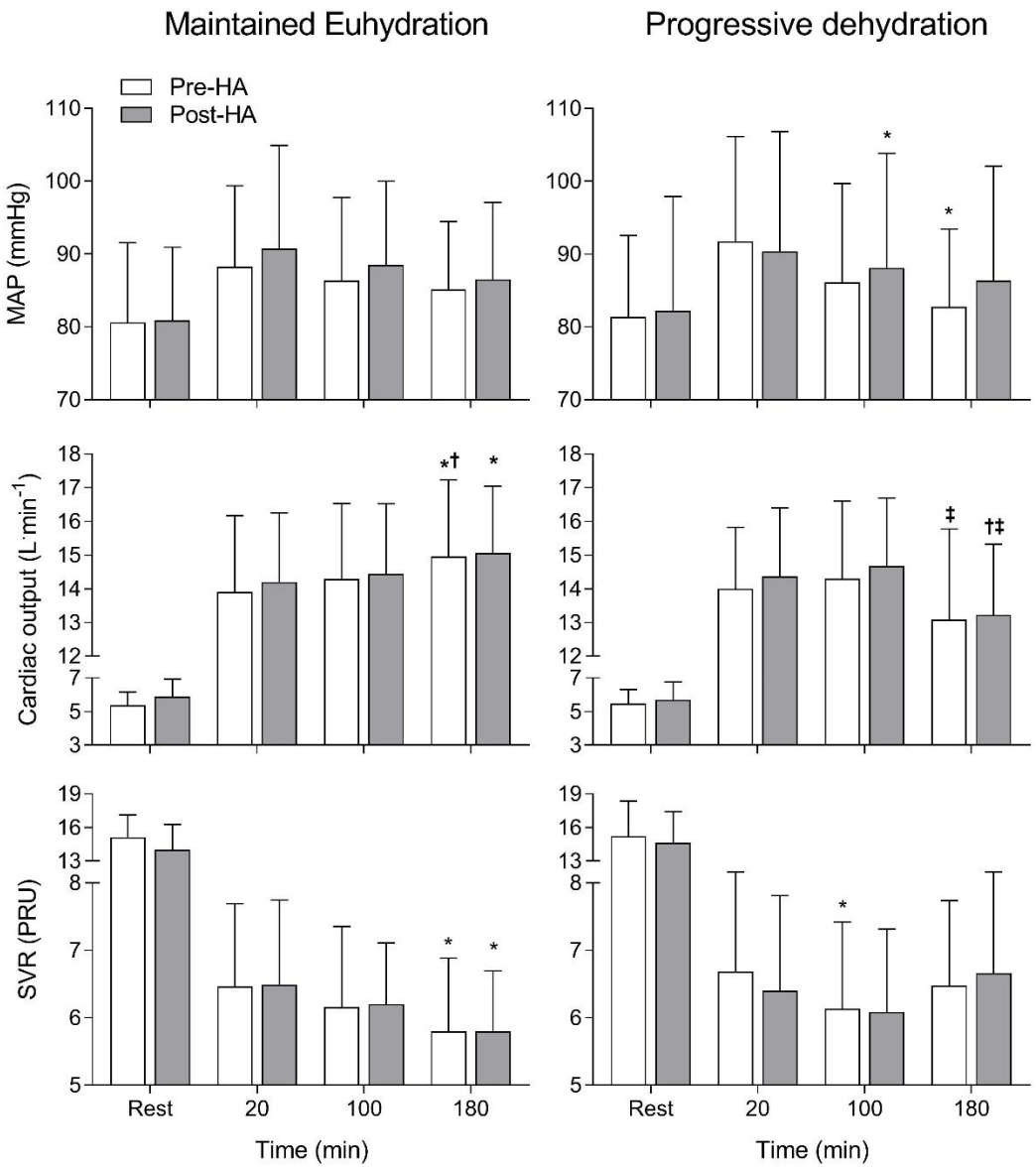


Figure 6.5: MAP, Q̇ and SVR at rest and during repeated bouts of semi-recumbent cycling with maintained euhydration (left) and progressive dehydration (right) via altered fluid ingestion. Clear and shaded bars are pre- and post-HA responses, respectively. * Significantly different from 20 min. † Significant difference from 100 min. ‡ Significantly different from euhydration.

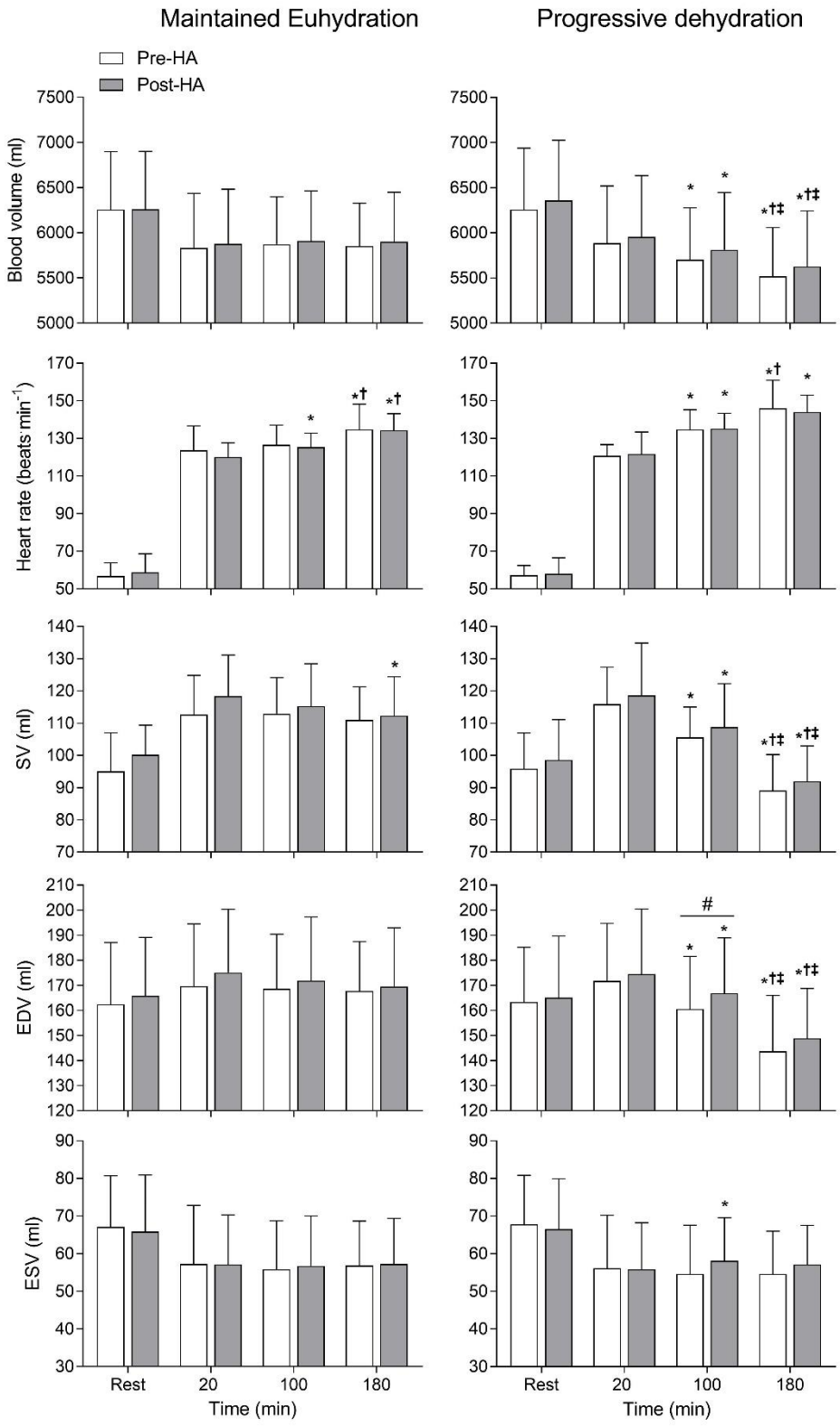


Figure 6.6: BV, HR and LV volumes at rest and during semi-recumbent cycling with maintained euhydration (left) or progressive dehydration (right). Open and filled bars are pre- and post-HA, respectively. # Significantly different to pre-HA. * Significantly different from 20 min. † Significantly different from 100 min. ‡ Significantly different from euhydration.

6.4 – Discussion

This study sought to determine the thermal, haematological and haemodynamic responses to acute exercise induced dehydration and heat stress following 10 days of dehydrated HA. An additional aim was to characterise these responses during prolonged exercise heat stress with maintained euhydration and therefore determine the influence of hydration status on global thermoregulatory function and LV volumes following HA. HA with controlled HR and fluid restriction resulted in similar daily body mass deficits of ~2.8%. Despite several significant adaptations – a lowering of exercise HR and T_{sk} and an increase in sweat rate – the intervention did not result in an expansion of PV. Furthermore, during semi-recumbent exercise in 33°C and 50% relative humidity, T_C, T_{sk} and HR responses before HA were unaltered following HA, regardless of hydration status. Acute dehydration ~3.6% was accompanied by significant haemoconcentration and a progressive decline in SV, MAP and $\dot{Q}$ after HA. These responses did not differ from those observed pre-HA. In the absence of changes in exercising BV or HR when euhydration was maintained pre- to post-HA, MAP, SV and $\dot{Q}$ were not different after 180 min of exercise. Therefore, after a 10-day dehydrated exercise HA intervention, exercise heat stress with progressive dehydration resulted in a significantly lower $\dot{Q}$ compared to maintained euhydration. This decrease was associated with a lower EDV. These findings highlight the persistent impairment in global cardiovascular and thermoregulatory function during prolonged exercise heat stress and dehydration following HA.

Considering these findings, the hypothesis that dehydrated HA would significantly improve thermoregulatory and cardiovascular responses to prolonged exercise in the heat while maintaining euhydration is rejected. However, the hypotheses that the significant haemoconcentration, hyperthermia and reductions in $\dot{Q}$ compared with exercise induced dehydration compared to euhydration would be similar before and after HA are accepted.

6.4.1 – Resting thermal, haematological and haemodynamic responses to dehydrated acclimation

Throughout HA there were slight (~5 beats·min^{-1}) reductions in resting HR. This change is somewhat smaller than the 8-18 beats·min^{-1} reductions observed by others using medium-term controlled hyperthermia with fluid restriction (Gibson et al., 2015a; Gibson et al., 2015b; Neal et al., 2016b; Patterson et al., 2004a) and the ~7 beats·min^{-1} reduction generally observed with heat adaptation (Tyler et al., 2016). However, resting HR in many of these studies is greater than 72 beats·min^{-1} prior to HA; somewhat larger than the ~60 beats·min^{-1} resting HR of participants in the current investigation. In addition, the present investigation did not result in an expansion of PV that has been reported to occur in the region of 7-15% following similar interventions (Gibson et al., 2015a; Neal et al., 2016b; Patterson et al., 2004b, 2014). Such an expansion has been hypothesised to enhance ventricular filling (Senay, 1986) and therefore support a maintained $\dot{Q}$ with reductions in HR via an increase in SV. Instead, this slight decrease in HR in the present study may be related to the tendency for resting T_C to decrease by ~0.2°C across HA. The slight changes in HR and T_C observed throughout HA were however not evident during the post-HA experimental trials. The reason for this is unclear but may also be related to the relatively low absolute pre-HA T_C of participants in the present study. However, others have reported 0.3-0.5°C reductions in T_C with HA from similar pre-HA values (Gibson et al., 2015a; Gibson et al., 2015b; Patterson et al., 2004b). Therefore, these results indicate that the present HA intervention did not consistently alter resting HR, T_C or PV.

For the reasons described above, since T_C and BV remained similar it is not entirely surprising that a large reduction in HR also did not occur with HA. Therefore, neither resting SV nor $\dot{Q}$ were altered by HA in the current study. A lack of a change in PV throughout or following dehydrated HA is somewhat unexpected but has been reported previously (Garrett et al., 2009; Neal et al., 2016a). The average degree of dehydration of the present study was similar to those imposed by others (Garrett et al., 2014; Neal et al., 2016b; Patterson

et al., 2004b, 2014). One difference may be due to the standardised dehydration stimulus between HA days used in the current investigation via alterations in fluid intake. Although slight, previous investigations have resulted in progressively greater body mass deficits throughout HA (Neal et al., 2016b; Patterson et al., 2004b). It is unknown whether such a progressive stimulus may be necessary to maintain fluid regulatory strain. Additionally, although participants were dehydrated by ~2.8% each day, this may not have resulted in the >2% change in plasma osmolality required to stimulate renal water conservation (Cheuvront & Kenefick, 2014) and expand PV.

6.4.2 – Effect of dehydrated acclimation on cardiovascular function

HA is thought to have the potential to offset some of the deleterious effects of dehydration on physiological strain during exercise in the heat (Periard et al., 2015). This may occur via increased voluntary fluid intake (Bean & Eichna, 1943) and, improved tolerance (Fleming & James, 2014), reducing the effects of hyperosmotic hypovolemia that diminish sweat output (Takamata et al., 2001), maintaining PV (Senay, 1972) and lowering HR (Sawka & Coyle, 1999). However, the present study demonstrates that matched levels of progressive dehydration during exercise heat stress was associated with remarkably similar thermal, haematological and haemodynamic responses before and after 10-days HA with dehydration. Relative ~9% reductions in PV were similar before and after HA with similar plasma sodium concentrations at the end of dehydrating exercise. It has previously been proposed that an increased sweating rate and reduced sweat sodium content would increase extracellular osmolality and potentially prevent reductions in PV during dehydration (Senay, 1972). However, recent evidence is contradictory to this theoretical defence of PV (Harrison et al., 1981; Patterson et al., 2014) as relatively larger reductions in PV occur post-HA, possibly via increased loss of albumin from the intravascular compartment (Patterson et al., 2014). Whilst the present findings may appear to extend these observations to more pronounced levels of dehydration post-HA, there are several key differences between the present data and those of Patterson et al. (2014). Firstly, the ~5% increase in sweat loss of the present study is somewhat smaller than the

~30% increase previously observed following dehydrated HA (Patterson et al., 2004b, 2014). Secondly, sweat composition was not determined in the present study. Therefore, it is not clear if there was a reduction of sweat sodium content that typically occurs with HA (Chinevere et al., 2008; Dill et al., 1938) that may be required to enhance extracellular osmolality. Finally, PV was not expanded by HA in the current study. Therefore, given the small increases in total body water loss pre- to post-HA and the relatively larger contributions of intracellular water and interstitial fluid that make up this loss with dehydration (Patterson et al., 2014), the similar ~350 ml reductions in PV pre- and post-HA in the present study is perhaps not surprising.

Together with the unaltered BV and PV responses to progressive dehydration following HA were similar thermal and haemodynamic adjustments to prolonged exercise heat stress. T_C and HR responses after 180 min were unaltered by HA, reaching ~38.8°C and 145 beats·min^{-1} before and after-HA, respectively. This was accompanied by similar ~27 ml reductions in SV and ~5 mmHg reductions in MAP during both dehydration trials. These results, as well as previous observations suggest that dehydration has the potential to offset the improved thermoregulatory and circulatory responses observed whilst euhydrated (Buskirk et al., 1958; Sawka et al., 1983c) and result in a similar $\dot{Q}$ response to exercise and heat stress (Ikegawa et al., 2011). Data from the present study and previous investigations indirectly support the possibility that SV responses to exercise with dehydration with HA may be improved via greater intravascular volume and a diminished hyperthermia-induced tachycardia. Sawka et al. (1983c) demonstrated that ~5% hypohydration via exercise heat stress and overnight fluid restriction resulted in smaller reductions in PV following HA compared to pre-HA responses (~6 *vs.* 11%). This was accompanied by a ~21 beats·min^{-1} reduction in HR during exercise in a hot-dry and hot-humid environment (Sawka et al., 1983c). In addition, when Ikegawa et al. (2011) returned PV and BV to pre-HA volumes via diuretics before 30 min exercise in the heat, SV, $\dot{Q}$ and MAP responses were similar despite a slight ~5 beats·min^{-1} reduction in HR (Ikegawa et al., 2011). The current study extends these findings further. Together with the unaltered BV, HR and T_C responses to dehydration pre- and post-HA, there

were similar decreases in EDV during exercise, as ESV was maintained. Taken together, these findings suggest that following HA dehydration results in similar MAP and $\dot{Q}$ responses to exercise. These may be related to a persistent reduction in SV that occurs second to similar significant increases in HR and T_C and reductions in BV negatively impacting on the filling of the LV.

Similar to the effects of HA on exercise with acute progressive dehydration, no differences were observed between pre- and post-HA responses to exercise with maintained euhydration. SV was unaltered between bouts of semi-recumbent cycling before and after HA as $\dot{Q}$ progressively increased throughout exercise. This observation is in line with several previous studies in unacclimated or partially acclimated humans (González-Alonso et al., 1998; González-Alonso et al., 1995; Montain & Coyle, 1992b; Nielsen et al., 1993). Other investigations have also shown that SV is similar during exercise in cool and hot conditions despite ~1-2.5 $L \cdot min^{-1}$ greater $\dot{Q}$ and several fold higher skin blood flows (González-Alonso et al., 1997; González-Alonso et al., 2000a; Nadel, Carfarelli, Roberts, & Wenger, 1979). The increase in $\dot{Q}$ is predominantly related to elevations in HR. In line with this, the 1.1 $L \cdot min^{-1}$ increase in $\dot{Q}$ between 20 and 180 min of euhydrated exercise was accompanied by 11 $beats \cdot min^{-1}$ elevations in HR while MAP was maintained over the same period. This additional $\dot{Q}$ may be in part due to redistribution of central blood to the cutaneous circulation to increase skin blood flow as there was also a ~1.1°C increase in T_C while SVR fell ~9% and T_{sk} was maintained ~34°C. Although skin blood flow was not measured in the present investigation, forearm and cutaneous blood flows tend to increase with elevations in T_C during exercise in the heat to a T_C ~38°C (Brengelmann et al., 1977; González-Alonso et al., 1999b).

The pre- to post-HA similarities in thermal and haematological responses to euhydrated exercise in the heat are however in contrast to several previous investigations. It is generally accepted that HA improves cardiovascular stability as reflected by the lowering of HR, an increase in SV and the maintenance of $\dot{Q}$ (Periard et al., 2015; Sawka et al., 2011). This stems from

the typical observation that a reduction in HR throughout the first 4-5 days of acclimation accompanies an increase in PV (Eichna et al., 1950; Wyndham, 1951; Wyndham et al., 1968a) that is proposed to enhance venous return during exercise (Wyndham et al., 1968a). The data of the present study indirectly supports this notion. Here SV was 111 and 112 ml after 180 min of exercise pre- and post-HA with maintained euhydration. Importantly, however this was second to similar responses in EDV and ESV with HA, suggesting no effect of HA on altered LV diastolic function or contractility. These observations were accompanied by similar end-exercise HR, T_C and. In contrast, Rowell et al. (1967) reported a ~30 beats·min^{-1} reduction in HR which was attributed to a general increase in SV and maintenance of $\dot{Q}$ following HA. This observation was accompanied by a ~1°C reduction in exercising T_C (Rowell et al., 1967). In another study, ~13% increases in PV and relatively smaller (~10 beats·min^{-1}) reduction in HR observed by Nielsen et al. (1993) were accompanied by a ~20 ml and ~2 L·min^{-1} increases in SV and $\dot{Q}$ after 40 min of exercise following HA. Taken together, these findings suggest that in the absence of an increased BV or lowered HR, exercising SV with euhydration in hot-dry heat is unaffected by dehydrated HA.

6.4.3 – Hydration status following acclimation

Similar to observations following euhydrated HA (Chapter 5), effects of HA with dehydration on responses to prolonged submaximal exercise heat stress with a given level of fluid intake were minimal. Therefore, progressive dehydration has the potential to result in significant thermoregulatory and cardiovascular strain which is attenuated with adequate fluid intake during exercise heat stress. Post-HA, intake of fluid to match 10% of expected sweat losses resulted in a ~2.5 L greater loss of total body water compared to replenishing 90% of this expected deficit. This was associated with ~0.5°C greater increase in T_C and ~5% and ~20 ml lower BV and SV, respectively after 180 min of exercise. Body water deficit is closely related to a fall in SV and $\dot{Q}$ during steady state exercise (Montain & Coyle, 1992b). The reductions in SV are greatest when dehydration is coupled with hyperthermia (González-Alonso et al., 1997). Greater increases in T_C occur as $\dot{Q}$, skin and muscle

blood flows are reduced during prolonged exercise in the heat compared to euhydrated exercise (González-Alonso et al., 1998; Montain & Coyle, 1992a). These reductions in SV are influenced by alterations in central BV an cardiac filling during exercise in the heat, since the decline in SV is attenuated when dehydrated exercise is performed in the cold (González-Alonso et al., 2000a) and arterial blood pressure and skin blood flows are restored during supine exercise (González-Alonso et al., 1999a). In line with this, during semi-recumbent cycling the lower SV with dehydration appeared to be predominately related to a diminished EDV as ESV remained similar compared to maintained euhydration at the end of exercise. Despite a decline in MAP throughout dehydrated exercise and a fall in SVR throughout euhydrated exercise, these responses did not differ significantly between trials at 180 min. However, $\dot{Q}$ was 1.8 $L \cdot min^{-1}$ lower with dehydration at the end of semi-recumbent exercise, and therefore likely resulted in lower systemic perfusion pressure or blood flow to exercising and non-exercising tissues compared to euhydration that occurs with upright cycling (González-Alonso et al., 1998). A reduction in exercising limb blood flow with dehydration may impair heat transport from the active muscle (González-Alonso, Quistorff, Krustrup, Bangsbo, & Saltin, 2000b) while relative decreases in skin blood flow reduces heat exchange to the environment (Montain & Coyle, 1992a; Nadel, Fortney, & Wenger, 1980). However, as skin blood flow was not determined in the present study, it is unclear whether this was reduced with fluid restriction compared to euhydration after HA. Regardless, the current data indicate significantly lower fluid intake following HA results in significant thermoregulatory and cardiovascular strain compared to maintained euhydration following HA.

6.4.4 – Limitations

It could be argued that the similar responses between pre- and post-HA trials with both euhydration and dehydration may be due to an apparent absence of HA in the participants of the present study. For instance, a decrease in HR and T_C at comparative time-points during exercise heat stress at a given workload are classic hallmarks of successful heat adaptation (Fox, Goldsmith,

& Hampton, 1967; Horvath & Shelley, 1946; Rowell et al., 1967). However, adaptive responses were evident throughout the HA period (Table 6.1) and during periods of upright cycling exercise in acclimated experimental trials. For example, over the last 5 min of upright cycling at 65% $\dot{V}O_{2max}$, HA resulted in decreases in T_C and HR of 0.3°C and 7 beats·min^{-1} with euhydration and 0.2°C and 5 beats·min^{-1} with dehydration compared to their respective pre-HA experimental trials (data not shown). On the other hand, it is unlikely that the lack of a clear effect of HA during exercise bouts is due to measurement error. The error associated with LV volume measurements during exercise in the current study is sensitive enough to have determined increases in SV during euhydrated exercise similar to those reported elsewhere (Lorenzo et al., 2010; Nielsen et al., 1993; Rowell et al., 1967). This is further highlighted by the consistent effect of dehydration to reduce SV by ~20 ml compared to euhydration pre- and post-HA; not dissimilar to values previously observed elsewhere with comparable body mass deficits (González-Alonso et al., 1998; Montain & Coyle, 1992b; Stöhr et al., 2011a). In addition, it is possible the shorter less-intense periods of semi-recumbent cycling may have masked some adaptive responses. The present protocol was chosen to promote dehydration during prolonged exercise heat stress. However, future studies should perhaps use a prolonged continuous exercise modality to fully determine the responses to euhydration and dehydration following HA.

6.4.5 – Conclusions

Progressive dehydration during exercise and heat stress is associated with a reduction in BV and greater elevations in T_C and HR compared to euhydrated exercise. The present data suggest that the reduced filling time and intravascular volume may contribute to impaired EDV and a fall in SV. When matched levels of dehydration occurred following a period of dehydrated HA with controlled HR, a similar relationship between the decline in EDV and SV was observed. Therefore, the declines in $\dot{Q}$ and MAP that occur with acute dehydration and exercise heat stress (González-Alonso et al., 1998; González-Alonso et al., 1995; González-Alonso et al., 2000a; Montain & Coyle, 1992b) persist following dehydrated HA.

CHAPTER 7

General Discussion and Conclusions

7.1 – Introduction

The main purposes of this thesis were to 1) characterise the adaptive responses to exercise HA with controlled HR and to explore the influence of hydration status on adaptation and endurance exercise performance, 2) determine the heat acclimated thermal, haematological and cardiovascular responses to prolonged exercise and heat stress while maintaining euhydration and with progressive dehydration, 3) explore the influence of hydration status on changes in cardiovascular function during prolonged exercise in the heat. In Chapter 4, intra-individual responses to both euhydrated and dehydrated HA with controlled HR were investigated to explore the rate and magnitude of adaptation. The effect of each intervention on self-paced exercise performance in hot-humid conditions was determined while the potential effect of HA on $\dot{V}O_{2max}$ in temperate conditions was also briefly explored. Chapter 5 looked to not only characterise the thermal and haematological responses at rest and during prolonged exercise in the heat following HA but to explore in further detail the effect of HA on central haemodynamic responses. In addition to euhydrated responses, the influence of matched levels of progressive dehydration pre- and post-HA had upon thermal, haematological and central haemodynamic adjustments to exercise was investigated. Finally, Chapter 6 extended these findings by exploring the influence a medium-term HA protocol with matched levels of mild dehydration had on acute responses to prolonged euhydrated and dehydrated exercise.

The following chapter will review the main findings of the thesis and highlight the novel contribution of these results in the context of existing literature. In addition, the limitations of both the findings and the procedures of the presented research will be discussed. Finally, recommendations for the application of these findings and future research will be made.

7.2 – Summary of main findings

The findings of this thesis indicate that several significant adaptations that are typical of the heat acclimated phenotype occur during a medium-term exercise HA intervention with controlled HR. However, neither maintained euhydration or daily dehydration interventions increased PV nor reduced resting T_C and HR. Across the euhydrated HA intervention there was a reduction in HR during exercise at a given workload, average T_{sk} was lowered and power output for a given exercising HR was increased. Several adaptations also appeared to be slightly more pronounced with euhydration compared to dehydration, since an increase in sweating rate and reduction in T_{sk} did not occur during dehydrated HA. Furthermore, the increases in workload to maintain exercising HR throughout HA were consistently greater when euhydration was maintained compared to progressive dehydration, suggesting there is a persistent effect for dehydration beyond ~2% of body mass to develop significant thermal and cardiovascular strain at a given exercise intensity despite HA. This finding was extended to the combined stressors of prolonged submaximal exercise and heat stress with and without fluid restriction. When euhydration was maintained, $\dot{Q}$ was maintained via an increase in HR as SV slightly declined with euhydration prior to HA. However, this response did not occur following HA. Instead, $\dot{Q}$ progressively increased together with unaltered BV or HR responses to exercise. The differences in these responses appeared to be due to a maintenance of SV that tended to be slightly higher post-HA. A functional diastolic improvement may be possible but appears negligible as the differences between trials was relatively small and similar responses did not occur with dehydrated HA. Therefore overall, HA *per se* appears to have had no significant effect on LV function. Hydration status however had a significant influence on thermal, haematological and haemodynamic responses to exercise in heat acclimated humans. A body mass deficit >3% consistently resulted in significant reductions in $\dot{Q}$ and elevations in T_C and HR compared to euhydrated exercise, while MAP declined during dehydrating submaximal exercise before and after HA.

Together the studies comprising this thesis indicate that exercise with controlled HR is a practical and effective method of inducing HA. However, euhydration may facilitate the adaptation process as well as improve 30-min self-paced exercise performance in the heat. Furthermore, dehydration following HA results in a diminished $\dot{Q}$ during prolonged exercise in the heat in a manner akin to un-acclimated responses in humans.

7.2.1 – Heat acclimation with controlled heart rate

Effective HA interventions are those which can be easily implemented in a safe manner and induce significant adaptation. Physiological adaptation is driven by repeated disturbance to the internal environment. However, the stimuli must induce sufficient homeostatic disturbance before adaptation ensues (Adolph, 1955). T_C responses are similar with exercise in conditions ranging from 5-30°C (Nielsen & Nielsen, 1962) and it is clear that heat adaptation does not occur by the simple elevation of T_C by exercise in a cool environment (Keiser et al., 2015). Furthermore, despite aerobic exercise training conferring some benefit to exercise heat tolerance, highly trained individuals have exhibited improvements to performance in hot environments within as little as 5 days of HA (e.g. Garrett et al., 2012). Therefore, to induce positive adaptations that benefit exercise responses during high external heat loads, repeated exogenous heat stress that induces whole-body temperature elevations (i.e. narrows temperature gradients between the skin and environment, stimulates sweating and challenges homeostasis) is necessary. In support of this, significant adaptation may be brought about by the repeated passive elevation in whole-body temperature via hot water immersion following typical exercise training in cool conditions (e.g. Zurawlew et al., 2016). Controlled hyperthermia HA applies the principle of progressive overload by maintaining the thermal stimulus for adaptation (Fox et al., 1963a). However, monitoring T_C lacks practicality and a somewhat arbitrary cut-off internal temperature of 38.5°C does not typically facilitate the exercising component of HA, which is considered an integral part of adaptation for athletes (Armstrong & Maresh, 1991).

Chapter 4 characterised the rate and magnitude of responses to euhydrated and dehydrated HA with controlled HR. This method is also proposed to induce a persistent stimulus to adapt, possibly by maintaining the physiological strain of exercise in the heat (Periard et al., 2015). Average T_C and T_{sk} throughout both interventions were 38.2 and 36.5°C, respectively. These responses may be expressed as whole-body temperatures considering 90% and 10% respective contributions of deep tissue (i.e. T_C) and average skin temperatures (Sawka, Wenger, & Pandolf, 1996). Therefore, euhydrated and dehydrated HA with controlled HR resulted in an average 1.8 and 1.9°C elevations in whole-body temperature with each 90-min heat exposure, respectively. Furthermore, during HR controlled exercise average whole-body temperature was ~38.2°C throughout both interventions (i.e. the average whole-body temperature for 12.5 h over a 10-day period). This represents a significant degree of thermal strain, compared to the same exercise in cool conditions (i.e. ~20°C with convective airflow) where similar T_C responses and a T_{sk} ~28-30°C may be expected. These thermal perturbations are similar to those previously invoked by other medium-term interventions (Neal et al., 2016b; Patterson et al., 2004a, 2014). Both present interventions led to several adaptive responses to exercise heat stress (Figure 7.1). Despite this, some typical responses (e.g. an increase in PV) were not observed. The use of a higher HR target would have led to greater elevations in T_C. However, previous controlled hyperthermia investigations did not observe additional benefit of a higher target T_C for adaptation (Gibson et al., 2015a). Therefore, the reasons for a relatively smaller magnitude of adaptive responses in these individuals compared to previous investigations, despite similar levels of whole-body heat stress, is currently unclear. This potential limitation is discussed later in further detail.

A stable exercising T_C was achieved over the last ~30 min of exercise via alterations in workload to maintain the target HR with both euhydration and dehydration, highlighting the intimate relationship between cardiovascular and thermoregulatory adjustments to exercise. This implies that exercising heat strain may be regulated regardless of hydration status and that prescribing exercise intensity with HR may be a relatively safe and practical approach to

HA. Future studies should seek to directly determine the efficacy of HR controlled HA against other types of interventions and address the need for appropriately matched temperate aerobic training to determine the contribution of heat stress *per se* on HA (Corbett et al., 2014).

The purpose of manipulating hydration status throughout HA was to determine the contribution of fluid regulatory strain on adaptation. Previous studies have used the controlled hyperthermia method to ensure the effect of dehydration is separated from a constant thermal stimulus (i.e. maintained T_C ~38.5°C) to adapt (Garrett et al., 2012; Neal et al., 2016a; Patterson et al., 2004b, 2014). However, few have directly assessed the influence of hydration on intra-individual responses (Garrett et al., 2014; Neal et al., 2016b). In addition, these studies did not alter fluid intakes across HA and therefore the levels of dehydration were not standardised as increased sweating rate altered the levels of daily body water deficits. Chapter 4 sought to extend our understanding of the role hydration has in HA by characterising the responses to matched levels of dehydration (daily body mass loss of 2.8%) over a 10-day period against euhydrated exercise with controlled HR in a balanced cross-over design. The effects of heat strain on adaptation were also indirectly controlled by design. The findings of Chapter 4 disagree with those of Garrett et al. (2014) that dehydration augments PV expansion. However, euhydrated HA did not result in a significant increase in PV either, also conflicting the findings of others using controlled hyperthermia (Garrett et al., 2014; Neal et al., 2016b). This is unlikely to be due to an insufficient adaptive stimulus produced by HA with controlled HR as others have observed significant consistent hypervolemia with a similar euhydrated protocol (Keiser et al., 2015). There are several possible alternative reasons for variable responses in PV with HA. These include a possible ‘ceiling effect’ in trained individuals (Neal et al., 2016a), or experimental artefact due to an insufficient adaptive stimulus (Patterson et al., 2014; Taylor, 2014). However, a mechanism underpinning this lack of a response was not determined by the present investigation. Without further studies that appropriately control of the timing or magnitude of a dehydrating stimulus for adaptation and the nutritional constitution of rehydrating food and fluid intake, evidence that dehydration

independently augments the haematological responses to HA remains unclear (Akerman et al., 2016).

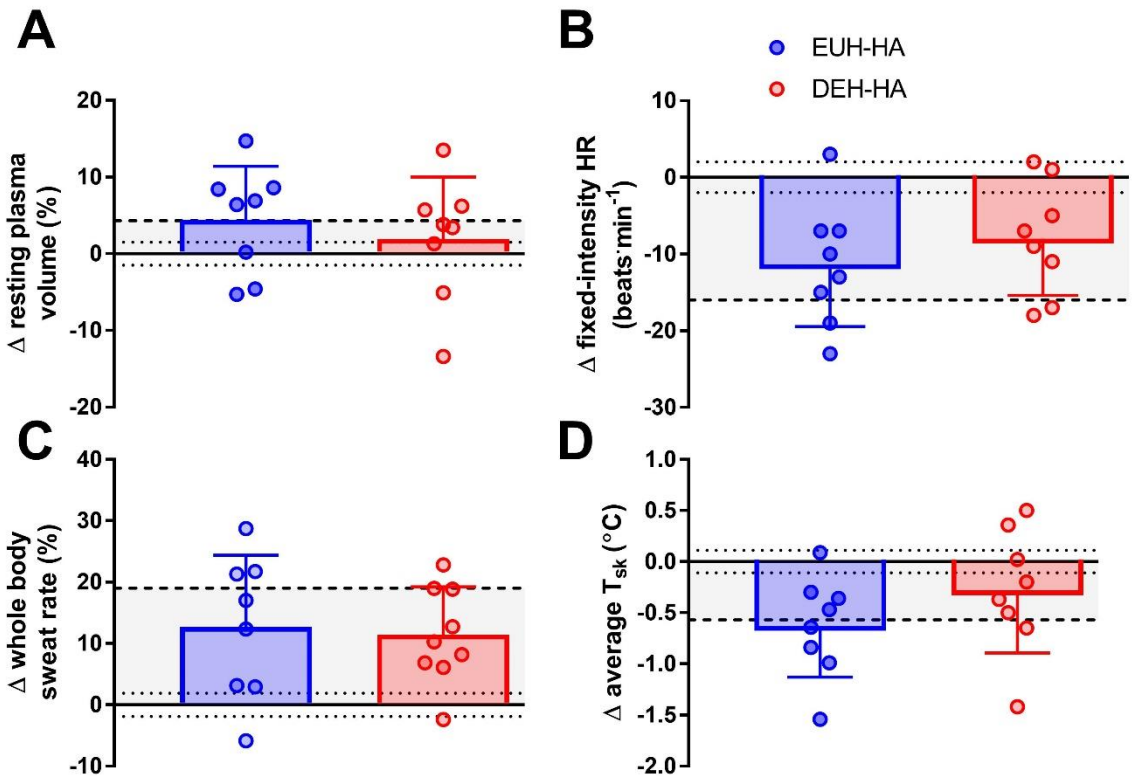


Figure 7.1: Average and individual changes in resting PV (A), fixed-intensity exercise HR (B), whole body sweat rate (C) and average exercise T_{sk} (D) in response to 10 days of both euhydrated and dehydrated HA with controlled HR (Chapter 4). Dotted lines represent smallest worthwhile change (i.e. 0.2 x SD; Hopkins, 2004). Dashed line and shaded area represents average change in each respective variable with medium-term HA from numerous studies (and methodologies) reported in a recent meta-analysis (Tyler et al., 2016).

7.2.2 – Heat acclimation effects on cardiovascular stability

Overall, the findings of Chapters 5 and 6 suggest HA with controlled HR, regardless of hydration strategy, does not alter the euhydrated or dehydrated $\dot{Q}$ and SV responses to prolonged exercise heat stress. A summary of the main findings of the present investigations with altered hydration status are summarised in Table 7.1 and Figure 7.2. With euhydrated exercise $\dot{Q}$ was ~15 $L \cdot min^{-1}$ after 180 min and was unaffected by HA. SV was also largely also unchanged by HA, with only a slight tendency for to be ~5 ml greater at the end of exercise following euhydrated HA. Similar responses were also observed with ~3.6% dehydration before and after HA, with $\dot{Q}$ and SV averaging ~13 $L \cdot min^{-1}$ and ~88 ml, respectively. However, both interventions were also coupled with similar thermal, haematological and HR responses pre- to post-HA (Table 7.1). It has long been considered that the early adjustments to HA are of cardiovascular origin and that these adaptations are integral for improved tolerance to exercise heat stress. Numerous studies, narrative reviews and consensus statements in the literature have suggested HA changes cardiovascular function predominantly via a reduction in HR and increase in PV that permits an enhanced ventricular filling (Garrett et al., 2014; Guy, Deakin, Edwards, Miller, & Pyne, 2015; Keiser et al., 2015; Lorenzo et al., 2010; Nadel et al., 1980; Periard et al., 2015; Periard et al., 2016; Racinais et al., 2015a; Rowell et al., 1967; Taylor, 2014; Taylor & Cotter, 2006; Tyler et al., 2016; Wyndham et al., 1976) and these examples are far from exhaustive. It was hypothesised that HA would indeed lower T_C and HR during exercise and expand PV (perhaps more-so with dehydrated HA). Therefore, this hypothesis that HA would improve cardiovascular stability via a lowered HR and increased SV at the same exercising workload following HA must be rejected.

Despite a lack of an effect of HA on cardiovascular stability from the interventions in Chapters 5 and 6, the mechanisms behind previously observed improvements that have been proposed by others may however be

indirectly supported by these observations. A classical observation following HA is a reduction in HR at a comparative time-point during exercise at a constant submaximal workload compared to un-acclimated responses (Gisolfi & Robinson, 1969; Horvath & Shelley, 1946; Strydom et al., 1966). However, altered HR alone gives relatively little mechanistic insight as to a change in cardiovascular function as this adaptive response is typically accompanied by reductions in thermal strain following HA. More in-depth investigations into altered function following HA have reported $\dot{Q}$ is either maintained (Goto et al., 2010; Rowell et al., 1967) or increased (Nielsen et al., 1993) during exercise in dry heat whilst SV is significantly increased. Therefore, increases in PV and BV along with an enhanced ventricular filling pressure have been proposed to enhance LV volumes during exercise and heat stress (Rowell et al., 1967; Senay et al., 1976) via an altered Frank-Starling mechanism.

The similar body temperatures, HR and BV responses between pre- and post-HA with euhydrated exercise heat stress was not associated with a significant increase in EDV or SV. This was a broadly common finding in both Chapters 5 and 6 which contrast with the observations of Rowell et al. (1967) and Nielsen et al. (1993). In each of these studies $\dot{Q}$ was either maintained (Rowell et al., 1967) or increased ~1 $L \cdot min^{-1}$ (Nielsen et al., 1993) and the increases in SV could be ascribed to reductions in HR and increase in PV. Additionally, the fall in T_C, T_{sk}, HR and increase in SV was observed by others to be related to initial increases in PV 4-5 days into HA and not to alterations in evaporative heat loss (Mitchell et al., 1976; Senay et al., 1976). Further evidence stems from the acute cardiovascular responses to exercise heat stress. For instance, during euhydrated exercise in the heat SV is maintained when significant elevations in T_C and HR do not occur (González-Alonso et al., 1998; González-Alonso et al., 1995; Montain & Coyle, 1992b). Additionally, acute manipulations in BV alter haemodynamic responses to exercise. Phlebotomy is proceeded by rapid adjustments of PV. However, following BV reduction $\dot{Q}$, HR and SV are maintained as skin blood flow is lowered (Fortney, Nadel, Wenger, & Bove, 1981b). In contrast, with acute PV expansion of ~13% $\dot{Q}$ is still maintained, however HR is lowered and SV is increased during submaximal exercise in the heat (Fortney, Nadel, Wenger, & Bove, 1981a;

Sawka, Hubbard, Francesconi, & Horstman, 1983a), suggesting that the HR and SV responses combine to maintain adequate $\dot{Q}$ when cardiac filling pressure is enhanced (Senay, 1986). A larger acute increase in PV (~540 ml) has also been shown to increase $\dot{Q}$ during incremental exercise following passive heating (Keiser et al., 2015). Taken together, the similarities observed across HA in the present studies and the adjustments that occur during acute manipulations of BV in humans support the notion that improved cardiovascular stability following HA may indeed be related to the interplay between BV, HR and body temperature that potentially alter ventricular filling.

Table 7.1: Percentage differences in cardiovascular haemodynamics and haematological responses from the pre-acclimation euhydrated condition of each respective study. Comparisons are during semi-recumbent cycling after 180 min of exercise heat stress with progressive dehydration pre-HA and maintained euhydration and progressive dehydration in heat acclimated individuals (post-HA). HA was achieved via 10 days of exercise with controlled HR with either maintained euhydration (Study 2) or progressive dehydration (Study 3) each day.

Study	Condition	MAP (mmHg, %)	EDV (ml, %)	ESV (ml, %)	SV (ml, %)	HR (beats·min^{-1}, %)	$\dot{Q}$ (L·min^{-1}, %)	SVR (PRU, %)	BV (ml, %)	Δ body mass (%)	T_C (°C)	T_{sk} (°C)
2 Euhydrated HA with controlled HR	Euhydration (Pre-HA)	91	146	42	103	139	14.4	6.4	5653	-0.4	38.4	34.1
	Dehydration (Pre-HA)	-6	-16*	-10	-18*	8*	-11*	5	-6*	-3.5*	39.0*	33.9
	Euhydration (Post-HA)	-2	5	-5	9	-3	5	-7	1	-0.4	38.3	34.0
	Dehydration (Post-HA)	-9	-16*	-13	-17*	9*	-10*	1	-5*	-3.8*	39.0*	34.0
3 Dehydrated HA with controlled HR	Euhydration (Pre-HA)	85	168	57	111	135	15.0	5.8	5852	-0.6	38.5	34.1
	Dehydration (Pre-HA)	-3	-14*	-4	-20*	8*	-13*	12	-6*	-3.6*	39.0*	34.1
	Euhydration (Post-HA)	2	1	1	1	0	1	0	1	-0.3	38.2	34.2
	Dehydration (Post-HA)	1	-11*	0	-17*	7*	-12*	15	-4*	-3.7*	38.8*	34.0

Values are means. * Significantly different from respective euhydrated pre-HA condition.

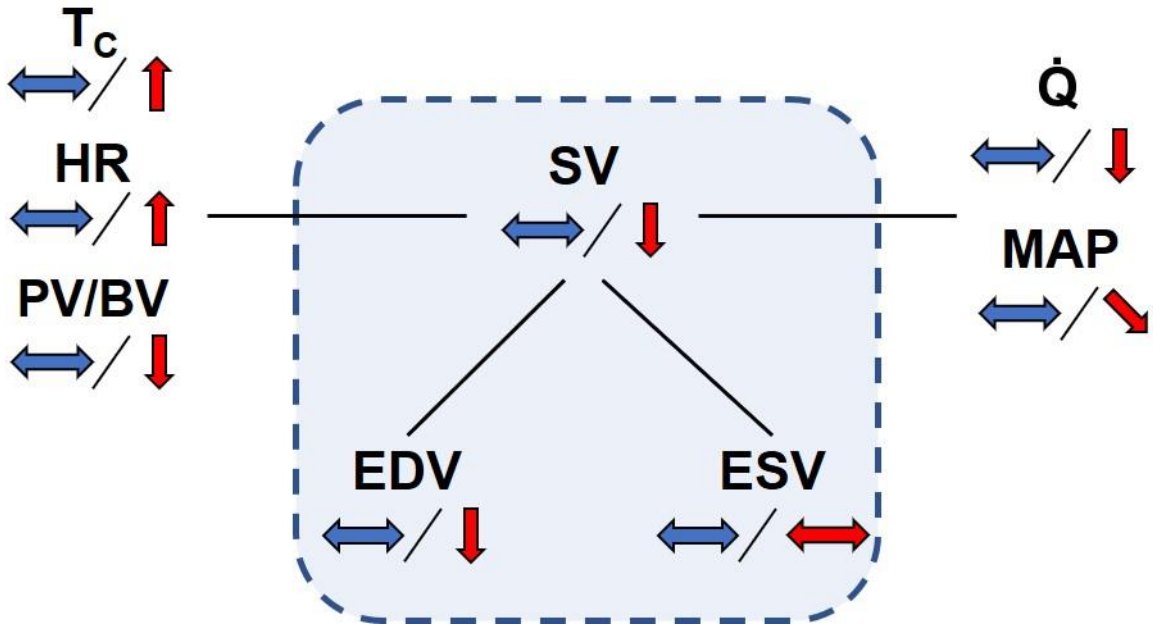


Figure 7.2: Summary of findings from Chapters 5 and 6 which explored the effects of HA and acute influence of hydration status on LV volumes during exercise heat stress in humans. The figure denotes the influence of HA (blue arrows) on thermal, cardiovascular and haemodynamic responses to semi-recumbent exercise and the effect of progressive dehydration compared to adequate fluid intake (i.e. maintained euhydration; red arrows) following HA.

7.2.3 – Acute progressive dehydration following heat acclimation

As previously highlighted, neither HA intervention increased PV or BV. This is in stark contrast to the generally accepted notion that HA increases PV and total body water (Patterson et al., 2004b, 2014). HA has also been shown to increase *ad libitum* fluid intake during exercise, minimising voluntary dehydration (Adolph, 1955; Bean & Eichna, 1943), better matching thirst sensation to body water needs. HA may also dampen the potential reductions in sweat output and skin blood flow that occur with acute dehydration (Takamata et al., 2001). Others have also demonstrated a potential for familiarisation to the sensation of dehydration and heat stress and improve exercise performance in the heat while dehydrated (Fleming & James, 2014). However, considering the wealth of compelling evidence indicating impairments in thermoregulatory and cardiovascular function during exercise, heat stress and dehydration (Montain & Coyle, 1992b; Nadel et al., 1980; Saltin, 1964; Stöhr et al., 2011a; Trangmar et al., 2014), relatively few studies have directly investigated the potential for these impairments following HA. Both the studies of Chapters 5 and 6 show that the development of progressive dehydration beyond 3% of body mass is associated with similar thermal, haematological and haemodynamic responses to exercise pre- and post-HA. Therefore, the hypotheses that dehydration offsets beneficial effects of HA and results in the development of significant thermoregulatory and cardiovascular strain compared to euhydrated exercise are accepted.

Previously, studies had induced hypohydration via heat exposure and/or overnight fluid restriction (Buskirk et al., 1958; Sawka et al., 1983c) and diuretics (Ikegawa et al., 2011) or used moderate exercise induced dehydration (~2.5%; Patterson et al., 2004; Patterson et al., 2014) to explore the effect of HA on thermoregulation, cardiovascular function and fluid regulation. Together, the findings of these studies suggest that dehydration has significant potential to, at least partially, offset the improved thermoregulatory, circulatory and haematological responses to exercise in the heat following HA. In Chapters 5 and 6, these observations were developed further by determining the haematological, haemodynamic, and thermal

responses to progressive exercise induced dehydration and maintained euhydration following HA. In both studies, $\dot{Q}$ (~12%) and BV (~5%) were significantly lower with dehydration compared to euhydration whilst there were also ~0.5°C and ~8% greater increases in T_C and HR, respectively. These alterations resulted in reduction in LV filling as EDV (~14%) was significantly lower than euhydration (Table 7.1). Redistribution of blood flow, reductions in BV and increases in body temperature and HR appear to have varying contributions on the impairment in LV filling and decline in SV. EDV is reduced at rest and during small muscle mass exercise despite enhanced diastolic LV function (Stöhr et al., 2011a), while reductions in BV seem to account for most of the decline in SV during exercise in the cold (González-Alonso et al., 2000a) or supine position (González-Alonso et al., 1999a) in dehydrated humans. The present studies extend these observations to heat acclimated humans (Figure 7.2).

Dehydration before and after HA resulted in remarkably similar reductions in PV and hence, BV. Although these findings do not fully match those of Patterson et al. (2014), data from Chapter 6 indicates that dehydrated HA did not alter the loss of PV or retention of plasma sodium during acute exercise induced dehydration. Therefore, despite consecutive daily body mass deficits of ~2.8% during exercise in the heat, it does not appear that dehydrated HA promoted a preferential defence of PV during acute dehydration as previously proposed (Senay et al., 1976).

Together, the responses to prolonged exercise heat stress with and without dehydration following HA provide insight into the mechanisms underpinning improvements in cardiovascular stability, as well as the persistent potential for dehydration to impair cardiovascular and thermoregulatory responses to sub-maximal exercise in the heat. Increased PV and reductions in HR that accompany decreases in thermal strain with HA seem to be integral to increased SV and $\dot{Q}$ in these circumstances. However, the importance of increases in PV, SV and $\dot{Q}$ with HA and their contribution to maximal exercise performance remains unclear.

7.2.4 – The ergogenic effect of heat acclimation

Chapter 4 explored the effect of medium-term HA with controlled HR on $\dot{V}O_{2max}$ in cool conditions and 30-min self-paced exercise performance in the heat. These performance effects were determined following both euhydrated and dehydrated HA. There is a wealth of evidence indicating HA improves both time to exhaustion and self-paced exercise performance in a hot environment (Castle, Mackenzie, Maxwell, Webborn, & Watt, 2011; Keiser et al., 2015; Lorenzo et al., 2010; Nielsen et al., 1993; Nielsen et al., 1997; Racinais, Periard, Karlsen, & Nybo, 2015b; Zurawlew et al., 2016). In addition, the concept that an environmental stressor may prove beneficial to performance in another environment (an ergogenic cross-adaptation) is not new. For example, altitude training is often used to improve sea-level exercise performance (Levine & Stray-Gundersen, 1997). However, whether HA benefits exercise capacity and performance in cool and temperate climates was the subject of recent debate (Minson & Cotter, 2016; Nybo & Lundby, 2016).

Here, 5 submaximal workloads (90-210 W) were maintained for 4 min each in a temperate environment (~19°C) before and after both HA interventions. Similar $\dot{V}O_2$, $\dot{V}CO_2$, respiratory exchange ratio, breathing frequency, minute ventilations, T_C and HR responses over the final minute of each workload were observed between trials. Furthermore, maximal aerobic power and $\dot{V}O_{2max}$ were similar at the end of ramp-incremental exercise to volitional fatigue following submaximal exercise. These findings suggest that HA with controlled HR did not alter submaximal or maximal responses to aerobic exercise in temperate conditions, reflecting the observations of others (Karlsen et al., 2015; Keiser et al., 2015; Zurawlew et al., 2016). However, contrasting effects have also been reported (Lorenzo et al., 2010; Sawka et al., 1983b) and the mechanisms behind a possible ergogenic effect of HA on performance in temperate conditions remain unclear.

An increase in PV has been implicated in the increase in lactate threshold during exercise in cool (~10°C) conditions (Lorenzo et al., 2010). However,

changes in plasma lactate concentrations may also be resultant from altered glucose metabolism (Febbraio et al., 1994; Young et al., 1985) or haemodilution via PV expansion. Additionally, a ~16 W improvement in lactate threshold has been observed in the absence of a PV expansion with HA (Neal et al., 2016a). In Chapter 4, $\dot{V}O_2$ and respiratory exchange ratios were not altered by HA during an incremental test to exhaustion in ~20°C. Similarly, neither intervention increased PV and during submaximal semi-recumbent exercise in the heat lactate concentration was not affected by HA (Chapter 6). However, there were also no differences in lactate accumulation between hydrated and dehydrated trials. These responses may specifically be due to the relatively brief periods of semi-recumbent cycling used in these experiments. A more prolonged bout of exercise may very well have been expected to alter this relationship (e.g. González-Alonso et al., 1998). Despite this, it does not appear that either of the current interventions significantly altered submaximal $\dot{V}O_2$ or substrate metabolism.

During maximal aerobic exercise on the other hand, an expanded PV may improve LV filling and muscle blood flow as SV and $\dot{Q}$ have been observed to be increased following HA (Lorenzo et al., 2010). However, more recent evidence suggests that increases in PV via HA are not ergogenic to exercise in a cool environment (Karlsen et al., 2015; Keiser et al., 2015). A plateau or decline in leg blood flow and vascular conductance occurs prior to the attainment of $\dot{V}O_{2max}$ during incremental and constant load maximal cycling (Mortensen, Damsgaard, Dawson, Secher, & González-Alonso, 2008; Mortensen et al., 2005). Although a greater PV may increase maximal $\dot{Q}$ and muscle blood flow, a relative dilution of arterial oxygen content may also occur and therefore the limitation of oxygen delivery during maximal exercise may persist at a similar workload (Kanstrup & Ekblom, 1984). Therefore, without concomitant increases in RCV and haemoglobin content an increase in $\dot{V}O_{2max}$ is not likely and was the case in the present study.

A further consideration is a possible training effect. Despite non-significant differences in $\dot{V}O_{2max}$, there were slight (~3 $ml \cdot kg^{-1}$) average increases pre- to post-HA. However, as previously stated this difference may be considered

variability in the measure as the pre-HA responses between interventions also exhibited a similar difference, while *a priori* analysis revealed the study was sufficiently powered to detect a significant change. Here, further analysis has also been conducted to account for a potential training effect and revealed no correlation between the total work done or the increase in power output for the same HR and the change in $\dot{V}O_{2max}$ with either HA intervention (Figure 7.3). Therefore, for the reasons described here and above it is concluded that, as hypothesised, neither of the interventions reported in Chapter 4 were ergogenic to temperate exercise performance.

In contrast to the findings in cooler ambient conditions, self-paced exercise in the heat was significantly improved by ~19 W or ~9% following euhydrated HA. However, 30-min time-trial performance was not significantly improved following dehydrated HA. Similar to the reasons highlighted earlier, the differences in performance in this instance are thought to be related to improved thermoregulatory and perceptual adjustments following HA, and not an increase in $\dot{V}O_{2max}$. Throughout these performance trials power output was progressively declining from 6-24 min while T_C and HR increased (Figure 4.6). This is in line with the suggestion that relative exercise intensity is maintained as the development of cardiovascular strain contributes to a progressive lowering of $\dot{V}O_{2peak}$ (Periard et al., 2011; Periard & Racinais, 2015). Following HA, these cardiovascular and thermoregulatory responses were similar while the pacing profiles also resembled pre-HA trials. As such, the changes in performance were due to a consistently greater power production throughout the 30 min effort and this was potentially facilitated via improved heat exchange with the environment following euhydrated HA.

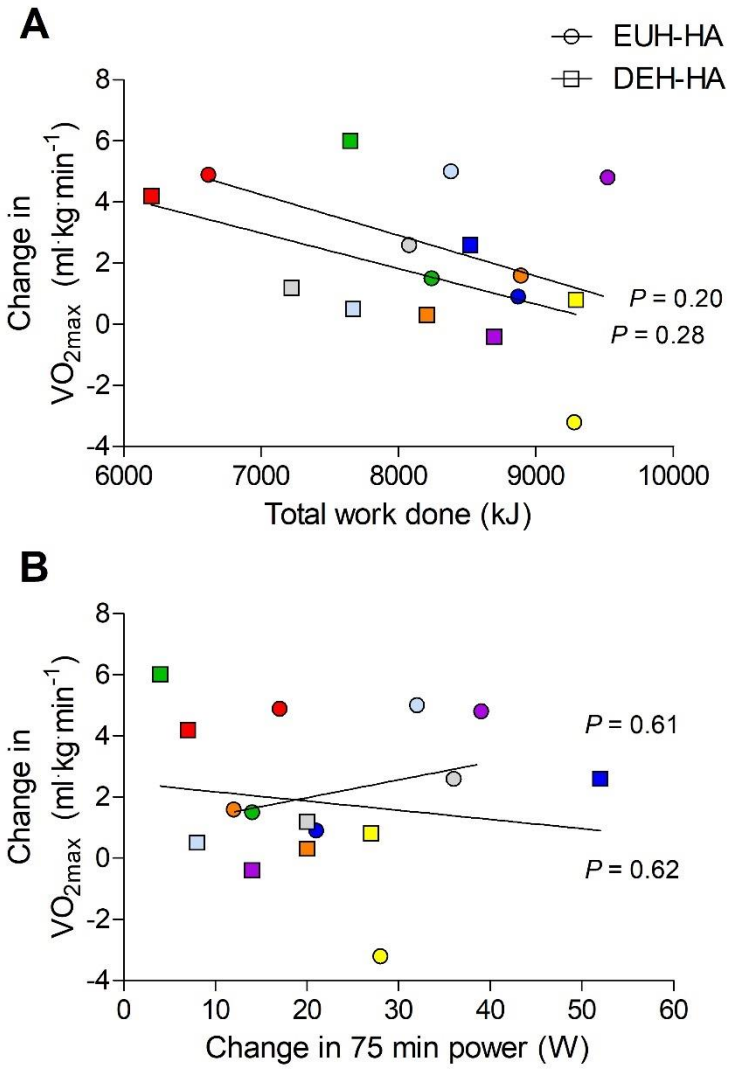


Figure 7.3: Relationships between the total work done during HA (A) and the increase in power for a given HR during HA (B) with the change in relative $\dot{V}O_{2max}$. Circles and squares represent HA-EUH and HA-DEH responses, respectively. Fill colour is unique to a given participant between interventions.

It is likely that a more prolonged self-paced effort may have demonstrated a significant improvement in performance following dehydrated HA. A 30-min time trial was chosen as it encompassed the ~20 min period of intense self-paced exercise that occurs in the heat before trained individuals exhibit a significant reduction in power output compared to cool conditions (Periard et al., 2011). Future work may wish to employ a more ecologically valid performance test such as a 1 h time-trial. Furthermore, given the small number

of studies conducted to date exploring the influence of both euhydrated and dehydrated HA to adaptation and exercise performance (Garrett et al., 2014; Neal et al., 2016b; Pethick et al., 2018; Philp et al., 2017; Schleh et al., 2018), or the relative independent contributions of exercise and heat stress to these responses (Corbett et al., 2014), further work is required to conclusively promote one particular intervention.

7.3 – Limitations

7.3.1 – Sample size

The subject sample sizes for each of the studies was relatively small (Chapter 4, n = 8; Chapter 5, n = 8; Chapter 6, n = 9 HA participants and 8 experimental participants) and therefore increased the possibility of Type II errors affecting the statistical analyses. Furthermore, this risk was also likely increased by use of the relatively conservative Holm-Bonferroni correction of family-wise error rate in the multiple comparisons conducted in each study. However, there are several reasons why this does not appear to have overly affected the results reported here. Firstly, the primary outcome data analysed in these studies consisted in changes in thermal, haematological and haemodynamic responses to exercise heat stress with manipulations in hydration. The changes observed in these parameters were consistent with those reported elsewhere in the literature and were measured independently of each other. Secondly, similar relationships between these independently measured variables were observed when euhydration was maintained.

The experimental designs of the studies in this thesis were well controlled. Although participants were not highly trained elite cyclists, a counterbalanced cross-over design was employed, and highly consistent performances were observed. In further support of the findings of HA on exercise performance in temperate and hot environments, post hoc analysis determined the experiments were more than sufficiently powered to detect changes in $\dot{V}O_{2max}$ and TT performance. However, it is also accepted that a larger cohort may have been beneficial for analysis of some other parameters. For instance, post

hoc analysis of the effects of HA had on PV identified the experiment was underpowered and a minimum sample size of 12 may have been necessary to observe a significant change. However, the complex and prolonged procedures required to conduct this study meant that recruitment was limited and the chance of participant drop-out or illness throughout the experiment was high, as was the case in Chapter 6.

7.3.2 – Development of the heat acclimated phenotype

The influence of HA on physiological strain during physical work in the heat has been extensively studied. The magnitude of adaptive responses of the participants in this study are relatively small compared to previous results (Figure 7.1) and therefore gives rise to the possibility that HA was not successfully induced by the interventions within this thesis. This is further illustrated by the apparent lack of either intervention to result in a lowered T_C or HR during euhydrated semi-recumbent cycling in 33°C. A major limitation of the present studies is that a work-matched control-arm of HR controlled exercise training was not conducted. While within-participant designs are useful for characterising adaptive responses, many of the adaptations to HA are also typical of aerobic exercise training. Much of the research in acclimation/acclimatisation conducted to date is flawed as matched relative training demands are not controlled (Corbett et al., 2014) and the benefit of heat adaptation *per se* beyond typical training is not yet fully established (Nybo & Lundby, 2016). However, as previously shown, the significant and consistent thermal strain produced by both protocols used here were not dissimilar to those induced by others employing a constant thermal forcing function for adaptation; controlled hyperthermia (Garrett et al., 2012; Garrett et al., 2014; Neal et al., 2016a; Neal et al., 2016b; Patterson et al., 2004b, 2014). Further evidence of the levels of experimental control and responses to HA seen in Chapter 4 are provided in the form of daily individual responses from each intervention in Appendix V.

Despite the lack of a work-matched control it does not seem likely that exercise heat stress with controlled HR *per se* is insufficient at inducing HA.

Instead, the reasons for these relatively small adaptive responses may be due to other methodological and logistical constraints. For instance, three of the eight participants recruited underwent both arms of HA between the months of May and September and all participants were residents of Qatar (southwest Asia, Latitude: 25° North). While every effort was taken to limit the confounding influence of natural acclimatisation it is possible this was a factor that contributed to the present responses. All participants recruited for this study were non-native residents of the Middle East who predominately originated from North America and Western Europe. Two participants were South African Caucasians. Participants had resided in Qatar for an average of 2.1 years prior to undertaking the first HA intervention (range 0.3 – 5.7 years). Although a ~3-week period of indoor training was observed prior to commencement of each intervention, participants may have been partially acclimatised and re-induction may have occurred rapidly (Daanen et al., 2018) with the onset of each arm of the study. Anecdotally however, due to the extremely high ambient temperatures and humidity in the region during the summer months, all participants reported typically either avoiding outdoor cycling (i.e. use of home ergometers), performing some form of cross-training (e.g. swimming), travel to cooler climates or a combination of the above. Furthermore, the local competitive cycling season occurs in Autumn and Winter (i.e. November to March).

To further determine whether residency in Qatar or a residual order effect of prior HA may have affected some of the responses observed in Chapter 4, several additional analyses were performed. Firstly, despite tropical natives exhibiting increased sweat rates with HA (Magalhães et al., 2010), it has been suggested that a thermoregulatory efficient very long-term adaptation to HA is a relatively lower sweating rate (Taylor, 2014). Secondly, inter-individual natural acclimatisation status (i.e. duration of residency and/or seasonal exposure) might influence pre-HA T_C, considering experiments were conducted at a similar time of day. Finally, insufficient washout of the first HA intervention might be expected to influence the rapid re-induction of Tc and HR responses during the second intervention (Daanen et al., 2018). However, there was no relationship between participants' duration of residency in Qatar

and the change in sweat rate with HA or absolute resting T_C on day one of their first intervention (Figure 7.4). In addition, comparison of resting and exercising T_C and HR responses between the first and second HA interventions on days two and three also demonstrated no apparent residual HA influenced re-induction in these parameters (Figure 7.5). Therefore, together with the use of a randomised counterbalanced cross-over design, the responses observed are likely specific to a given intervention and suggests residency in a hot climate does not fully account for the responses seen and that prior HA did not induce a more rapid re-induction of these adaptations.

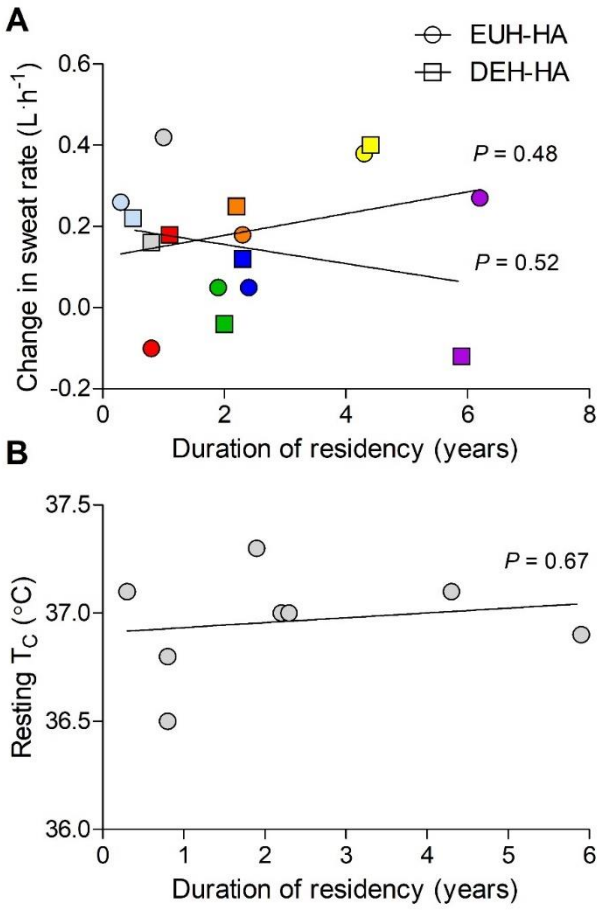


Figure 7.4: Relationships between the duration of participant's residency in Qatar and (A) change in sweat rate with HA and (B) resting T_C on day one of the first HA intervention, regardless of condition. Colours in panel A are representative of individual participants while circles and squares represent EUH- and DEH-HA, respectively.

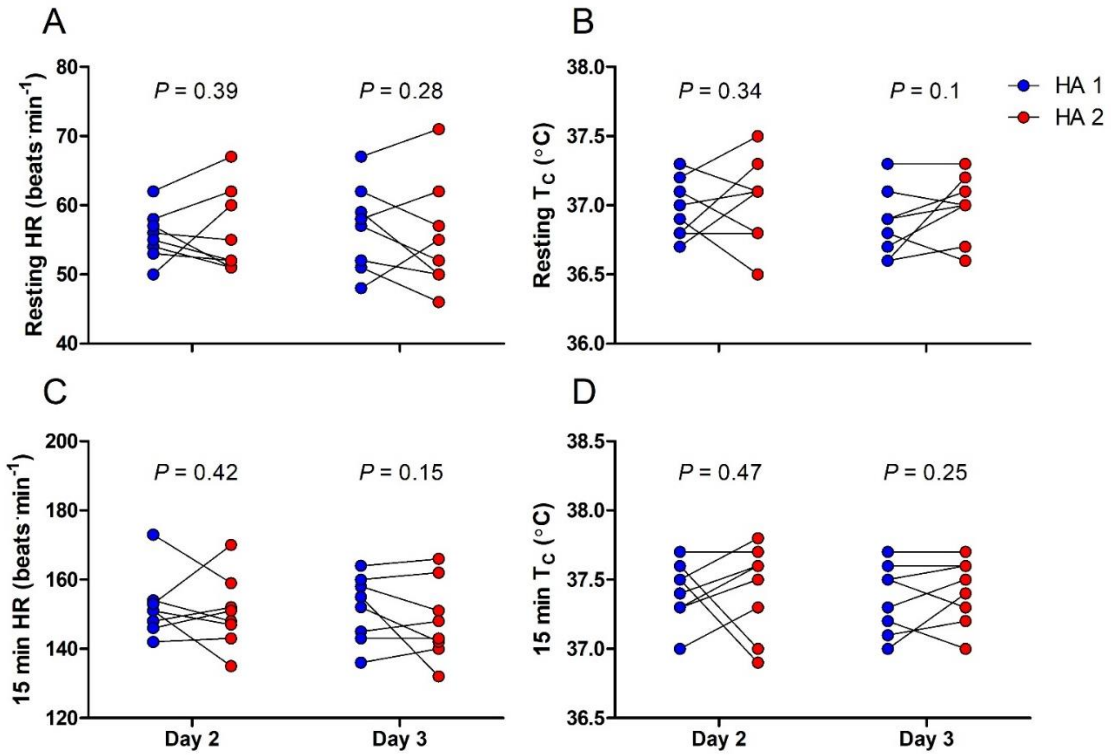


Figure 7.5: Individual HR and T_C at rest (A & B) and after 15 min of exercise (C & D) on days two and three of the first (HA 1) and second (HA 2) intervention undertaken, regardless of condition.

7.3.3 – Determination of ventricular, blood and plasma volumes

The non-invasive measurement of LV volumes provides additional insight as to the mechanisms underpinning cardiovascular stability during exercise, heat stress, dehydration and HA. However, the use of echocardiography for the measurement of LV volume is prone to several sources of error. Firstly, 2D imaging of the 4- and 2-chamber views may be influenced by slight changes in isonation angle that could potentially foreshorten the ventricle and alter the endocardial border independently of changes in ventricular function. However, care was taken to ensure images were standardised to anatomical landmarks, visible throughout each cardiac cycle (Lang et al., 2015). Furthermore, when conducted correctly the use of Simpsons Bi-Plane method of disk summation accounts for the entire length of the LV cavity, providing greater accuracy to alternative methods that require anatomical assumptions (e.g. Teicholz, 1976). Secondly, images were recorded at a maximum of 60 Hz due to

technical limitations of the device used. This potentially introduces both slight under- and over-estimations of diastolic and systolic LV volumes as HR becomes significantly elevated due the unobserved movement of the myocardium between recorded frames. However, this represents the minimum recommended frame rate used elsewhere (Helle-Valle et al., 2005; Stöhr et al., 2011a) and the differences observed between LV volumes during euhydrated and dehydrated exercise are consistent with those reported previously in the literature.

A vast majority of studies in the literature have determined the effects of HA on relative changes in PV using the methods of Van Beaumont (1972) or Dill and Costill (1974). While these techniques are minimally invasive and easily implemented, they do not account for changes in RCV throughout an intervention. In this thesis, a dual-baseline measurement of Hb_{mass} was performed prior to each study and this measurement was repeated ~24 h after the final HA exposure. The co-efficient of variation for this technique was favourable in comparison to other published measurements (Gore et al., 2005) however, these tests were not performed on the same day as other experimentation. This was to prevent any confounding effect of residual carbon monoxide bound to haemoglobin on the metabolic responses to exercise trials. Therefore, values presented are under the assumption of an accurate and stable haemoglobin content between measurements. Others have observed stable (Gibson et al., 2015a; Neal et al., 2016b) and slight increases (Scoon et al., 2007) in Hb_{mass} throughout similar time-frames with HA. Therefore, it is likely the timing of the tests conducted appropriately controlled for any potential influence of HA on haematological variables measured.

Venous samples were collected via venepuncture and not a cannula throughout HA. This was due to the limited availability of assistance with the appropriate technical skills. As such sampling was conducted during supine rest prior to and immediately following exercise to avoid potential complications of performing venepunctures on individuals exercising on the ergometer. Therefore, the samples obtained are influenced by the termination

of exercise and changes in posture and may not entirely reflect the influence of the exercising HA intervention. Future studies should consider obtaining exercising venous samples to further elucidate the specific effects of exercising heat stress and hydration on adaptive responses to HA.

7.4 – Directions for future research

Several results from this thesis as well as other recent investigations (Neal et al., 2016b; Pethick et al., 2018; Schleh et al., 2018) have observed no influence of hydration on the haematological and thermoregulatory responses to HA. However, it is currently unclear what contribution non-standardised habitual activity and food, or beverage intake has upon adaptation to dehydrated HA. Changes in posture, the timing and constituent carbohydrate and protein content of food and beverages, and dietary sodium intake have all been shown to influence the haematological responses to exercise in the heat (Kamijo et al., 2012; Nagashima et al., 1999; Okazaki et al., 2009) . Whilst further control of daily activity and dietary intake throughout interventions may have little practical application to athletic performance, such methodological considerations might provide further mechanistic insight to the influence of hydration status on haematological adaptations to HA.

Similar to the proposed improvements in cardiovascular stability with HA via an improvement in ventricular filling, various other observations have been applied to heat acclimated humans with little direct evidence. The concept of acquired thermal tolerance in mammals has been elucidated in the rodent model (Horowitz, 1998) and may share a common heat shock response with HA (Kuennen et al., 2011). In addition, based on the findings of animal studies LV compliance may be increased and systolic pressure generation and myocardial $\dot{V}O_2$ may be lowered by HA (Horowitz, 1998; Levy et al., 1997). However, very few studies have directly determined the effects of exercise HA in humans on intra-cellular heat shock protein levels that are commonly implicated in these pathways (HSP-72 and HSP-90; McClung et al., 2008). Furthermore, direct evidence of altered myocardial function with HA is yet to be demonstrated in humans. Developments in imaging technology may

provide non-invasive insights into possible human cardiac adaptations to HA during thermal, pharmacological or exercise stressors. These include diffusion tensor MRI, 3D-echocardiography, speckle tracking measurement of myocardial strain and torsion and Doppler ultrasound. Recent studies using echocardiography have shown various responses of cardiac diastolic and systolic function and contractility to a reduction in preload via whole body heat stress (Brothers et al., 2009; Stöhr et al., 2011b), dehydration (Stöhr et al., 2011a) and BV reduction (Lord et al., 2018). The use of similar techniques during a standardised stressor before and after a period of HA could provide evidence of altered cardiac mechanics in humans and their relationship with changes in BV and HR.

Finally, further work is necessary to establish the efficacy of HR controlled exercise in the heat for adaptation. As with other techniques used to induce HA, the contribution of exercise, independent of heat stress, for adaptation is relatively unknown. Future studies should attempt to control for the potential relative differences in exercise intensity between hot and cold environments (Corbett et al., 2014), possibly via work-matched exercise stimuli. In addition, the concept that HR controlled exercise with and without dehydration standardises internal load and cardiovascular strain (Periard et al., 2015) requires further exploration and validation. Acutely, dehydration during exercise heat stress results in substantial reductions in workload to achieve a stable HR while thermal responses are comparable to those seen with maintained euhydration (Chapter 4). However, there are also significant reductions in SV, $\dot{Q}$ and $\dot{V}O_2$ during controlled HR exercise with dehydration (Ng, Dobbs, & Wingo, 2018; Wingo & Cureton, 2006), while presumably preload and afterload are altered in this scenario as dehydration develops. Therefore, hydrated and dehydrated exercise at the same HR represents contrasting physiological states beyond that of merely total body water. Future studies may wish to further explore the acute and chronic relationships to heat stress, hydration and relative exercise intensity to determine the mechanisms that may contribute to exercise HA.

References

ACSM's Guidelines for Exercise Testing and Prescription. (2014). (B. Ferguson Ed. 9 ed.): Lippincot Willaims & WIlkins.

Adolph, E. F. (1947). *Physiology of man in the desert*. New York, USA: Interscience.

Adolph, E. F. (1955). General and specific characteristics of physiological adaptation. *Am J Physiol, 184*, 18-28.

Akerman, A. P., Tipton, M., Minson, C. T., & Cotter, J. D. (2016). Heat stress and dehydration in adapting for performance: Good, bad, both, or neither? *Temperature (Austin), 3*(3), 412-436. doi: 10.1080/23328940.2016.1216255

Alpert, J. S., Petersen, P., & Godtfredsen, J. (1988). Atrial fibrillation: natural history, complications and management. *Ann Rev Med, 39*, 41-52.

Armstrong, L. E. (2005). Hydration assessment techniques. *Nutr Rev, 63*(6 Pt 2), S40-54.

Armstrong, L. E., & Maresh, C. M. (1991). The induction and decay of heat acclimatization in trained athletes. *Sports Med, 12*(5), 302-312.

Armstrong, L. E., Maresh, C. M., Castellani, J. W., Bergeron, M. F., Kenefick, R. W., LaGasse, K. E., & Riebe, D. (1994). Urinary indices of hydration status. *Int J Sport Nutr, 4*(3), 265-279.

Balligand, J. L., Feron, O., & Dessy, C. (2009). eNOS activation by physical forces: from short-term regulation of contraction to chronic remodeling of cardiovascular tissues. *Physiol Rev, 89*(2), 481-534. doi: 10.1152/physrev.00042.2007

Barnes, R. J., Bower, E. A., & Rink, T. J. (1986). Haemodynamic responses to stimulation of the splanchnic and cardiac synpathetic nerves in the anaesthetized cat. *J Physiol, 378*(14), 417-436.

Bass, D. E., Kleeman, C. R., Quinn, M., Henschel, A., & Hegnauer, A. H. (1955). Mechanisms of acclimatization to heat in man. *Medicine, 34*(3), 323-380.

Bazett, H. C., Sunderman, F. W., Doupe, J., & Scott, J. C. (1940). Climatic effects on the volume and composition of blood in man. *Am J Physiol, 129*(1), 69-83. doi: 10.1152/ajplegacy.1940.129.1.69

Bean, W. B., & Eichna, L. W. (1943). Performance in relation to environmental temperature. Reactions of normal young men to simulated desert environment. *Fed Proc, 2*(3), 144-158.

Beaudin, A. E., Walsh, M. L., & White, M. D. (2012). Central chemoreflex ventilatory responses in humans following passive heat acclimation. *Respir Physiol Neurobiol, 180*(1), 97-104. doi: 10.1016/j.resp.2011.10.014

Bedford, T. (1936). The warmth factor in comfort at work *MRC Industrial Health Board Report*.

Beladen, C. C., Călin, A., Roşca, M., Ginghină, C., & Popescu, G. A. (2014). Left ventricular twist dynamics: principles and applications. *Heart, 100*(9), 731-740. doi: 10.1136/heartjnl-2012302064

Bergeron, M. F., Bahr, R., Bartsch, P., Bourdon, L., Calbet, J. A., Carlsen, K. H., . . . Engebretsen, L. (2012). International Olympic Committee consensus statement on thermoregulatory and altitude challenges for high-level athletes. *Br J Sports Med, 46*(11), 770-779. doi: 10.1136/bjsports-2012-091296

Bers, D. M. (2002). Cardiac excitation-contraction coupling. *Nature, 415*, 198-205. doi: 10.1038/415198a

Beyar, R., & Seideman, S. (1985). Effect of the twisting motion of the nonuniformities of transmyocardial fiber mechanics and energy demand - A theoretical study. *IEE Trans Biomed Eng, 32*(10), 764-769.

Birk, G. K., Dawson, E. A., Atkinson, C., Haynes, A., Cable, T., Thijssen, D. H. J., & Green, D. J. (2012). Brachial artery adaptation to lower limb exercise training: role of shear stress. *J Appl Physiol, 112*, 1653-1658. doi: 10.1152/japplphysiol.01489.2011

Borg, G. A. V. (1982). Psychophysical bases of perceived exertion. *Med Sci Sports Exerc, 14*(5), 377-381.

Bradford, C. D., Lucas, S. J., Gerrard, D. F., & Cotter, J. D. (2015). Swimming in warm water is ineffective in heat acclimation and is non-ergogenic for swimmers. *Scand J Med Sci Sports, 25 Suppl 1*, 277-286. doi: 10.1111/sms.12351

Brengelmann, G. L., Johnson, J. M., Hermansen, L., & Rowell, L. B. (1977). Altered control of skin blood flow during exercise at high internal temperature. *J Appl Physiol Respirat Environ Exercise Physiol, 43*(5), 790-794.

Brothers, R. M., Bhella, P. S., Shibata, S., Wingo, J. E., Levine, B. D., & Crandall, C. G. (2009). Cardiac systolic and diastolic function during whole body heat stress. *Am J Physiol Heart Circ Physiol, 296*(4), H1150-1156. doi: 10.1152/ajpheart.01069.2008

Brouet, A., Sonveaux, P., Dessy, C., Balligand, J. L., & Feron, O. (2001). Hsp90 ensures the transition from the early Ca2+-dependent to the late phosphorylation-dependent activation of the endothelial nitric-oxide synthase in vascular endothelial growth factor-exposed endothelial cells. *J Biol Chem, 276*(35), 32663-32669. doi: 10.1074/jbc.M101371200

Bruce, E. N., & Bruce, M. C. (2003). A multicompartment model of carboxyhemoglobin and carboxymyoglobin responses to inhalation of carbon monoxide. *J Appl Physiol, 95*, 1235-1247. doi: 10.1152/japplphysiol.00217.2003

Buckberg, G. D., Nanda, N. C., Nguyen, C., & Kocica, M. J. (2018). What Is the Heart? Anatomy, Function, Pathophysiology, and Misconceptions. *J Cardiovasc Dev Dis, 5*(2). doi: 10.3390/jcdd5020033

Buguet, A., Gati, R., Soubiran, G., Straboni, J. P., Hanniquet, A. M., Livecchi-Gonnot, G., & Bittel, J. (1988). Seasonal changes in circadian rhythms of body temperatures in humans living in a dry tropical climate. *Eur J Appl Physiol, 58*(3), 334-339.

Bulmer, M. G., & Forwell, G. D. (1956). The concentration of sodium in thermal sweat. *J Physiol, 132*(1), 115-122.

Buono, M. J., Ball, K. D., & Kolkhorst, F. W. (2007). Sodium ion concentration vs. sweat rate relationship in humans. *J Appl Physiol (1985), 103*(3), 990-994. doi: 10.1152/japplphysiol.00015.2007

Buono, M. J., Martha, S. L., & Heaney, J. H. (2009). Peripheral sweat gland function is improved with humid heat acclimation. *Journal of Thermal Biology, 34*(3), 127-130. doi: 10.1016/j.jtherbio.2008.11.006

Burge, C. M., & Skinner, S. L. (1995). Determination of hemoglobin mass and blood volume with CO: evaluation and application of a method. *J Appl Physiol (1985), 79*(2), 623-631. doi: 10.1152/jappl.1995.79.2.623

Buskirk, E. R., Iampietro, P. F., & Bass, D. E. (1958). Work performance after dehydration: Effects physical conditioning and heat acclimatization. *J Appl Physiol, 12*(2), 189-194.

Carter, Robert, Cheuvront, Samuel N., Williams, Jeffrey O., Kolka, Margaret A., Stephenson, Lou A., Sawka, Michael N., & Amoroso, Paul J. (2005). Epidemiology of Hospitalizations and Deaths from Heat Illness in Soldiers. *Medicine & Science in Sports & Exercise, 37*(8), 1338-1334. doi: 10.1249/01.mss.0000174895.19639.ed

Castle, P., Mackenzie, R. W., Maxwell, N., Webborn, A. D., & Watt, P. W. (2011). Heat acclimation improves intermittent sprinting in the heat but additional pre-cooling offers no further ergogenic effect. *J Sports Sci, 29*(11), 1125-1134. doi: 10.1080/02640414.2011.583673

Charkoudian, N., & Stachenfeld, N. S. (2014). Reproductive hormone influences on thermoregulation in women. *Compr Physiol, 4*(2), 793-804. doi: 10.1002/cphy.c130029

Cheuvront, S. N., & Kenefick, R. W. (2014). Dehydration: physiology, assessment, and performance effects. *Compr Physiol, 4*(1), 257-285. doi: 10.1002/cphy.c130017

Cheuvront, S. N., Kenefick, R. W., Montain, S. J., & Sawka, M. N. (2010). Mechanisms of aerobic performance impairment with heat stress and dehydration. *J Appl Physiol, 109*, 1989-1995. doi: 10.1152/japplphysiol.00367.2010

Chiesa, S. T., Trangmar, S. J., & Gonzalez-Alonso, J. (2016). Temperature and blood flow distribution in the human leg during passive heat stress. *J Appl Physiol (1985), 120*(9), 1047-1058. doi: 10.1152/japplphysiol.00965.2015

Chinevere, T. D., Kenefick, R. W., Cheuvront, S. N., Lukaski, H. C., & Sawka, M. N. (2008). Effect of heat acclimation on sweat minerals. *Med Sci Sports Exerc, 40*(5), 886-891. doi: 10.1249/MSS.0b013e3181641c04

Clarkson, P., Montgomery, H. E., Mullen, M. J., Donald, A. E., Powe, A. J., Bull, T., . . . Deanfield, J. E. (1999). Exercise training enhances endothelial function in young men. *J Am Coll Cardiol, 33*(5), 1379-1385.

Climate & Weather Averages in Doha, Qatar. (2018). from https://www.timeanddate.com/weather/qatar/doha/climate

Cohen, J. S. (1988). *Statistical power analysis for the behavioural sciences* (2 ed.). New York: Academic Press.

Cohen, J. S., & Gisolfi, C. V. (1982). Effects of interval training on work-heat tolerance of young women. *Med Sci Sports Exerc, 14*(1), 46-52.

Cohen, O., Kanana, H., Zoizner, R., Gross, C., Meiri, U., Stern, M. D., . . . Horowitz, M. (2007). Altered Ca2+ handling and myofilament desensitization underlie cardiomyocyte performance in normothermic and hyperthermic heat-acclimated rat hearts. *J Appl Physiol, 103*(1), 266-275. doi: 10.1152/japplphysiol.01351.2006

Corbett, J., Neal, R. A., Lunt, H. C., & Tipton, M. J. (2014). Adaptation to heat and exercise performance under cooler conditions: a new hot topic. *Sports Med, 44*(10), 1323-1331. doi: 10.1007/s40279-014-0212-8

Coyle, E. F., Hopper, M. K., & Coggan, A. R. (1990). Maximal oxygen uptake relative to plasma volume expansion. *Int J Sports Med, 11*(2), 116-119. doi: 10.1055/s-2007-1024774

Crandall, C. G. (2000). Carotid baroreflex responsiveness in heat-stressed humans. *Am J Physiol Heart Circ Physiol, 279*(4), H1955-1962. doi: 10.1152/ajpheart.2000.279.4.H1955

Crandall, C. G. (2008). Heat stress and baroreflex regulation of blood pressure. *Med Sci Sports Exerc, 40*(12), 2063-2070. doi: 10.1249/MSS.0b013e318180bc98

Crandall, C. G., Etzel, R. A., & Farr, D. B. (1999). Cardiopulmonary baroreceptor control of muscle sympathetic nerve activity in heat-stressed humans. *Am J Physiol, 277*(6 Pt 2), H2348-2352.

Crandall, C. G., & González-Alonso, J. (2010). Cardiovascular function in the heat-stressed human. *Acta Physiol (Oxf), 199*(4), 407-423. doi: 10.1111/j.1748-1716.2010.02119.x

Crandall, C. G., Wilson, T. E., Marving, J., Vogelsang, T. W., Kjaer, A., Hesse, B., & Secher, N. H. (2008). Effects of passive heating on central blood volume and ventricular dimensions in humans. *The Journal of Physiology, 586*(1), 293-301. doi: 10.1113/jphysiol.2007.143057

Daanen, H. A. M., Racinais, S., & Periard, J. D. (2018). Heat Acclimation Decay and Re-Induction: A Systematic Review and Meta-Analysis. *Sports Med, 48*(2), 409-430. doi: 10.1007/s40279-017-0808-x

Dill, D. B., & Costill, D. L. (1974). Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol, 37*(2), 247-248.

Dill, D. B., Hall, F. G., & Edwards, H. T. (1938). Changes in composition of sweat during acclimatization to heat. *am J Physiol, 123*, 412-419.

Doppler, C. J. (1842). *On the coloured light of the binary stars and some other stars of the heavens - Attempt at a general theory including Bradley's*

theorem as an integral part (Vol. 5). Prague: Proceedings of the Royal Bohemian Society of Sciences.

Eichna, L. W., Park, C. R., Nelson, N., Horvath, S. M., & Palmes, E. D. (1950). Thermal regulation during acclimatization in a hot, dry (desert type) environment. *AM J Physiol, 163*, 585-597.

Ely, B. R., Blanchard, L. A., Steele, J. R., Francisco, M. A., Cheuvront, S. N., & Minson, C. T. (2018). Physiological Responses to Overdressing and Exercise-Heat Stress in Trained Runners. *Med Sci Sports Exerc, 50*(6), 1285-1296. doi: 10.1249/MSS.0000000000001550

Ely, B. R., Cheuvront, S. N., Kenefick, R. W., & Sawka, M. N. (2010). Aerobic performance is degraded, despite modest hyperthermia, in hot environments. *Med Sci Sports Exerc, 42*(1), 135-141. doi: 10.1249/MSS.0b013e3181adb9fb

Ely, B. R., Lovering, A. T., Horowitz, M., & Minson, C. T. (2014). Heat acclimation and cross tolerance to hypoxia: Bridging the gap between cellular and systemic responses. *Temperature (Austin), 1*(2), 107-114. doi: 10.4161/temp.29800

Ely, M. R., Cheuvront, S. N., Roberts, W. O., & Montain, S. J. (2007). Impact of weather on marathon-running performance. *Med Sci Sports Exerc, 39*(3), 487-493. doi: 10.1249/mss.0b013e31802d3aba

Febbraio, M. A., Snow, R. J., Hargreaves, M. , Stathis, C. G., Martin, I. K, & Carey, M. F. (1994). Muscle metabolism during exercise and heat stress in trained men: effect of acclimation. *J Appl Physiol, 76*(2), 589=597.

Feigenbaum, H., Armstrong, W. F., & Ryan, T. (2005). *Feigenbaum's Echocardiography* (6 ed.). Philadelphia, USA: Lippincot Williams & Wilkins.

Fleming, J., & James, L. J. (2014). Repeated familiarisation with hypohydration attenuates the performance decrement caused by hypohydration during treadmill running. *Appl Physiol Nutr Metab, 39*(2), 124-129. doi: 10.1139/apnm-2013-0044

Fortney, S. M., Nadel, E. R., Wenger, C. B., & Bove, J. R. (1981a). Effect of acute alterations of blood volume on circulatory performance in humans. *J Appl Physiol Respir Environ Exerc Physiol, 50*(2), 292-298. doi: 10.1152/jappl.1981.50.2.292

Fortney, S. M., Nadel, E. R., Wenger, C. B., & Bove, J. R. (1981b). Effect of blood volume on sweating rate and body fluids in exercising humans. *J Appl Physiol Respir Environ Exerc Physiol, 51*(6), 1594-1600. doi: 10.1152/jappl.1981.51.6.1594

Fox, R. H., Goldsmith, R., & Hampton, I .F. G. (1967). Heat acclimatization by controlled hyperthermia in hot-dry and hot-wet climates *J Appl Physiol, 22*(1), 39-46.

Fox, R. H., Goldsmith, R., Hampton, I .F. G., & lewis, H. E. (1964). The nature of increase in sweating capacity produced by heat acclimatization. *J Physiol, 171*, 368-376.

Fox, R. H., Goldsmith, R., Kidd, D. J., & Lewis, H. E. (1963a). Acclimatization to the heat in man by controlled elevation of body temperature. *J Physiol, 166*, 530-547.

Fox, R. H., Goldsmith, R., Kidd, D. J., & Lewis, H. E. (1963b). Blood flow and other thermoregulatory changes with acclimatization to heat. *J Physiol, 166*(3), 548-562.

Fritzsche, R. G., Switzer, T. W., Hodgkinson, B. J., & Coyle, E. F. (1999). Stroke volume decline during prolonged exercise is influenced by the increase in heart rate. *J Appl Physiol (1985), 86*(3), 799-805. doi: 10.1152/jappl.1999.86.3.799

Fujii, N., Honda, Y., Ogawa, T., Tsuji, B., Kondo, N., Koga, S., & Nishiyasu, T. (2012). Short-term exercise-heat acclimation enhances skin vasodilation but not hyperthermic hyperpnea in humans exercising in a hot environment. *Eur J Appl Physiol, 112*(1), 295-307. doi: 10.1007/s00421-011-1980-6

Funder, J W. (1993). Aldosterone Action. *Annual Review of Physiology, 55*(1), 115-130. doi: 10.1146/annurev.ph.55.030193.000555

Gagnon, D., Schlader, Z. J., & Crandall, C. G. (2015). Sympathetic activity during passive heat stress in healthy aged humans. *J Physiol, 593*(9), 2225-2235. doi: 10.1113/JP270162

Galloway, S. D., & Maughan, R. J. (1997). Effects of ambient temperature on the capacity to perform prolonged exercise cycle exercise in man. *Med Sci Sports Exerc, 29*(9), 1240-1249.

Ganio, M. S., Overgaard, M., Seifert, T., Secher, N. H., Johansson, P. I., Meyer, M. A. S., & Crandall, C. G. (2012). Effect of heat stress on cardiac output and systemic vascular conductance during simulated hemorrhage to presyncope in young men. *Am J Physiol Heart Circ Physiol, 302*, H1756-H1761. doi: 10.1152/ajpheart.00941.2011

Garrett, A. T., Creasy, R., Rehrer, N. J., Patterson, M. J., & Cotter, J. D. (2012). Effectiveness of short-term heat acclimation for highly trained athletes. *Eur J Appl Physiol, 112*(5), 1827-1837. doi: 10.1007/s00421-011-2153-3

Garrett, A. T., Goosens, N. G., Rehrer, N. J., Patterson, M. J., & Cotter, J. D. (2009). Induction and decay of short-term heat acclimation. *Eur J Appl Physiol, 107*(6), 659-670. doi: 10.1007/s00421-009-1182-7

Garrett, A. T., Goosens, N. G., Rehrer, N. J., Patterson, M. J., Harrison, J., Sammut, I., & Cotter, J. D. (2014). Short-term heat acclimation is effective and may be enhanced rather than impaired by dehydration. *Am J Hum Biol, 26*(3), 311-320. doi: 10.1002/ajhb.22509

Garrett, A. T., Rehrer, N. J., & Patterson, M. J. (2011). Induction and decay of short-term heat acclimation in moderately and highly trained athletes. *Sports Med, 41*(9), 757-771. doi: 10.2165/11587320-000000000-00000

Garrey, W. E., & Townsend, S. E. (1948). Neural responses and reactions of the heart of a human enbryo. *Am J Physiol, 152*(2), 219-224.

George, K., Whyte, G., Stephenson, C., Shave, R., Dawson, E., Edwards, B., . . . Collinson, P. (2004). Postexercise left ventricular function and cTnT in recreational marathon runners. *Med Sci Sports Exerc, 36*(10), 1709-1715.

Gibson, O. R., Mee, J. A., Tuttle, J. A., Taylor, L., Watt, P. W., & Maxwell, N. S. (2015a). Isothermic and fixed intensity heat acclimation methods induce similar heat adaptation following short and long-term timescales. *J Therm Biol, 49-50*, 55-65. doi: 10.1016/j.jtherbio.2015.02.005

Gibson, O. R., Turner, G., Tuttle, J. A., Taylor, L., Watt, P. W., & Maxwell, N. S. (2015b). Heat acclimation attenuates physiological strain and the HSP72, but not HSP90a, mRNA response to acute normobaric hypoxia. *J Appl Physiol, 119*, 889-899. doi: 10.1152/japplphysiol.00332.2015

Ginsburg, K. S., & Bers, D. M. (2004). Modulatin of excitation-contraction coupling by isoproterenol in cardiomyocytes with controlled SR Ca2+ load and Ca2+ current trigger. *J Physiol, 556*(2), 463-480.

Gisolfi, C., & Robinson, S. . (1969). Relations between physical training acclimatization and heat tolerance. *J Appl Physiol, 26*(5), 530-534.

Gisolfi, C. V., & Wenger, C. B. (1984). Temperature regulation during exercise: old concepts, new ideas. *Exerc Sport Sci Rev, 12*, 339-372.

Goldberg, A. L. (2003). Protein degradation and protection against misfolded or damaged proteins. *Nature, 426*(6968), 895-899. doi: 10.1038/nature02263

González-Alonso, J. (1998). Separate and combined influences of dehydration and hyperthermia on cardiovascular responses to exercise. *Int J Sports Med, 19 Suppl 2*, S111-114.

González-Alonso, J. (2007). Hyperthermia impairs brain, heart and muscle function in exercising humans. *Sports Med, 37*(4-5), 371-373. doi: 10.2165/00007256-200737040-00025

González-Alonso, J., Calbet, J. A. L., & Nielsen, B. (1998). Muscle blood flow is reduced with dehydration during prolonged exercise in humans. *J Physiol, 513*(3), 895-905.

González-Alonso, J., & Calbet, José A. L. (2003). Reductions in Systemic and Skeletal Muscle Blood Flow and Oxygen Delivery Limit Maximal Aerobic Capacity in Humans. *Circulation, 107*(6), 824-830. doi: 10.1161/01.cir.0000049746.29175.3f

González-Alonso, J., Mora-Rodríguez, R., Below, P. R., & Coyle, E. F. (1995). Dehydration reduces cardiac output and increases systemic and cutaneous vascular resistance during exercise. *J Appl Physiol, 79*(5), 1487-1496.

González-Alonso, J., Mora-Rodríguez, R., Below, P. R., & Coyle, E. F. (1997). Dehydration markedly impairs cardiovascular function in hyperthermic endurance athletes during exercise. *J Appl Physiol, 82*(4), 1229-1236.

González-Alonso, J., Mora-Rodríguez, R., & Coyle, E. F. (1999a). Supine exercise restores arterial blood pressure and skin blood flow despite dehydration and hyperthermia. *Am J Physiol Heart Circ Physiol, 46*, H576-H583.

González-Alonso, J., mora-Rodríguez, R., & Coyle, E. F. (2000a). Stroke volume during exercise: interaction of environment and hydration. *Am J Physiol Heart Circ Physiol, 278*, H321-H330.

González-Alonso, J., Quistorff, B., Krustrup, P., Bangsbo, J., & Saltin, B. (2000b). Heat production in human skeletal muscle at the onset of intense dynamic exercise. *J Physiol, 524*(2), 603-615.

González-Alonso, J., Teller, C., Andersen, S. L., Jensen, F .B., Hyldig, T., & Nielsen, B. (1999b). Influence of body temperature on the development of fatigue during prolonged exercise in the heat. *J Appl Physiol, 86*(3), 1032-1039.

Gonzalez, R. R., & Gagge, A. P. (1976). Warm discomfort and associated thermoregulatory changes during dry, and humid-heat acclimatization. *Isr J Med Sci, 12*, 804-807.

Good, D. W. (2007). Nongenomic actions of aldosterone on the renal tubule. *Hypertension, 49*(4), 728-739. doi: 10.1161/01.HYP.0000259797.48382.b2

Gore, C. J., Hopkins, W. G., & Burge, C. M. (2005). Errors of measurement for blood volume parameters: a meta-analysis. *J Appl Physiol, 99*, 1745-1758. doi: 10.1152/japplphysiol.00505.2005

Gorman, A. J., & Proppe, D. W. (1984). Mechanisms producing tachycardia in conscious baboons during environmental heat stress. *J Appl Physiol Respir Environ Exerc Physiol, 56*(2), 441-446. doi: 10.1152/jappl.1984.56.2.441

Goto, C., Higashi, Y., Kimura, M., Noma, K., Hara, K., Nakagawa, K., . . . Nara, I. (2003). Effect of different intensities of exercise on endothelium-dependent vasodilation in humans: role of endothelium-dependent nitric oxide and oxidative stress. *Circulation, 108*(5), 530-535. doi: 10.1161/01.CIR.0000080893.55729.28

Goto, M., Okazaki, K., Kamijo, Y., Ikegawa, S., Masuki, S., Miyagawa, K., & Nose, H. (2010). Protein and carbohudrate supplementation during 5 day aerobic training enhanced plasma volume expansion and thermoregulatory adaptation in young men. *J Appl Physiol, 109*, 1247-1255. doi: 10.1152/japplphysiol.00577.2010

Gottdiener, J. S. (2001). Overview of stress echocardiography: uses, advantages, and limitations. *Prog Cardiovasc Dis, 43*(4), 315-334. doi: 10.1053/pcad.2001.20502

Greenbaum, R. A., Ho, S. Y., Gibson, D. G., Becker, A. E., & Anderson, R. H. (1981). Left ventricular fibre architecture in man. *Br Heart J, 45*(3), 248-263.

Greenleaf, J. E. (1964). Lack of artificial acclimatization to the heat in physically fit subjects. *Nature, 203*, 1072.

Guy, J. H., Deakin, G. B., Edwards, A. M., Miller, C. M., & Pyne, D. B. (2015). Adaptation to hot environmental conditions: an exploration of the performance basis, procedures and future directions to optimise opportunities for elite athletes. *Sports Med, 45*(3), 303-311. doi: 10.1007/s40279-014-0277-4

Guyton, A. C., Jones, C. E., & Coleman, T. G. (1973). *Circulatory physiology: Cardiac output and its regulation* (2 ed.). Philadelphia, USA: W. B. Saunders.

Halliwill, J. R., Sieck, D. C., Romero, S. A., Buck, T. M., & Ely, M. R. (2014). Blood pressure regulation X: what happens when the muscle pump is lost? Post-exercise hypotension and syncope. *Eur J Appl Physiol, 114*(3), 561-578. doi: 10.1007/s00421-013-2761-1

Harrison, M. H., Edwards, R. J., Graveney, M. J., Cochrane, L. A., & Davies, J. A. (1981). Blood volume and plasma protein responses to heat acclimatization in humans. *J Appl Physiol Respirat Environ Exercise Physiol, 50*(3), 597-604.

Heinicke, K., Wolfarth, B., Winchenbach, P., Bierman, B., Schmid, A., Huber, G., . . . Schmidt, W. (2001). Blood volume and hemoglobin mass in elite athletes of different disciplines. *Int J Sports Med, 22*, 504-512.

Helle-Valle, T., Crosby, J., Edvardsen, T., Lyseggen, E., Amundsen, B. H., Smith, H. J., . . . Smiseth, O. A. (2005). New noninvasive method for assessment of left ventricular rotation: speckle tracking echocardiography. *Circulation, 112*(20), 3149-3156. doi: 10.1161/CIRCULATIONAHA.104.531558

Henane, R., Flandrois, R., & Charbonner, J. P. (1977). Increase in sweating sensitivity by endurance conditioning in man. *J Appl Physiol Respir Environ Exerc Physiol, 43*(5), 822-828.

Higashi, Y., Sasaki, S., Kurisu, S., Yoshimizu, A., Sasaki, N., Matsuura, H., . . . Oshima, T. (1999). Regular aerobic exercise augments endothelium-dependent vascular relaxation in normotensive as well as hypertensive subjects - role of endothelium-derived nitric oxide. *Circulation, 100*, 1194-1202.

Higginbotham, M. B., Morris, K. G., Williams, R. S., McHale, P. A., Coleman, R. E., & Cobb, F. R. (1986). Regulation of stroke volume during submaximal and maximal upright exercise in normal man. *Circ Res, 58*(2), 281-291.

Hogan, M. C., Bebout, D. E., Gray, A. T., Wagner, P. D., West, J. B., & Haab, P. E. (1990). Muscle maximal O2 uptake at constant O2 delivery with and without CO in the blood. *J Appl Physiol, 69*(3), 830-866.

Hopkins, W. G. (2000). Measures of reliability in sports medicine and science. *Sports Med, 30*(1), 1-15.

Hopkins, W. G. (2004). How to interpret changes in an athletic performance test. *Sportsscience, 8*, 1-7.

Horowitz, M. (1998). Do cellular heat acclimation responses modulate central thermoregulatory activity? *News Physiol Sci, 13*, 218-225.

Horowitz, M. (2002). From molecular and cellular to integrative heat defense during exposure to chronic heat. *Comp Biochem Physiol, Part A*(131), 475-483.

Horvath, S. M., & Shelley, W. B. (1946). Acclimatization to extreme heat and its effect on the ability to work in less severe environments *Am J Physiol, 146*, 336-343.

Houmard, J. A., Costill, D. L., Davis, J. A., Mitchell, J. B., Pascoe, D. D., & Robergs, R. A. (1990). The influence of exercise intensity on heat acclimation in trained subjects. *Med Sci Sports Exerc, 22*(5), 615-620.

Ikegawa, S., Kamijo, Y., Okazaki, K., Masuki, S., Okada, Y., & Nose, H. (2011). Effects of hypohydration on thermoregulation during exercise before and after 5-day aerobic training in a warm environment in young men. *J Appl Physiol, 110*, 972-980. doi: 10.1152/japplphysiol.01193.2010

Inoue, y., Havenith, G., Kenney, L. W., Loomis, J. L., & Buskirk, E. R. (1999). Exercise- and methylcholine-induced sweating responses in older and younger men: effect of heat acclimation and aerobic fitness. *Int J Biometerol, 42*(2), 210-216.

Inoue, Y., Nakao, M., Okudaira, S., Ueda, H., & Araki, T. (1995). Seasonal variation in sweating response of older and younger men. *Eur J Appl Physiol, 70*(1), 6-12.

Jay, O., Bain, A. R., Deren, T. M., Sacheli, M., & Cramer, M. N. (2011). Large differences in peak oxygen uptake do not independently alter changes in core temperature and sweating during exercise. *Am J Physiol Regul Integr Comp Physiol, 301*, R832-R841. doi: 10.1152/ajpregu.00257.2011

Johnson, J. M., & Park, M. K. (1982). Effect of heat stress on cutaneous vascular responses to the initiation of exercise. *J Appl Physiol Respirat Environ Exercise Physiol, 53*(3), 744-749.

Jose, A. D. (1966). Effect of combined sympathetic and parasympathetic blockade on heart rate and cardiac function in man. *Am J Cardiol, 18*(3), 476-478.

Jose, A. D., Stitt, F., & Collison, D. (1970). The effects of exercise and changes in body temperature on the intrinsic heart rate in man. *Am Heart J, 79*(4), 488-498.

Kamijo, Y., Ikegawa, S., Okada, Y., Masuki, S., Okazaki, K., Uchida, K., . . . Nose, H. (2012). Enhanced renal Na+ reabsorption by carbohydrate in beverages during restitution from thermal and exercise-induced dehydration in men. *Am J Phsyiol Regul Integr Comp Physiol, 303*, R824-R833. doi: 10.1152/ajpregu.00588.2011

Kanstrup, I. L., & Ekblom, B. (1984). Blood volume and hemoglobin concentration as determinants of maximal aerobic power. *Med Sci Sports Exerc, 16*(3), 256-262.

Karlsen, A., Racinais, S., Jensen, M. V., Norgaard, S. J., Bonne, T., & Nybo, L. (2015). Heat acclimatization does not improve VO2max or cycling

performance in a cool climate in trained cyclists. *Scand J Med Sci Sports, 25 Suppl 1*, 269-276. doi: 10.1111/sms.12409

Keiser, S., Fluck, D., Huppin, F., Strava, A., Hilty, M. P., & Lundby, C. (2015). Heat training increases exercise capacity in hot but not in temperate conditions: a mechanistic counter-balanced cross-over study. *Am J Physiol Heart Circ Physiol, 309*, H750-H761. doi: 10.1152/ajpheart.00138.2015

Kellogg, D. L., Johnson, J. M., & Kosiba, W. A. (1991). Competition between cutanous active vasoconstriction and active vasodilation during exercise in humans. *Am J Physiol Heart Circ Physiol, 261*(30), H1184-H1189.

Kenefick, R. W., Maresh, C. M., Armstrong, L. E., Riebe, D., Echegaray, M. E., & Castellani, J. W. (2007). Rehydration with fluid of varying tonicities: effects on fluid regulatory hormones and exercise performance in the heat. *J Appl Physiol, 102*, 1899-1905. doi: 10.1152/japplphysiol.00920.2006

Kenney, L. W., Stanhewicz, A. E., Bruning, R. S., & Alexander, L. M. (2014). Blood pressure regulation III: what happens when one system must serve two masters: temperature and pressure regulation? *Eur J Appl Physiol, 114*(3), 467-479. doi: 10.1007/s00421-013-2652-5

Kenney, W. L., & Johnson, J. M. (1992). Control of skin blood flow during exercise. *Med Sci Sports Exerc, 24*(3), 303-312.

Kenney, W. L., Tankersley, C. G., Newswanger, D. L., & Puhl, S. M. (1991). Alpha 1-adrenergic blockade does not alter control of skin blood flow during exercise. *Am J Phsyiol Heart Circ Physiol, 260*(2), H855-H861.

Kirby, C. R., & Convertino, V. A. (1986). Plasma aldosterone and sweat sodium concentrations after exercise and heat acclimation. *J Appl Physiol, 61*(3), 367-370.

Klabunde, R. E. (2012). *Cardiovascular Physiology Concepts* (2 ed.). Philadelphia, USA: Lippincott Williams & Wilkins.

Kuennen, M., Gillum, T., Dokladny, K., Bedrick, E., Schneider, S., & Moseley, P. (2011). Thermotolerance and heat acclimation may share a common mechanism in humans. *Am J Physiol Regul Integr Comp Physiol, 301*, R524-R533. doi: 10.1152/ajpregu.00039.2011.

Ladell, W. S. S. (1951). Assessment of group acclimatization to heat and humidity. *J Physiol, 115*, 296-312.

Ladell, W. S. S. (1955). Physiological observations on men working in supposedly limiting environments in a west african gold mine. *Brit J Industr Med, 12*, 111-125.

Lang, R. M., Badano, L. P., Mor-Avi, V., Afilalo, J., Armstrong, A., Ernande, L., . . . Voigt, J. U. (2015). Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *Eur Heart J Cardiovasc Imaging, 16*(3), 233-270. doi: 10.1093/ehjci/jev014

Lang, R. M., Bierig, M., Devereux, R. B., Flachskampf, F. A., Foster, E., Pellikka, P. A., . . . European Association of Echocardiography, European Society of Cardiology. (2006). Recommendations for chamber quantification. *Eur J Echocardiogr, 7*(2), 79-108. doi: 10.1016/j.euje.2005.12.014

Laughlin, M. H., Davis, M. J., Secher, N. H., van Lieshout, J. J., Arce-Esquivel, A. A., Simmons, G. H., . . . Duncker, D. J. (2012). Peripheral circulation. *Compr Physiol, 2*(1), 321-447. doi: 10.1002/cphy.c100048

Laughlin, M. H., Newcomer, S. C., & Bender, S. B. (2008). Importance of hemodynamic forces as signals for exercise-induced changes in endothelial cell phenotype. *J Appl Physiol (1985), 104*(3), 588-600. doi: 10.1152/japplphysiol.01096.2007

Levi, E., Vivi, A., Hasin, Y., Tassini, M., Navon, G., & Horowitz, M. (1993). Heat acclimation improves cardiac mechanics and metabolic performance during ischemia and reperfusion. *J Appl Physiol, 75*(2), 833-839.

Levine, B. D., & Stray-Gundersen, J. (1997). "Living high-training low": effect of moderate altitude acclimatization with low altitude training on performance. *J Appl Physiol, 83*(1), 102-112.

Levy, E., Hasin, Y., Navon, G., & Horowitz, M. (1997). Chronic heat improves mechanical and metabolic response of trained rat heart on ischemia and reperfusion. *Am J Physiol, 272*(5 Pt 2), H2085-2094. doi: 10.1152/ajpheart.1997.272.5.H2085

Levy, M. N. (1979). The cardiac and vascular factors that determine systolic blood flow. *Circ Res, 44*(6), 739-747.

Levy, W. C., Cerquiera, M. D., Abrass, I. B., Schwartz, R. S., & Stratton, J. R. (1993). Endurance exercise training augments diastolic filling at rest and during exercise in healthy young and older men. *Circulation, 88*(1), 116-126.

Lippi, G., Salvagno, G., Solero, G., & Guidi, G. (2006). The Influence of the Tourniquet Time on Hematological Testing for Antidoping Purposes. *International Journal of Sports Medicine, 27*(5), 359-362. doi: 10.1055/s-2005-865749

Lord, R., MacLeod, D., George, K., Oxborough, D., Shave, R., & Stembridge, M. (2018). Reduced left ventricular filling following blood volume extraction does not result in compensatory augmentation of cardiac mechanics. *Exp Physiol, 103*(4), 495-501. doi: 10.1113/EP086761

Lorenzo, S., Halliwell, J. R., Sawka, M. N., & Minson, C. T. (2010). Heat acclimation improves exercise performance. *J Appl Physiol, 109*, 1140-1147. doi: 10.1152/japplphysiol.00495.2010

Lorenzo, S., & Minson, C. T. (2010). Heat acclimation improves cutaneous vascular function and sweating in trained cyclists. *J Appl Physiol, 109*, 1736-1743. doi: 10.1152/japplphysiol.00725.2010

Low, D. A., Keller, D. M., Wingo, J. E., Brothers, R. M., & Crandall, C. G. (2011). Sympathetic nerve activity and whole body heat stress in

humans. *J Appl Physiol, 111*(5), 1329-1334. doi: 10.1152/japplphysiol.00498.2011

Machado-Moreira, C. A., D., Magalhaes F., Vimiero-Gomes, A. C., Lima, N. R. V., & Rodrigues, L. O. C. (2005). Effects of heat acclimation on sweating during graded exercise until exhaustion. *J Therm Biol, 30*(6), 437-442. doi: 10.1016/j.therbio.2005.05.002

Magalhães, Flávio C., Passos, Renata L. F., Fonseca, Michele A., Oliveira, Kenya P. M., Ferreira-Júnior, João B., Martini, Angelo R. P., . . . Rodrigues, Luiz O. C. (2010). Thermoregulatory Efficiency is Increased after Heat Acclimation in Tropical Natives. *Journal of PHYSIOLOGICAL ANTHROPOLOGY, 29*(1), 1-12. doi: 10.2114/jpa2.29.1

Magalhães, F. C., Machado-Moreira, C. A., Vimieiro-Gomes, A. C., Silami-Garcia, E., Lima, N. R., & Rodrigues, L. O. (2006). Possible biphasic sweating response during short-term heat acclimation protocol for tropical natives. *J Physiol Anthropol, 25*(3), 215-219.

Maloyan, A., Palmon, A., & Horowitz, M. (1999). Heat acclimation increases the basal HSP72 level and alters its production dynamics during heat stress. *Am J Physiol, 276*(45), R1506-R1515.

Maw, G. J., Mackenzie, I. L., & Taylor, N. A. (1998). Human body-fluid distribution during exercise in hot, temperate and cool environments. *Acta Physiol Scand, 163*(3), 297-304. doi: 10.1046/j.1365-201x.1998.00380.x

McClung, J. P., Hasday, J. D., Ju-ren, H, Montain, S. J., Cheuvront, S. N., Sawka, M. N., & Singh, I. S. (2008). Excerise-heat acclimation in humans alters baseline lovels and ex vivo heat inducibilty of HSP72 and HSP90 in peripheral blood mononuclear cells. *Am J Physiol Regul Integr Comp Physiol, 294*, R185-R191. doi: 10.1152/ajpregu.00532.2007

McGowan, J. H., & Cleland, J. G. (2003). Reliability of reporting left ventricular systolic function by echocardiography: a systematic review of 3 methods. *Am Heart J, 146*(3), 388-397. doi: 10.1016/s0002-8703(03)00248-5

McNamara, T. C., Keen, J. T., Simmons, G. H., Alexander, L. M., & Wong, B. J. (2014). Endothelial nitric oxide synthase mediates the nitric oxide component of reflex cutaneous vasodilatation during dynamic exercise in humans. *J Physiol, 592*(23), 5317-5326. doi: 10.1113/jphysiol.2014.272898

Mee, J. A., Gibson, O. R., Doust, J., & Maxwell, N. S. (2015). A comparison of males and females' temporal patterning to short- and long-term heat acclimation. *Scand J Med Sci Sports, 25 Suppl 1*, 250-258. doi: 10.1111/sms.12417

Mee, J. A., Peters, S., Doust, J. H., & Maxwell, N. S. (2018). Sauna exposure immediately prior to short-term heat acclimation accelerates phenotypic adaptation in females. *J Sci Med Sport, 21*(2), 190-195. doi: 10.1016/j.jsams.2017.02.024

Merry, T. L., Ainslie, P. N., & Cotter, J. D. (2010). Effects of aerobic fitness on hypohydration-induced physiological strain and exercise impairment. *Acta Physiol (Oxf), 198*(2), 179-190. doi: 10.1111/j.1748-1716.2009.02051.x

Minson, C. T., & Cotter, J. D. (2016). CrossTalk proposal: Heat acclimatization does improve performance in a cool condition. *J Physiol, 594*(2), 241-243. doi: 10.1113/JP270879

Minson, C. T., Wladkowski, S. L., Cardell, A. F., Pawelczyk, J. A., & Kenney, W. L. (1998). Age alters the cardiovascular response to direct passive heating. *J Appl Physiol (1985), 84*(4), 1323-1332. doi: 10.1152/jappl.1998.84.4.1323

Mitchell, D., Senay, L. C., Wyndham, C. H., Van Rensburg, A. J., Rogers, G. G., & Strydom, N. B. (1976). Acclimatization in a hot, humid environment: energy exchange, body temperature and sweating. *J Appl Physiol, 40*(5), 786-778.

Montain, S. J., & Coyle, E. F. (1992a). Fluid ingestion during exercise increases skin blood flow independent of increases in blood volume. *J Appl Physiol (1985), 73*(3), 903-910. doi: 10.1152/jappl.1992.73.3.903

Montain, S. J., & Coyle, E. F. (1992b). Influence of graded dehydration on hyperthermia and cardiovascular drift during exercise. *J Appl Physiol, 73*(4), 1340-1350.

Montain, S. J., Sawka, M. N., Latzka, W. A., & Valeri, C. R. (1998). Thermal and cardiovascular strain from hypohydration: influence of exercise intensity. *Int J Sports Med, 19*(2), 87-91. doi: 10.1055/s-2007-971887

Mortensen, S. P., Damsgaard, R., Dawson, E. A., Secher, N. H., & González-Alonso, J. (2008). Restrictions in systemic and locomotor skeletal muscle perfusion, oxygen supply and VO2 during high-intensity whole-body exercise in humans. *J Physiol, 586*(10), 2621-2635. doi: 10.1113/jphysiol.2007.149401

Mortensen, S. P., Dawson, E. A., Yoshiga, C. C., Dalsgaard, M. K., Damsgaard, R., Secher, N. H., & González-Alonso, J. (2005). Limitations to systemic and locomotor limb muscle oxygen delivery and uptake during maximal exercise in humans. *J Physiol, 566*(Pt 1), 273-285. doi: 10.1113/jphysiol.2005.086025

Moseley, P. L. (1997). Heat shock proteins and heat adaptation of the whole organism. *J Appl Physiol, 83*(5), 1413-1417.

Mould, R. F. (2006). Pierre Curie, 1859-1906. *Current Oncology, 14*(2), 74-82.

Munch, G. D., Svendsen, J. H., Damsgaard, R., Secher, N. H., Gonzalez-Alonso, J., & Mortensen, S. P. (2014). Maximal heart rate does not limit cardiovascular capacity in healthy humans: insight from right atrial pacing during maximal exercise. *J Physiol, 592*(2), 377-390. doi: 10.1113/jphysiol.2013.262246

Myers, K., & Clough, A. M. (2014). *Practical Vascular Ultrasound - An Illustrated Guide*. Florida, USA: Taylor & Francis.

Nadel, E. R. (1979). Control of sweating rate while exercising in the heat. *Med Sci Sports, 11*(1), 31-35.

Nadel, E. R., Carfarelli, E., Roberts, M. F., & Wenger, C. B. (1979). Circulatory regulation during exercise in different ambient temperatures. *J Appl Physiol Respirat Environ Exercise Physiol, 46*(3), 430-437.

Nadel, E. R., Fortney, S. M, & Wenger, C. B. (1980). Effect of hydration state on circulatory and thermal regulations. *J Appl Physiol, 49*(4), 715-721.

Nadel, E. R., Pandolf, K. B., Roberts, M. F., & Stolwijk, J. A. (1974). Mechanisms of thermal acclimation to exercise and heat. *J Appl Physiol, 37*(4), 515-520.

Naef, N., Steiner, T., & Wehrlin, J. P. (2015). Replicate Measurements of Haemoglobin Mass during a Single Day are Feasible and Precise. *Int J Sports Med*. doi: 10.1055/s-0034-1398583

Nagashima, K., Mack, G. M., Haskell, A., Nishiyashu, T., & Nadel, E. R. (1999). Mechanism for the posture-specific plasma volume increase after a single intense exercise protocol. *J Appl Physiol, 86*(3), 867-873.

Nagashima, K., Wu, J., Kavouras, S. A., & Mack, G. W. (2001). Increased renal tubular sodium reabsorption during exercise-induced hypervolemia in humans. *J Appl Physiol (1985), 91*(3), 1229-1236. doi: 10.1152/jappl.2001.91.3.1229

Naylor, L. H., Carter, H., Fitzsimons, M. G., Cable, T. N., Thijssen, D. H. J., & Green, D. J. (2011). Repeated increases in blood flow, independent of exercise, ehnahce conduit artery vasodilator function in humans. *Am J Physiol Heart Circ Physiol, 300*, H664-H669. doi: 10.1152/ajpheart.00985.2010

Neal, R. A., Corbett, J., Massey, H. C., & Tipton, M. J. (2016a). Effect of short-term heat acclimation with permissive dehydration on thermoregulation and temperate exercise performance. *Scand J Med Sci Sports, 26*(8), 875-884. doi: 10.1111/sms.12526

Neal, R. A., Massey, H. C., Tipton, M. J., Young, J. S., & Corbett, J. (2016b). Effect of Permissive Dehydration on Induction and Decay of Heat Acclimation, and Temperate Exercise Performance. *Front Physiol, 7*, 564. doi: 10.3389/fphys.2016.00564

Nelson, M. D., Altamirano-Diaz, L. A., Petersen, S. R., DeLorey, D. S., Stickland, M. K., Thompson, R. B., & Haykowsky, M. J. (2011). Left ventricular systolic and diastolic function during tilt-table positioning and passive heat stress in humans. *Am J Physiol Heart Circ Physiol, 301*(2), H599-608. doi: 10.1152/ajpheart.00127.2011

Nelson, M. D., Haykowsky, M. J., Petersen, S. R., DeLorey, D. S., Cheng-Baron, J., & Thompson, R. B. (2010a). Increased left ventricular twist, untwisting rates, and suction maintain global diastolic function during passive heat stress in humans. *Am J Physiol Heart Circ Physiol, 298*(3), H930-H937. doi: 10.1152/ajpheart.00987.2009

Nelson, M. D., Haykowsky, M. J., Petersen, S. R., DeLorey, D. S., Stickland, M. K., Cheng-Baron, J., & Thompson, J. (2010b). Aerobic fitness does

not influence the biventricular response to whole body heat stress. *J Appl Physiol (1985), 109*(5), 1545-1551. doi: 10.1152/japplphysiol.00769.2010

Ng, J., Dobbs, W. C., & Wingo, J. E. (2018). Effect of Ice Slurry Ingestion on Cardiovascular Drift and VO2max during Heat Stress. *Med Sci Sports Exerc*. doi: 10.1249/mss.0000000000001794

Nielsen, B., Hales, J. R. S., Strange, S., Christensen, N. J., Warberg, J., & Saltin, B. (1993). Human circulatory and thermoregulatory adaptations with heat acclimation and exercise in a hot, dry environment. *J Physiol, 460*, 467-485.

Nielsen, B., Savard, G., Richter, E. A., Hargreaves, M., & Saltin, B. (1990). Muscle blood flow and muscle metabolism during exercise and heat stress. *J Appl Physiol, 69*(3), 1040-1046.

Nielsen, B., Sjogaard, G., Ugelvig, J., Knudsen, B., & Dohlmann, B. (1986). Fluid balance in exercise dehydration and rehydration with different glucose-electrolyte drinks. *Eur J Appl Physiol Occup Physiol, 55*(3), 318-325.

Nielsen, B., Strange, S., Christensen, N. J., Warberg, J., & Saltin, B. (1997). Acute and adaptive responses in humans to exercise in a warm, humid environment. *Pflugers Arch, 434*, 49-56.

Nielsen, Bodil, & Nielsen, Marius. (1962). Body Temperature During Work at Different Environmental Temperatures. *Acta Physiologica Scandinavica, 56*(2), 120-129. doi: doi:10.1111/j.1748-1716.1962.tb02489.x

Nose, H., Mack, G. W., Shi, X. R., & Nadel, E. R. (1988a). Involvement of sodium retention hormones during rehydration in humans. *J Appl Physiol (1985), 65*(1), 332-336. doi: 10.1152/jappl.1988.65.1.332

Nose, H., Mack, G. W., Shi, X. R., & Nadel, E. R. (1988b). Shift in body fluid compartments after dehydration in humans. *J Appl Physiol, 65*(1), 318-324.

Notley, S. R., Park, J., Tagami, K., Ohnishi, N., & Taylor, N. A. S. (2017). Variations in body morphology explain sex differences in thermoeffector function during compensable heat stress. *Exp Physiol, 102*(5), 545-562. doi: 10.1113/ep086112

Notomi, Y., Martin-Miklovic, M. G., Oryszak, S. J., Shiota, T., Deserranno, D., Popovic, Z. B., . . . Thomas, J. D. (2006). Enhanced ventricular untwisting during exercise: a mechanistic manifestation of elastic recoil described by Doppler tissue imaging. *Circulation, 113*(21), 2524-2533. doi: 10.1161/CIRCULATIONAHA.105.596502

Nottin, S., Doucende, G., Schuster, I., Tanguy, S., Dauzat, M., & Obert, P. (2009). Alteration in left ventricular strains and torsional mechanics after ultralong duration exercise in athletes. *Circ Cardiovasc Imaging, 2*(4), 323-330. doi: 10.1161/CIRCIMAGING.108.811273

Nybo, L., & Lundby, C. (2016). CrossTalk opposing view: Heat acclimatization does not improve exercise performance in a cool condition. *J Physiol, 594*(2), 245-247. doi: 10.1113/JP270880

Ogawa, T., Asayama, M., & Miyagawa, T. (1982). Effects of sweat gland training by repeated local heating. *Jpn J Physiol, 32*(6), 971-981.

Okazaki, K., Hayase, H., Ichinose, T., Mitono, H., Doi, T., & Nose, H. (2009). Protein and carbohydrate supplementation after exercise increases plasma volume and albumin content in older and young men. *J Appl Physiol, 107*, 770-779. doi: 10.1152/japplphysiol.91264.2008

Ooue, A., Ichinose, T. K., Inoue, Y., Nishiyasu, T., Koga, S., & Kondo, N. (2008). Changes in blood flow in conduit artery and veins of the upper arm during leg exercise in humans. *Eur J Appl Physiol, 103*(3), 367-373. doi: 10.1007/s00421-008-0706-x

Oxborough, D. (2008). A practical approach to transthoracic echocardiography. *British Journal of Cardiac Nursing, 3*(4), 163-169. doi: 10.12968/bjca.2008.3.4.28922

Pandolf, K. B. (1998). Time course of heat acclimation and its decay. *Int J Sports Med, 19 Suppl 2*, S157-160. doi: 10.1055/s-2007-971985

Pandolf, K. B., Burse, R. L., & Goldman, R. F. (1977). Role of physical fitness in heat acclimatisation, decay and reinduction. *Ergonomics, 20*(4), 399-408. doi: 10.1080/00140137708931642

Parks, R. J., & Howlett, S. E. (2013). Sex differences in mechanisms of cardiac excitation-contraction coupling. *Pflugers Arch, 465*(5), 747-763. doi: 10.1007/s00424-013-1233-0

Patterson, M. J., Stocks, J. M., & Taylor, N. A. (2004a). Humid heat acclimation does not elicit a preferential sweat redistribution toward the limbs. *Am J Physiol Regul Integr Comp Physiol, 286*, R512-R518. doi: 10.1152/ajpregu.00359.2003

Patterson, M. J., Stocks, J. M., & Taylor, N. A. (2004b). Sustained and generalized extracellular fluid expansion following heat acclimation. *J Physiol, 559*(Pt 1), 327-334. doi: 10.1113/jphysiol.2004.063289

Patterson, M. J., Stocks, J. M., & Taylor, N. A. (2014). Whole-body fluid distribution in humans during dehydration and recovery, before and after humid-heat acclimation induced using controlled hyperthermia. *Acta Physiol (Oxf), 210*(4), 899-912. doi: 10.1111/apha.12214

Patterson, S. W., Piper, H., & Starling, E. H. (1914). The regulation of the heart beat. *J Physiol, 48*(6), 465-513.

Pearson, J. D., Low, D. A., Stöhr, E. J., Kalsi, K. K., Ali, L., Barker, H., & González-Alonso, J. (2011). Hemodynamic responses to heat stress in the resting and exercising human leg: insight into the effect of temperature on skeletal muscle blood flow. *Am J Physiol Regul Integr Comp Physiol, 300*, R663-R673. doi: 10.1152/ajpregu.00662.2010

Periard, J. D., Cramer, M. N., Chapman, P. G., Caillaud, C., & Thompson, M. W. (2011). Cardiovascular strain impairs prolonged self-paced exercise

in the heat. *Exp Physiol, 96*(2), 134-144. doi: 10.1113/expphysiol.2010.054213

Periard, J. D., & Racinais, S. (2015). Self-paced excerise in hot ad cool conditions is associated with the maintenance of % VO2peak within a narrow range. *J Appl Physiol, 118*, 1258-1265. doi: 10.1152/japplphysiol.00084.2015.

Periard, J. D., Racinais, S., & Sawka, M. N. (2015). Adaptations and mechanisms of human heat acclimation: Applications for competitive athletes and sports. *Scand J Med Sci Sports, 25 Suppl 1*, 20-38. doi: 10.1111/sms.12408

Periard, J. D., Travers, G. J. S., Racinais, S., & Sawka, M. N. (2016). Cardiovascular adaptations supporting human exercise-heat acclimation. *Auton Neurosci, 196*, 52-62. doi: 10.1016/j.autneu.2016.02.002

Pethick, W. A., Murray, H. J., McFadyen, P., Brodie, R., Gaul, C. A., & Stellingwerff, T. (2018). Effects of hydration status during heat acclimation on plasma volume and performance. *Scand J Med Sci Sports*. doi: 10.1111/sms.13319

Philp, C. P., Buchheit, M., Kitic, C. M., Minson, C. T., & Fell, J. W. (2017). Does Short-Duration Heat Exposure at a Matched Cardiovascular Intensity Improve Intermittent-Running Performance in a Cool Environment? *Int J Sports Physiol Perform, 12*(6), 812-818. doi: 10.1123/ijspp.2016-0072

Quinones, M. A., Waggoner, A. D., Reduto, L. A., Nelson, J. G., Young, J. B., Winters, W. L., Jr., . . . Miller, R. R. (1981). A new, simplified and accurate method for determining ejection fraction with two-dimensional echocardiography. *Circulation, 64*(4), 744-753.

Racinais, S., Alonso, J. M., Coutts, A. J., Flouris, A. D., Girard, O., González-Alonso, J., . . . Periard, J. D. (2015a). Consensus recommendations on training and competing in the heat. *Scand J Med Sci Sports, 25 Suppl 1*, 6-19. doi: 10.1111/sms.12467

Racinais, S., Periard, J. D., Karlsen, A., & Nybo, L. (2015b). Effect of heat and heat acclimatization on cycling time trial performance and pacing. *Med Sci Sports Exerc, 47*(3), 601-606. doi: 10.1249/MSS.0000000000000428

Ramanathan, N. L. (1964). A new weighting system for mean surface temperature of the human body. *J Appl Physiol, 19*(3), 531-533.

Regan, J. M., Macfarlane, D. J., & Taylor, N. A. (1996). An evaluation of the role of skin temperature during heat adaptation. *Acta Physiol Scand, 158*, 365-375.

Reuter, H., Pott, C., Goldhaber, J. I., Henderson, S. A., Philipson, K. D., & Schwinger, R. H. (2005). Na(+)--Ca2+ exchange in the regulation of cardiac excitation-contraction coupling. *Cardiovasc Res, 67*(2), 198-207. doi: 10.1016/j.cardiores.2005.04.031

Richardson, R. S., Noyszewski, E. A., Saltin, B., & González-Alonso, J. (2002). Effect of mild carboxy-hemoglobin on exercising skeletal muscle: intravascular and intracellular evidence. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 283*(5), R1131-R1139. doi: 10.1152/ajpregu.00226.2002

Rickards, C. A., Johnson, B. D., Harvey, R. E., Convertino, V. A., Joyner, M. J., & Barnes, J. N. (2015). Cerebreal blood velocity regulation during progressive blood loss compared with lowered body negative pressure in humans. *J Appl Physiol (1985), 119*, 677-685.

Roberts, M. F., Wenger, C. B., Stolwijk, J. A., & Nadel, E. R. (1977). Skin blood flow and sweating changes following exercise training and heat acclimation. *J Appl Physiol Respir Environ Exerc Physiol, 43*(1), 133-137. doi: 10.1152/jappl.1977.43.1.133

Robinson, S., Turell, E. S., Belding, H. S., & Horvath, S. M. (1943). Rapid acclimatization to work in hot climates. *Am J Physiol, 140*, 168-176.

Rowell, L. B. (1974). Human cardiovascular adjustments to exercise and thermal stress. *Physiol Rev, 54*(1), 75-159. doi: 10.1152/physrev.1974.54.1.75

Rowell, L. B. (1986). *Human Circulation: Regulation during physical stress*. New York: Oxford University Press.

Rowell, L. B. (1990). Hyperthermia: a hyperadrenergic state. *Hypertension, 15*(5), 505-507.

Rowell, L. B. (1993). *Human Cardiovascular Control*. New York: Oxford University Press, Inc.

Rowell, L. B., Brengelmann, G. L., Blackmon, J. R., & Murray, J. A. (1970). Redistribution of blood flow during sustained high skin temperature in resting man. *J Appl Physiol, 28*(4), 415-420. doi: 10.1152/jappl.1970.28.4.415

Rowell, L. B., Brengelmann, G. L., & Murray, J. A. (1969a). Cardiovascular responses to sustained high skin temperature in resting man. *J Appl Physiol, 27*(5), 673-680. doi: 10.1152/jappl.1969.27.5.673

Rowell, L. B., Detry, J. R., Profant, G. R., & Wyss, C. (1971). Splanchnic vasoconstriction in hyperthermic man - role of falling blood pressure. *J Appl Physiol, 31*(6), 864-869.

Rowell, L. B., Kraning II, K. K., Kennedy, J. W., & Evans, T. O. (1967). Central circulatory responses to work in dry heat before and after acclimatization. *J Appl Physiol, 22*(3), 509-518.

Rowell, L. B., Marx, H. J., Bruce, R. A., Conn, R. D., & Kusumi, F. (1966). Reductions in cardiac output, central blood volume, and stroke volume with thermal stress in normal men during exercise. *J Clin Invest, 45*(11), 1801-1816.

Rowell, L. B., Murray, J. A., Brengelmann, G. L., & Kraning, K. K., 2nd. (1969b). Human cardiovascular adjustments to rapid changes in skin temperature during exercise. *Circ Res, 24*(5), 711-724.

Saltin, Bengt. (1964). Circulatory response to submaximal and maximal exercise after thermal dehydration. *Journal of Applied Physiology, 19*(6), 1125-1132. doi: 10.1152/jappl.1964.19.6.1125

Sandstrom, M. E., Madden, L. A., Taylor, L., Siegler, J. C., Lovell, R. J., Midgley, A., & McNaughton, L. (2009). Variation in basal heat shock protein 70 is correlated to core temperature in human subjects. *Amino Acids, 37*(2), 279-284. doi: 10.1007/s00726-008-0144-4

Sandstrom, M. E., Siegler, J. C., Lovell, R. J., Madden, L. A., & McNaughton, L. (2008). The effect of 15 consecutive days of heat-exercise acclimation on heat shock protein 70. *Cell Stress Chaperones, 13*(2), 169-175. doi: 10.1007/s12192-008-0022-8

Sato, F., Owen, M., Meatthes, R., Sato, K., & Gisolfi, C. V. (1990). Functional and morphological changes in the eccrine sweat gland with heat acclimation. *J Appl Physiol, 69*(1), 232-236.

Sato, K., & Sato, F. (1983). Individual variations in structure and function of human eccrine sweat gland. *Am J Physiol, 245*(2), R203-208. doi: 10.1152/ajpregu.1983.245.2.R203

Savard, G. K., Nielsen, B., Laszynska, J., Larsen, B. E., & Saltin, B. (1988). Muscle blood flow is not reduced in humans during moderate exercise heat stress. *J Appl Physiol, 61*(2), 649-657.

Sawka, M. N., Burke, L. M., Eichner, E. R., Maughan, R. J., Montain, S. J., & Stachenfeld, N. S. (2007). American College of Sports Medicine position stand. Exercise and fluid replacement. *Med Sci Sports Exerc, 39*(2), 377-390. doi: 10.1249/mss.0b013e31802ca597

Sawka, M. N., & Coyle, E. F. (1999). Influence of body water and blood volume on thermoregulatory and exercise performance in the heat. *Exerc Sport Sci Rev, 27*, 167-218.

Sawka, M. N., Hubbard, R. W., Francesconi, R. P., & Horstman, D. H. (1983a). Effects of acute plasma volume expansion on altering exercise-heat performance. *Eur J Appl Physiol Occup Physiol, 51*(3), 303-312.

Sawka, M. N., Leon, L. R., Montain, S. J., & Sonna, L. A. (2011). Integrated physiological mechanisms of exercise performance, adaptation, and maladaptation to heat stress. *Compr Physiol, 1*(4), 1883-1928. doi: 10.1002/cphy.c100082

Sawka, M. N., Pandolf, K. B., Avellini, B. A., & Shapiro, Y. (1983b). Does heat acclimation lower the rate of metabolism elicited by muscular exercise? *Aviat Space Environ Med, 54*(1), 27-31.

Sawka, M. N., Toner, M. M., Francesconi, R. P., & Pandolf, K. B. (1983c). Hypohydration and exercise: effects of heat acclimation, gender, and environment. *J Appl Physiol: Resprat. Environ. Exercise Physiol, 55*(4), 1147-1153.

Sawka, M. N., Wenger, C. B., & Pandolf, K. B. (1996). Thermoregulatory responses to acute exercise-heat stress and heat acclimation. In M. J. Fregly & C. M. Blatteis (Eds.), *Handbook of Physiology* (pp. 157-185). New York: Oxford University Press.

Sawka, M. N., Young, A. J., Latzka, W. A., Neufer, P. D., Quigley, M. D., & Pandolf, K. B. (1992). Human heat tolerance to heat strain during exercise: influence of hydration. *J Appl Physiol, 73*(1), 368-375.

Schiller, Nelson B., Shah, Pravin M., Crawford, Michael, DeMaria, Anthony, Devereux, Richard, Feigenbaum, Harvey, . . . Tajik, A. Jamil. (1989). Recommendations for Quantitation of the Left Ventricle by Two-Dimensional Echocardiography. *Journal of the American Society of Echocardiography, 2*(5), 358-367. doi: 10.1016/s0894-7317(89)80014-8

Schleh, Michael W., Ruby, Brent C., & Dumke, Charles L. (2018). Short term heat acclimation reduces heat stress, but is not augmented by dehydration. *Journal of Thermal Biology, 78*, 227-234. doi: 10.1016/j.jtherbio.2018.10.004

Schmidt, W., & Prommer, N. (2005). The optimised CO-rebreathing method: a new tool to determine total haemoglobin mass routinely. *Eur J Appl Physiol, 95*(5-6), 486-495. doi: 10.1007/s00421-005-0050-3

Schmit, C., Le, M. Y., Duffield, R., Robach, P., Oussedik, N., Coutts, A. J., & Hausswirth, C. (2015). Heat-acclimatization and pre-cooling: a further boost for endurance performance? *Scand J Med Sci Sports, 27*(1), 55-65.

Scoon, G. S., Hopkins, W. G., Mayhew, S., & Cotter, J. D. (2007). Effect of post-exercise sauna bathing on the endurance performance of competitive male runners. *J Sci Med Sport, 10*(4), 259-262. doi: 10.1016/j.jsams.2006.06.009

Selye, H. (1973). The evolution of the stress concept. *Am Sci, 61*(6), 692-699.

Senay, L. C. (1986). An inquiry into the role of cardiac filling pressure in acclimatization to heat. *Yale J Biol Med, 59*, 247-256.

Senay, L. C., Jr. (1972). Changes in plasma volume and protein content during exposures of working men to various temperatures before and after acclimatization to heat: separation of the roles of cutaneous and skeletal muscle circulation. *J Physiol, 224*(1), 61-81.

Senay, L. C., Mitchell, D., & Wyndham, C. H. (1976). Acclimatization in a hot, humid environment: body fluid adjustments. *J Appl Physiol, 40*(5), 786-796.

Sengupta, P. P., Khandheria, B. K., Korinek, J., Jahangir, A., Yoshifuku, S., Milosevic, I., & Belohlavek, M. (2007). Left ventricular isovolumic flow sequence during sinus and paced rhythms: new insights from use of high-resolution Doppler and ultrasonic digital particle imaging velocimetry. *J Am Coll Cardiol, 49*(8), 899-908. doi: 10.1016/j.jacc.2006.07.075

Sengupta, P. P., Khandheria, B. K., Korinek, J., Wang, J., Jahangir, A., Seward, J. B., & Belohlavek, M. (2006a). Apex-to-base dispersion in regional timing of left ventricular shortening and lengthening. *J Am Coll Cardiol, 47*(1), 163-172. doi: 10.1016/j.jacc.2005.08.073

Sengupta, P. P., Korinek, J., Belohlavek, M., Narula, J., Vannan, M. A., Jahangir, A., & Khandheria, B. K. (2006b). Left ventricular structure and function: basic science for cardiac imaging. *J Am Coll Cardiol, 48*(10), 1988-2001. doi: 10.1016/j.jacc.2006.08.030

Shaffrath, J. D., & Adams, W. C. (1984). Effects of airflow and work load on cardiovascular drift and skin blood flow. *J Appl Physiol Respirat Environ Exercise Physiol, 56*(5), 1411-1417.

Shapiro, Y., Hubbard, R. W., Kimbrough, C. M., & Pandolf, K. B. (1981). Physiological and hematologic responses to summer and winter dry-heat acclimation. *J Appl Physiol Respir Environ Exerc Physiol, 50*(4), 792-798. doi: 10.1152/jappl.1981.50.4.792

Shutt, R. H., & Howlett, S. E. (2008). Hypothermia increases the gain of excitation-contraction coupling in guinea pig ventricular myocytes. *Am J Physiol Cell Physiol, 295*, C692-C700. doi: 10.1152/ajpcell.00287.2008

Shvartz, E., Saar, E., Meyerstein, N., & Benor, D. (1973). A comparison of three methods of acclimatization to dry heat. *J Appl Physiol, 34*(2), 214-219.

Shvartz, E., Shapiro, Y., Magazanik, A., Meroz, A., Birnfeld, H., Mechtinger, A., & Shibolet, S. (1977). Heat acclimation, physical fitness, and responses to exercise in temperate and hot environments. *J Appl Physiol Respirat Environ Exercise Physiol, 43*(4), 678-683.

Simmons, G. H., Padilla, J., Young, C. N., Wong, B. J., Lang, J. A., Davis, M. J., . . . Fadel, P. J. (2011). Increased brachial artery retrograde shear rate at exercise onset is abolished during prolonged cycling: role of thermoregulatory vasodilation. *J Appl Physiol (1985), 110*(2), 389-397. doi: 10.1152/japplphysiol.00936.2010

Sonnenblick, Edmund H. (1962). Force-velocity relations in mammalian heart muscle. *American Journal of Physiology-Legacy Content, 202*(5), 931-939. doi: 10.1152/ajplegacy.1962.202.5.931

Spotnitz, H. M. (2000). Macro design, structure and mechnics in mammalian heart muscle. *J Thorac Cadiovasc Surg, 119*(5), 1053-1077. doi: 10.1067/mtc.2000.105654

Stanley, J., Halliday, A., D'Auria, S., Buchheit, M., & Leicht, A. S. (2015). Effect of sauna-based heat acclimation on plasma volume and heart rate variability. *Eur J Appl Physiol, 115*(4), 785-794. doi: 10.1007/s00421-014-3060-1

Stickland, M. K., Welsh, R. C., Petersen, S. R., Tyberg, J. V., Anderson, W. D., Jones, R. L., . . . Haykowsky, M. J. (2006). Does fitness level modulate the cardiovascular haemodynamic response to exercise? *J Appl Physiol, 100*, 1895-1901. doi: 10.1152/japplphysiol.01485.2005

Stöhr, E. J. (2010). *The effect of heat stress, dehydration and exercise on global left ventricular function and mechanics in healthy humans.* (Doctor of Philosophy), Brunel University London.

Stöhr, E. J., González-Alonso, J., Pearson, J., Low, D. A., Ali, L., Barker, H., & Shave, R. (2011a). Dehydration reduces left ventricular filling at rest and during exercise independent of twist mechanics. *J Appl Physiol, 111*, 891-897. doi: 10.1152/japplphysiol.00528.2011

Stöhr, E. J., González-Alonso, J., Pearson, J., Low, D. A., Ali, L., Barker, H., & Shave, R. (2011b). Effects of graded heat stress on global left ventricular function and twist mechanics at rest and during exercise in healthy humans. *Exp Physiol, 96*(2), 114-124. doi: 10.1113/expphysiol.2010.055137

Stöhr, E. J., González-Alonso, J., & Shave, R. (2011c). Left ventricular mechanical limitations to stroke volume in healthy humans during incremental exercise. *Am J Physiol Heart Circ Physiol, 301*(2), H478-487. doi: 10.1152/ajpheart.00314.2011

Stöhr, E. J., McDonnell, B., Thompson, J., Stone, K., Bull, T., Houston, R., . . . Shave, R. (2012). Left ventricular mechanics in humans with high aerobic fitness: adaptation independent of structural remodelling, arterial haemodynamics and heart rate. *J Physiol, 590*(9), 2107-2119. doi: 10.1113/jphysiol.2012.227850

Strydom, N. B., Wyndham, C. H., Williams, C. G., Morrison, J. F., Bredell, G. A. G., Benade, A. J. S., & Von Rahden, M. (1966). Acclimatization to humid heat and the role of physical conditioning. *J Appl Physiol, 21*(2), 636-642.

Sunderland, C., Morris, J. G., & Nevill, M. E. (2008). A heat acclimation protocol for team sports. *Br J Sports Med, 42*(5), 327-333.

Takamata, A., Yoshida, T., Nishida, N., & Morimoto, T. (2001). Relationship of osmotic inhibition in thermoregulatory response and sweat sodium concentration in humans. *Am J Physiol Regul Integr Comp Physiol, 280*, R623-R629.

Tanaka, H., Shimizu, S., Ohmori, F., Muraoka, Y., Kumagai, M., Yoshizawa, M., & Kagaya, A. (2006). Increases in blood flow and shear stress to nonworking limbs during incremental exercise. *Med Sci Sports Exerc, 38*(1), 81-85.

Taylor, H. L., Henschel, A. F., & Keys, A. (1943). Cardiovascular adjustments of man in rest and work during exposure to dry heat. *Am J Phsyiol, 139*(4), 583-591.

Taylor, N. A. S. (2014). Human heat adaptation. *Compr Physiol, 4*(1), 325-365. doi: 10.1002/cphy.c130022

Taylor, N. A. S., & Cotter, J. D. (2006). Heat adaptation: guidelines for the optimisation of human performance. *International SpotMed Journal, 7*(1), 33-57.

Taylor, Nigel A. S. (2006). Ethnic differences in thermoregulation: Genotypic versus phenotypic heat adaptation. *Journal of Thermal Biology, 31*(1-2), 90-104. doi: 10.1016/j.jtherbio.2005.11.007

Teichholz, L. E., Kreulen, T., Herman, M. V., & Gorlin, R. (1976). Problems in echocardiographuc volume determinations: Echocardiographic-

angiographic correlations in the presence or absence of asynergy. *Am J Cardiol, 37*, 7-11.

Tortora, G. J., & Derricksen, B. (2012). *Principles of Anatomy & Physiology* (13 ed.). New Jersey, USA: John WIley & Sons, Inc.

Trangmar, S. J., Chiesa, S. T., Stock, C. G., Kalsi, K. K., Secher, N. H., & González-Alonso, J. (2014). Dehydration affects cerebral blood flow but not its metabolic rate for oxygen during maximal exercise in trained humans. *J Physiol, 592*(14), 3143-3160. doi: 10.1113/jphysiol.2014.272104

Trinity, J. D., Pahnhe, M. D., Lee, J. F., & Coyle, E. F. (2010). Interaction of hyperthermia and heart rate on stroke volume during prolonged exercise. *J Appl Physiol, 109*, 745-751. doi: 10.1152/japplphysiol.00377.2010

Tyler, C. J., Reeve, T., Hodges, G. J., & Cheung, S. S. (2016). The Effects of Heat Adaptation on Physiology, Perception and Exercise Performance in the Heat: A Meta-Analysis. *Sports Med, 46*(11), 1699-1724. doi: 10.1007/s40279-016-0538-5

Uhrenholt, T. R., Schjerning, J., Hansen, P. B., Norregaard, R., Jensen, B. L., Sorensen, G. L., & Skott, O. (2003). Rapid inhibition of vasoconstriction in renal afferent arterioles by aldosterone. *Circ Res, 93*(12), 1258-1266. doi: 10.1161/01.RES.0000106135.02935.E1

Ulrich, G., Strunz, J., Frese, F., Bartsch, P., & Friedmann-Bette, B. (2012). Dependence of hemoglobin mass estimation with the optimized CO-rebreathing method on different spectrophotometers. *Scand J Med Sci Sports, 22*(2), 224-231. doi: 10.1111/j.1600-0838.2010.01247.x

Van Beaumont, W. (1972). Evaluation of hemoconcentration from hematocrit measurements. *J Appl Physiol, 31*(5), 712-713.

van Dalen, B. M., Vletter, W. B., Soliman, O. I., ten Cate, F. J., & Geleijnse, M. L. (2008). Importance of transducer position in the assessment of apical rotation by speckle tracking echocardiography. *J Am Soc Echocardiogr, 21*(8), 895-898. doi: 10.1016/j.echo.2008.02.001

Viatchenko-Karpinski, S., & Györke, S. (2001). Modulation of the Ca2+ induced Ca2+ release cascade by β-adrenergic stimulation in rat ventricular myocytes. *J Physiol, 533*(3), 837-848.

Waterhouse, J., Drust, B., Weinert, D., Edwards, B., Gregson, W., Atkinson, G., . . . Reilly, T. (2005). The circadian rhythm of core temperature: origin and some implications for exercise performance. *Chronobiol Int, 22*(2), 207-225.

Weller, A. S., Linnane, D. M., Jonkman, A. G., & Daanen, H. A. (2007). Quantification of the decay and re-induction of heat acclimation in dry-heat following 12 and 26 days without exposure to heat stress. *Eur J Appl Physiol, 102*(1), 57-66. doi: 10.1007/s00421-007-0563-z

Wenger, C. B. (1988). Human heat acclimatization. In K. B. Pandolf, R. R. Gonzalez & M. N. Sawka (Eds.), *Human Performance Physiology and*

Environmental Medicine at Terrestrial Extremes (pp. 153-197). Indianapolis, IL: Benchmark.

Whitney, R. J. (1953). The measurement of volume changes in human limbs. *J Physiol, 121*(1), 1-27.

Wilson, T. E., Brothers, R. M., Tollund, C., Dawson, E. A., Nissen, P., Yoshiga, C. C., . . . Crandall, C. G. (2009). Effect of thermal stress on Frank-Starling relations in humans. *J Physiol, 587*(Pt 13), 3383-3392. doi: 10.1113/jphysiol.2009.170381

Wilson, T. E., & Crandall, C. G. (2011). Effect of thermal stress on cardiac function. *Exerc Sport Sci Rev, 39*(1), 12-17. doi: 10.1097/JES.0b013e318201eed6

Wilson, T. E., Tollund, C., Yoshiga, C. C., Dawson, E. A., Nissen, P., Secher, N. H., & Crandall, C. G. (2007). Effects of heat and cold stress on central vascular pressure relationships during orthostasis in humans. *J Physiol, 585*(Pt 1), 279-285. doi: 10.1113/jphysiol.2007.137901

Wingo, J. E., & Cureton, K. J. (2006). Maximal oxygen uptake after attenuation of cardiovascular drift during heat stress. *Aviat Space Environ Med, 77*, 687-694.

Wingo, J. E., Ganio, M. S., & Cureton, K. J. (2012). Cardiovascular drift during heat stress: implications for exercise prescription. *Exerc Sport Sci Rev, 40*(2), 88-94. doi: 10.1097/JES.0b013e31824c43af

Wyndham, C. H. (1951). Effect of acclimatization on circulatory responses to high environmental temperatures. *J Appl Physiol, 4*, 383-395.

Wyndham, C. H. (1967). Effect of acclimatiztion on the sweat rate/rectal temperature relationship. *J Appl Physiol, 22*(1), 27-30.

Wyndham, C. H., Benade, A. J. A., Williams, C. G., Strydom, N. B., Goldin, A., & Heyns, A. J. A. (1968a). Changes in central circulation and body fluid spaces during acclimatization to heat. *J Appl Physiol, 25*(5), 586-593.

Wyndham, C. H., Rogers, G. G., Senay, L. C., & Mitchell, D. (1976). Acclimization in a hot, humid environment: cardiovascular adjustments. *J Appl Physiol, 40*(5), 779-785. doi: 10.1152/jappl.1976.40.5.779

Wyndham, C. H., Williams, C. G., Morrison, J. F., Heyns, A. J., & Siebert, J. (1968b). Tolerance of very hot humid environments by highly acclimatized Bantu at rest. *Br J Ind Med, 25*(1), 22-39.

Yamada, P. M., Amorim, F. T., Moseley, P., Robergs, R., & Schneider, S. M. (2007). Effect of heat acclimation on heat shock protein 72 and interleukin-10 in humans. *J Appl Physiol (1985), 103*(4), 1196-1204. doi: 10.1152/japplphysiol.00242.2007

Yamazaki, F., & Hamasaki, K. (2003). Heat acclimation increases skin vasodilation and sweating but not cardiac baroreflex responses in heat-stressed humans. *J Appl Physiol (1985), 95*(4), 1567-1574. doi: 10.1152/japplphysiol.00063.2003

Young, A. J., Sawka, M. N., Levine, L., Cadarette, B. S., & Pandolf, K. B. (1985). Skeletal muscle metabolism during exercise is influenced by heat acclimation. *J Appl Physiol, 59*(6), 1929-1935.

Zurawlew, M. J., Walsh, N. P., Fortes, M. B., & Potter, C. (2016). Post-exercise hot water immersion induces heat acclimation and improves endurance exercise performance in the heat. *Scand J Med Sci Sports, 26*(7), 745-754. doi: 10.1111/sms.12638

Appendices

I. Ethical approval letters and relevant training

Anti- Doping Lab Qatar Institutional Review Board

Tel: 44132988
Fax: 44132997
Email: ADL_IRO@adlqatar.com

IRB SCH Registration: SCH-ADL-070
SCH Assurance: SCH-ADL-A-071

APPROVAL NOTICE

Date	28/10/2015
Lead Principal Investigator	**Gavin Travers**
IRB Application #	**F2015000105**
Protocol Title	**Influence of a heat acclimation regime with fixed cardiovascular strain on cardiac function and cycling time trial performance**
Submission Type	**Initial Application**
Review Type	**Full Board Review**
Approval Period	28/10/2015– 27/10/2016

The Anti-Doping Lab Qatar Institutional Review Board has reviewed and approved the above referenced protocol.
As the Principal Investigator of this research project, you are responsible for:

- Ethical Compliance and protection of the rights, safety and welfare of human subjects involved in this research project.
- To follow the policies and procedures as set by ADLQ-IRB in any matters related to the project, following the ADLQ-IRB approval (i.e., with regards to obtaining prior approval of any deviation of protocol, reporting of unanticipated events, and submission of progress reports).
- To inform the ADLQ-RO of the date of commencement of the research*.

Director – ORS/ADLQ (Office of Research Support)
Ms. Noor Al Mohawa

*For Commencement of Research, Protocol Deviation Reporting, Unanticipated Problem Reporting & Research Progress Annual Report, please contact - Education & Research Office, Anti-Doping Lab Qatar.

Anti- Doping Lab Qatar Institutional Review Board

Tel: 44132988
Fax: 44132997
Email: ADLQ-RO@adlqatar.com

IRB SCH Registration: SCH-ADL-070
SCH Assurance: SCH-ADL-A-071

APPROVAL NOTICE
[Ethics Approval Renewal & Protocol Deviation]

Date	09/10/2016
Lead Principal Investigator	Gavin Travers
IRB Application #	F2015000105
Protocol Title	Influence of a heat acclimation regime with fixed cardiovascular strain on cardiac function and cycling time trial performance
Submission Type	Ethics Approval Renewal & Protocol Deviation
Review Type	Full Board Review
Approval Period	28/10/2016 – 27/10/2017

The Anti-Doping Lab Qatar Institutional Review Board has reviewed and approved the above referenced protocol.
As the Principal Investigator of this research project, you are responsible for:

- Ethical Compliance and protection of the rights, safety and welfare of human subjects involved in this research project.
- To follow the policies and procedures as set by ADLQ-IRB in any matters related to the project, following the ADLQ-IRB approval (i.e., with regards to obtaining prior approval of any deviation of protocol, reporting of unanticipated events, and submission of progress reports).
- To inform the ADLQ-RO of the date of commencement of the research*.

Director – ORS/ADLQ (Office of Research Support)
Ms. Noor AlMotawa

مختبر مكافحة المنشطات قطر
Anti Doping Lab Qatar

*For Commencement of Research, Protocol Deviation Reporting, Unanticipated Problem Reporting & Research Progress Annual Report, please contact - Education & Research Office, Anti-Doping Lab Qatar.

Anti- Doping Lab Qatar Institutional Review Board

Tel: 44132988
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Email: ADLQ-RO@adlqatar.com

IRB MoPH Registration: SCH-ADL-070
MoPH Assurance: MOPH-A-ADL-Q-071

Approval Notice
[Ethics Approval Renewal]

Date	3rd Jan 2018
Lead Principal Investigator	Gavin Travers, Aspetar
Co-PI	Julien Periard, Prof. José Gonzalez-Alonso, David Nichols
IRB Application #	F2015000105
Sites	Aspetar, Brunel University - London
Funding Entity	Aspetar, Brunel University - London
Protocol Title	Influence of a heat acclimation regime with fixed cardiovascular strain on cardiac function and cycling time trial performance
Submission Type	Ethics Approval Renewal
Review Type	Full Board Review
Approval Period	28th Oct 2017 – 27th Oct 2018

The Anti-Doping Lab Qatar Institutional Review Board has reviewed and approved the above referenced protocol.
As the Principal Investigator of this research project, you are responsible for:

- Ethical compliance and protection of the rights, safety and welfare of human subjects involved in this research project.
- To follow the policies and procedures as set by ADLQ-IRB in any matters related to the project, following the ADLQ-IRB approval which includes:-
  - Obtaining prior approval of any modifications to the approved protocol including the change of research team members.
  - Reporting deviations and unanticipated events; major deviations within 24 hours.
  - Renewing Ethics annually or every six months if IRB requires it.
  - Submission of progress reports annually
  - Informing the ADLQ-RO of the date of commencement of the research.

ADLQ IRB ORS (Office of Research Support)
Ms. Noor Al Motawa

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Anti Doping Lab Qatar

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IRB MoPH Registration: SCH-ADL-070
MoPH Assurance: MOPH-A-ADL-Q-071

Approval Notice
[Ethics Approval Renewal]

Date	29th Oct, 2018
Lead Principal Investigator	Gavin Travers, Aspetar
Co-PI	Julien Periard, Prof. José Gonzalez-Alonso, David Nichols
IRB Application #	F2015000105
Sites	Aspetar, Brunel University - London
Funding Entity	Aspetar, Brunel University - London
Protocol Title	Influence of a heat acclimation regime with fixed cardiovascular strain on cardiac function and cycling time trial performance
Submission Type	Ethics Approval Renewal
Review Type	Full Board Review
Approval Period	29th Oct, 2018 – 28th Oct, 2019

The Anti-Doping Lab Qatar Institutional Review Board has reviewed and approved the above referenced protocol.
As the Principal Investigator of this research project, you are responsible for:

- Ethical compliance and protection of the rights, safety and welfare of human subjects involved in this research project.
- To follow the policies and procedures as set by ADLQ-IRB in any matters related to the project, following the ADLQ-IRB approval which includes:-
  - Obtaining prior approval of any modifications to the approved protocol including the change of research team members.
  - Reporting deviations and unanticipated events; major deviations within 24 hours.
  - Renewing Ethics annually or every six months if IRB requires it.
  - Submission of progress reports annually
  - Informing the ADLQ-RO of the date of commencement of the research.
- LPI may use the content of the approved Informed Consent form in their own organizational letter head, if it deems fit for the nature of the project.

ADLQ IRB ORS (Office of Research Support)
Ms. Noor Al Motawa

مختبر مكافحة المنشطات قطر
Anti Doping Lab Qatar

*For Commencement of Research, Protocol Deviation Reporting, Unanticipated Problem Reporting & Research Progress Annual Report, please contact - Education & Research Office, Anti-Doping Lab Qatar.

Certificate of Completion

The National Institutes of Health (NIH) Office of Extramural Research certifies that **Gavin Travers** successfully completed the NIH Web-based training course "Protecting Human Research Participants".

Date of completion: 10/28/2014

Certification Number: 1603491

CITI PROGRAM

Completion Date 25-Sep-2017
Expiration Date 24-Sep-2020
Record ID 24744425

This is to certify that:

Gavin Travers

Has completed the following CITI Program course:

Biomedical Research - Basic/Refresher (Curriculum Group)
Biomedical Research - Basic/Refresher (Course Learner Group)
1 - Basic Course (Stage)

Under requirements set by:

Ministry of Public Health, Qatar

CITI
Collaborative Institutional Training Initiative

Verify at www.citiprogram.org/verify/?w6ca0095d-e12c-4ad7-a95e-af5c61c1e51d-24744425

II. Informed consent form

مختبر مكافحة المنشطات قطر
Anti Doping Lab Qatar

ADLQ RESEARCH OFFICE
P.O.BOX 27775
Email: ADLQ-RO@adlqatar.com

CONSENT FORM

Participant's Statement
Documentation of Permission

Principal investigator
As a representative of this study, I have explained the purpose, the procedures, the possible benefits and risks that are involved in this research study. Any questions that have been raised have been answered to the individual's satisfaction.

Signature of Person Obtaining Consent _____ Date of Signature _____

Research Participant / Authorized Persons

I, the undersigned have been informed about this study's purpose, procedures, possible benefits and risks, and I have received a copy of this consent. I have been given the opportunity to ask questions before I sign, and I have been told that I can ask other questions at any time. I voluntarily agree to be in this study. I am free to stop being in the study at any time without need to justify my decision and if I stop being in the study I understand it will not in any way affect the benefits I normally have. I agree to cooperate with (name of principal investigator) and the research staff and to tell them immediately if I experience any unexpected or unusual symptoms.

Participant's Signature _____ Date of Signature _____

Signature of Witness _____ Date of Signature _____

Signature of Legally Authorized Representative (When Appropriate) _____ Date of Signature _____

Relationship to Participant (When Appropriate) _____ Date of Signature _____

IRB APPROVAL STAMP

مختبر مكافحة المنشطات قطر
Anti Doping Lab Qatar

III. Health questionnaire

Medical History Questionnaire

Name:_____

Address:_____

_____

_____

Phone: ( ) _____ (W)

Phone: ( ) _____ (H)

Age: _____

Today's Date: _____

Date of Birth: _____

1. **Family History.** Indicate if any of your immediate family (parents, brothers, sisters, grandparents) have experienced any of the following, the age at which diagnosis occurred and the person's relationship to you.

Relationship and Age

High Blood Pressure _____

High Cholesterol _____

Heart Disease _____

Stroke _____

Diabetes _____

Cancer _____

Clotting or Bleeding Disorders _____

Coeliac Disease _____

2. **Personal Medical History.** Indicate symptoms that apply to you.

- ☐ Pain or discomfort in chest at rest and following exercise, eating or exposure to cold
- ☐ Frequent heart palpitations or flutter
- ☐ Pain in lower legs when walking or climbing stairs
- ☐ Unusual shortness of breath
- ☐ Very poor exercise tolerance
- ☐ Frequent dizziness
- ☐ Chronic cough
- ☐ Frequent colds or flu
- ☐ Frequent headaches
- ☐ Frequent aches or pains in joints
- ☐ Frequent backache
- ☐ Unusual bleeding or blood clots
- ☐ No symptoms

Are you presently experiencing, or have you ever been treated by a doctor for any of the following?

3. **Lung Problems** (Asthma/Emphysema/Bronchitis/ Shortness of Breath/Other)
   - ☐ Yes
   - ☐ No

   Details_____

4. **Heart Problems** (Rheumatic Fever/Chest Pains/ Palpitations/Ankle Swelling/Other)
   - ☐ Yes
   - ☐ No

   Details_____

5. **Blood Pressure Problems**
   - ☐ Yes
   - ☐ No

   Details_____

6. **Gut Problems** (Coeliac Disease/Ulcer/Abdominal Pain/Diarrhoea/ Constipation/Hernia/Other)
   - ☐ Yes
   - ☐ No

   Details_____

7. **Urinary Problems** (Renal Insufficiency/Burning/ Difficulty with control of urine)
   - ☐ Yes
   - ☐ No

   Details_____

8. **Blood Loss** (In vomit/Sputum/Bowel Action/Urine)
   - ☐ Yes
   - ☐ No

   Details_____

9. **Malignant hyperthermia.** Do you have this disorder?
   - ☐ Yes
   - ☐ No

   Details_____

10. **Heat illness.** Have you ever suffered from heat illness or heat stroke after exercise?
    - ☐ Yes
    - ☐ No

    Details_____

11. **Fitting, Fainting, Blackouts, Loss of consciousness, Muscle weakness, Loss of sensation.**
    - ☐ Yes
    - ☐ No

    Details_____

12.Bone or Joint Injury
(Back/Knee/Ankle/Hip/Shoulders)

- ☐ Yes
- ☐ No

Details _____

13.Have you had an operation recently?

- ☐ Yes
- ☐ No

Details _____

14.Medication.

(a) Are you taking any medication prescribed by your Doctor or other Health Care providor (including non-steroidal anti-inflammatory drugs)?
If so, list details, ie, type of drugs, dosage.

_____

_____

(b) How often do you take over the counter medications such as aspirin, nurofen etc.

- ☐ Daily
- ☐ Weekly
- ☐ Occasionally
- ☐ Never

15.Physical Activity. How many times per week do you exercise for at least 20 - 30 minutes continuously?

- ☐ Do not have a regular program
- ☐ Once per week
- ☐ 2 - 3 times per week
- ☐ 4 - 5 times per week
- ☐ more than 5 times per week

16. Alcohol Consumption.

(a) In the past two weeks list how many days you consumed an alcoholic beverage.

- ☐ Did not drink in the past 6 months
- ☐ Did not drink in the past two weeks
- ☐ 1 - 2 days
- ☐ 3 – 4 days
- ☐ 5 – 7 days
- ☐ 8 – 10 days
- ☐ 11 – 14 days

Signature: _____

IV. Food and fluid intake diary

Participant food diary

Below is a table for you to record your food and fluid intake over the 24 hour period before visiting the lab. This shall be photocopied and returned to you for you to use as a guide and so that you can replicate your diet as best you can before you complete the experiment.

Remember to refrain from drinking alcohol during this period and consuming caffeine in the 12 hours before your visit.

NAME:..... DATE:.....

TIME (When did you eat/drink?)	**FOOD/DRINK** (What is it?)	**Amount** (If possible, weight or volume?)

V. Individual daily responses to heat acclimation

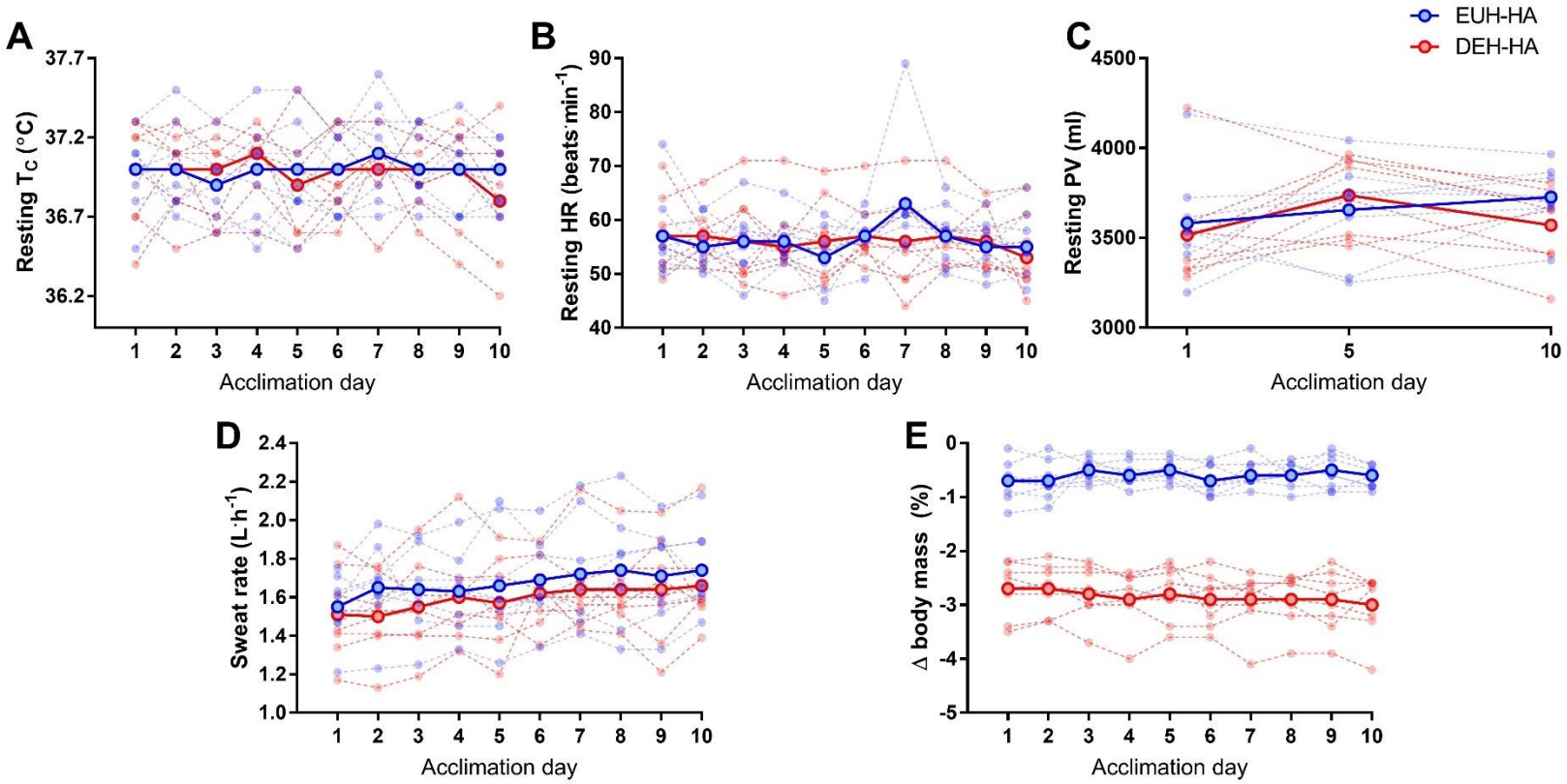

Figure 8.1: Individual resting core temperature (A), heart rate (B) and blood volume (C) and exercising sweat rate (D) and change in body mass (E) with each day of acclimation. Transparent lines are responses of individuals (n = 8) who completed both euhydrated (blue) and dehydrated (red) interventions in Chapter 4. Solid lines are daily group averages.

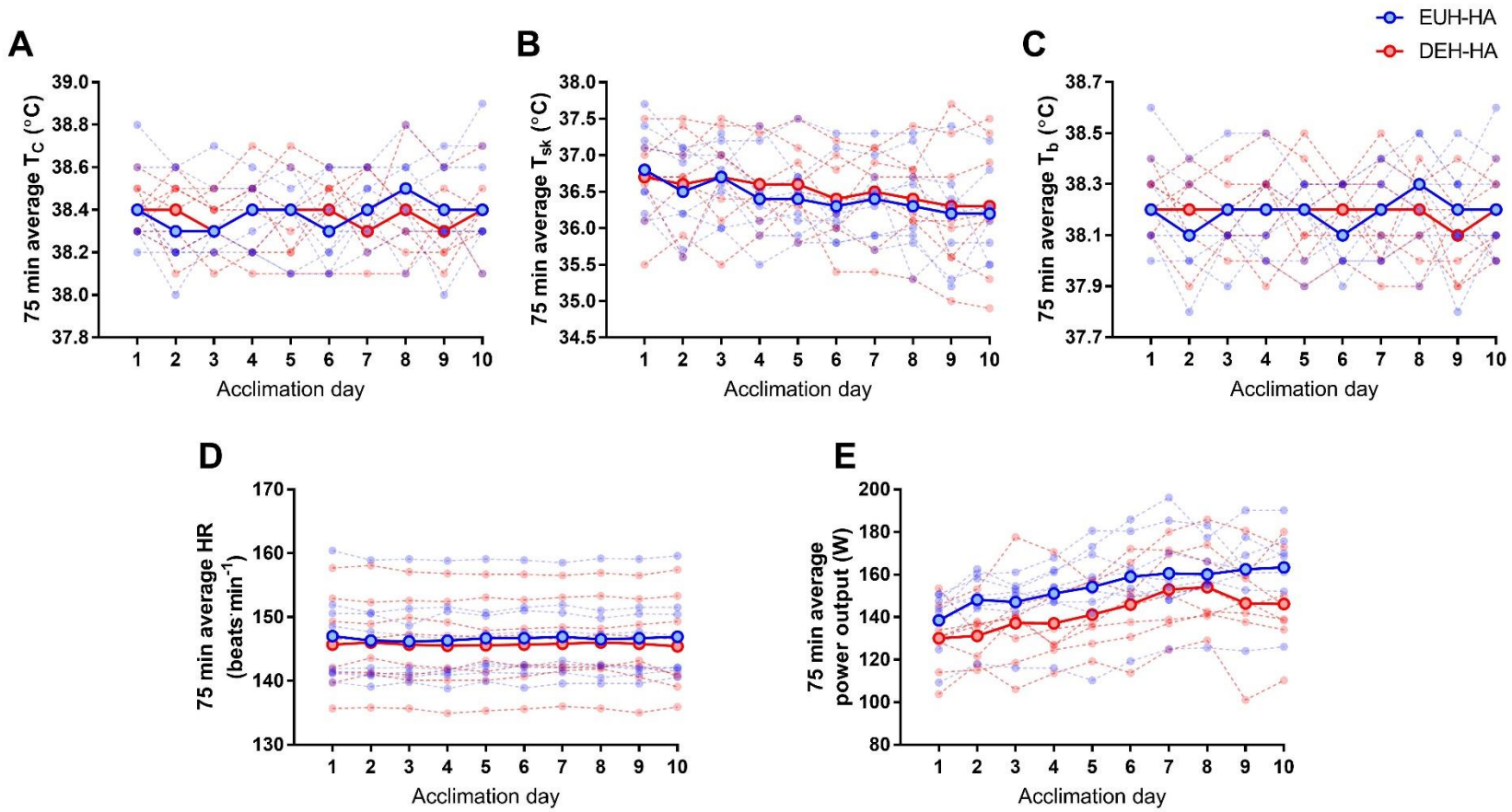

Figure 8.2: Average core temperature (A), skin temperature (B), calculated whole-body temperature (T_b; C), heart rate (D) and power output (E) for the final 75 minutes of heart rate controlled exercise during each day of acclimation. Transparent lines are responses of individuals (n = 8) who completed both euhydrated (blue) and dehydrated (red) interventions in Chapter 4. Solid lines are daily group averages.

VI. Conference abstract

HEAT ACCLIMATION WITH CONTROLLED HEART RATE: EFFECTS OF HYDRATION ON ADAPTATIONS AND SELF-PACED EXERCISE IN A HOT HUMID ENVIRONMENT

Oral presentation, ECSS 2018, Dublin, Ireland

Travers G, [1,2]. Nichols D, [1]. Riding N, [1]. González-Alonso J, [2]. Périard J. [1,3].

1: AHP (Qatar) 2: Brunel University London (UK) 3: UCRISE (Australia)

Introduction

During heat acclimation (HA) with controlled hyperthermia dehydration may enhance adaptations to HA independently of heat stress via a fluid regulatory response, resulting in a protracted expansion of the vascular compartment (1). However, recent findings comparing the additional dehydration stimulus in HA on haematological, thermoregulatory and cardiovascular adaptations are equivocal (2, 3). We therefore sought to characterise the adaptations to HA with and without dehydration and their effects on self-paced exercise performance.

Methods

Seven males underwent two 10-day HA interventions (40°C, 40% RH) separated by ~six weeks in a randomised order. Each 90-min exposure consisted of 15-min cycling at 65% VO_{2max} (174±22 W) followed by automated alterations in work-rate to maintain heart rate at the value associated with this intensity (145±7 bpm). Fluid intake was prescribed to either maintain body mass (-0.6±0.3%; HA-EUH) or elicit similar levels of dehydration (-2.9±0.5%; HA-DEH) each day. Changes in blood (BV), plasma (PV) and red cell (RCV) volumes were determined via CO rebreathing prior to each HA intervention and measured on days 1, 5 and 10 via venepuncture. A VO_{2max} test (~20°C) and 30-min cycling time trial (35°C, 60% RH) were completed to determine the effects on exercise capacity and performance.

Results

Resting BV, PV and RCV did not change across either HA intervention ($p>0.05$), but BV and PV were acutely reduced after DEH-HA exposures ($p<0.05$). 15-min heart rate and 90-min average skin temperature decreased ($p<0.05$), while mean heart rate and core temperature (38.4±0.2°C) were similar during the final 75-min of each day, regardless of hydration. Sweat lost increased with HA and power output during fixed heart rate exercise was lower in HA-DEH than HA-EUH ($p<0.05$). HA significantly increased VO_{2max} (+3.2±0.4%, $p<0.05$), while 30-min time trial power output increased significantly in HA-EUH (+16±11 W, $p<0.05$), but not HA-DEH (+18±28 W, $p=0.18$). Heart rate, core and skin temperature responses and rating of perceived exertion were similar across all time trials, while sweat lost increased following both HA interventions.

Discussion

The present findings demonstrate that moderate, transient dehydration with exercising heat stress do not enhance HA adaptations. Improvements in exercise performance may be due to the ability to maintain a similar relative intensity and evaporative heat loss for a greater level of metabolic heat production during self-paced exercise in the heat.

References

1. Patterson et al (2004). *J Physiol 559*, 327-334.
2. Garrett et al (2014). *Am J Human Biol 26*, 311-320.
3. Neal et al (2016). *Front. Physiol 7*, 564