CIRCULATORY LIMITATIONS TO EXERCISE CAPACITY IN HUMANS: THE IMPACT OF HEAT STRESS AND DEHYDRATION ON BRAIN AND MUSCLE BLOOD FLOW AND METABOLISM

A thesis submitted for the degree of
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ABSTRACT

Heat stress and dehydration pose a severe challenge to physiological function and the capability to perform physical work. There is, however, limited knowledge on the regional haemodynamic and metabolic responses to strenuous exercise in environmentally stressful conditions. The primary aim of this thesis was to examine whether dehydration and heat stress compromise brain, muscle and systemic blood flow and metabolism, and whether depressed brain and muscle oxygen delivery underpin reduced exercise capacity during graded incremental and prolonged exercise. This thesis makes an original contribution to the knowledge by showing for the first time that dehydration markedly accelerates the decline in cerebral blood flow during maximal incremental (Chapter 4) and prolonged sub-maximal exercise (Chapter 5) in the heat. Cerebral metabolism, however, is preserved by compensatory increases in substrate extraction. Falling carbon dioxide tension underpinned the decline in CBF. However, a distinct regional distribution of blood flow across the head was observed, suggesting that different mechanisms are responsible for the regulation of regional blood flow within the head. A reduced cerebral metabolism is therefore an unlikely factor explaining the compromised exercise capacity in physiologically stressful hot environments. Rather, restrictions in active muscle blood flow and oxygen supply, which are not apparent during sub-maximal exercise, may explain the reduced maximal aerobic power in heat stressed conditions. For the first time we have manipulated skin and core temperature to show that combined internal and skin hyperthermia reduces maximal aerobic power in association with restrictions in limb, brain and systemic blood flow and skeletal muscle metabolism (Chapter 6). Overall, the findings of the present thesis provide novel information on how circulatory limitations across contracting skeletal muscle, brain and systemic tissues and organs might underpin the impairment in exercise capacity in physiologically taxing environments evoking significant dehydration and hyperthermia.

“For a person in a winter climate, to drink 4000 cc. of fluids per day is a distinct discomfort…for a person in the desert, to drink less is a violent discomfort.”

(Adolph & Dill, 1938)

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DEFINITION OF TERMS

Arterial to venous difference: the difference in content of a given substance in arterial and venous blood. Differences allow for calculation of exchange of molecules across a given tissue e.g. brain, limb. In this thesis a-v differences were obtained for oxygen, carbon dioxide, lactate, glucose, ATP and catecholamines.

Basilar artery: major artery of the brain which supplies the cerebellum, pons and the majority of the posterior lobes.

Blood velocity ($cm \cdot s^{-1}$): the speed of blood through the lumen of the vessel under observation.

Carbon dioxide reactivity (% change in CBF·mmHg change in CO_2): the extent to which cerebral vessel diameter increases or decreases in response to alterations in the partial pressure of blood CO_2.

Cardiac Output ($\dot{Q}$, $l \cdot min^{-1}$): the volume (in litres) of blood ejected by the left ventricle in one minute.

Cardiovascular strain: alterations in systemic cardiovascular function associated with a given intervention. In the present thesis this refers to the impact of heat stress and dehydration during exercise. Strain in this context is often observed as progressive reductions in SV, CBV, $\dot{Q}$ and MAP, concomitant with a marked increase in HR.

Cerebral autoregulation (CA): theoretical observation of the maintenance of CBF over wide range of cerebral perfusion pressures. Act to prevent under/over perfusion, thus limiting the risk of haemorrhage or ischemia.

Cerebral blood flow (CBF, $l \cdot min^{-1}$): rate of perfusion of the cerebral tissue. Normal value = ~50 $ml \cdot min^{-1} \cdot 100g$ (or ~750 $ml \cdot min^{-1}$).

Cerebrovascular conductance (CVC, $ml \cdot min^{-1} \cdot mmHg$): ratio of ICA blood flow or MCA V_{mean} to mean arterial blood pressure.

Cerebral glucose uptake/Cerebral metabolic rate for glucose ($CMR_{[Glu]}$, $mmol \cdot min^{-1}$): rate of glucose uptake by the brain.

Cerebral lactate uptake/Cerebral metabolic rate for lactate ($CMR_{[La]}$, $mmol \cdot min^{-1}$): rate of lactate uptake by the brain.

Cerebral metabolic rate for oxygen ($CMRO_2$, $ml \cdot min^{-1}$): rate of oxygen consumed by the brain.

Cerebrovascular resistance (CVR, $mmHg \cdot ml \cdot min^{-1}$): the inverse of CVC.

Common carotid artery (CCA): major conduit artery supplying blood to the head. In the present thesis, measurements of the CCA were made in the right CCA which originates at the brachiocephalic trunk from the aortic arch.

Compensable heat stress: conditions whereby thermal balance (i.e. core temperature) is established by appropriate heat loss mechanisms, despite increased metabolic heat production.

Content of oxygen in blood (ctO_2/CaO_2/C_vO_2, $ml \cdot l^{-1}$): the sum of the concentration of haemoglobin-bound oxygen and physically dissolved oxygen in arterial/venous blood.

Content of carbon dioxide in blood ($ctCO_2$/CaO_2/$CvCO_2$, $ml \cdot l^{-1}$): the sum of the concentration of bound and unbound carbon dioxide in arterial/venous blood.

Dehydration (DE): the process of excessive water loss from the body through sweating and to a minor extent respiration.

Diastole: phase of cardiac relaxation during the cardiac cycle.

End-tidal CO_2 ($P_{ET}CO_2$, mmHg): an index of P_aCO_2 as measured in the expired air at the level of the mouth.

External carotid artery (ECA): major branch of the CCA, supplying tissues of the head (except for the brain itself).

Finometer: non-invasive method of assessing arterial blood pressure via the recreated brachial pressure waveform from finger plethysmography.

Haemoconcentation: a consequence of dehydration, defined as a reduction in the ratio of plasma volume to red blood cell volume; thus an increase in circulating red blood cells per unit of blood volume.

Haemodynamics: the study of blood flow to/through a given tissue.

Heat stress: Exposure to a markedly hot exogenous temperature. In the present thesis this is achieved through the application of a hot water perfused suit.

Hypercapnia: elevated P_aCO_2. Results in vasodilation of the cerebral arteries (see P_aCO_2).

Hyperthermia: a high core body temperature, usually described as 1°C increase from normal resting body temperature.

Hypervolaemia: abnormal increase in blood volume.

Hypocapnia: reduction in P_aCO_2 in the blood. At the cerebral level reductions in P_aCO_2 cause cerebral arteriole/pial vessel vasoconstriction and reductions in CBF.

Hypohydration: state of body water deficit which can be the result of dehydration.

Hypovolaemia: abnormal decrease in blood volume.

Internal carotid artery (ICA): the second branch of the common carotid artery. Supplies blood to the cerebrum (~75-85%) and lateral parts of the temporal, parietal and frontal lobes. Subsequent branches include the ophthalmic artery (blood supply to the eyes), anterior and middle cerebral arteries.

Leg blood flow (LBF, ml·min^{-1}): the rate of blood flowing to the whole leg vasculature per unit time.

Leg oxygen uptake (Leg $\dot{V}O_2$, ml·min^{-1}): the volume of oxygen taken up by the leg (primarily skeletal muscle during exercise).

Limb vascular conductance (ml·mmHg^{-1}·min^{-1}): measure of the ease at which blood flows through a given vessel. Calculated as limb blood flow/limb perfusion pressure.

Mean arterial pressure (MAP, mmHg): the average blood pressure in the arterial system during the cardiac cycle. Estimated as one third systolic and two thirds diastolic pressure.

Middle cerebral artery (MCA): intra-cranial branch of the internal carotid artery.

MCA blood flow velocity (MCA V_{mean}, cm·s^{-1}): an estimation of cerebral blood flow. The MCA perfuses 75% of the cerebellum.

Model flow analysis: *Wessling* method of estimating stroke volume from the reconstructed brachial artery pressure waveform from finger plethysmography.

Neurovascular coupling (NVC): mechanism of matching local flow to local metabolic needs.

Noradrenaline spillover: an inferred reflection of regional sympathetic nerve activity derived from concentration difference of noradrenaline in the artery and vein. In the present thesis, noradrenaline spillover across the brain was estimated from the [NA] difference in the brachial artery and left internal jugular vein.

Partial pressure of CO_2 (PCO_2, mmHg): a measure of free, i.e. unbound, CO_2 in the plasma. CO_2 buffered (to HCO^{3-}) or bound in the form of carboxyhaemoglobin do not exert a pressure within the blood vessel. Additionally, PCO_2 plays a major role in cerebral blood flow regulation (see hypo/hyper-capnia).

Partial pressure of O_2 (PO_2, mmHg): a measure of free, i.e. unbound, O_2 in the plasma. The vast majority (~99%) of O_2 is bound to haemoglobin and by extension does not exert a pressure within the blood vessel.

Perfusion pressure or perfusion pressure gradient (mmHg): measure of the pressure differential between the arterial and the venous circulations, required to supply blood to a given region. Calculated as the difference between mean arterial pressure and local venous pressure (e.g. internal jugular and femoral venous pressure).

Posterior cerebral artery (PCA): cerebral artery perfusing the posterior portion of the brain including the occipital lobe and thalamus.

Pulse-wave Doppler: mode of ultrasound used for the assessment of blood velocity.

Q_{10} temperature coefficient (Arrhenius activation law): describes the increase in rate of biological reactions for a given increase in temperature. The $CMRO_2$ is thought to increase by 5-10%/1 °C increase in core temperature (Bain *et al.* 2014).

Specific heat: physical quantity of heat energy required to change the temperature of an object by a given amount (energy required to increase temperature by 1 °C measured in Joules).

Specific gravity: ratio of a given substance to a standard equivalent. The specific gravity of blood and water/saline are used in the present thesis.

Stroke volume (SV, ml): volume of blood ejected by the left ventricle in one heartbeat.

Systemic haemodynamics: umbrella term for whole-body blood displacement. Parameters include heart rate (HR), stroke volume (SV), cardiac output (Q) and, systolic, diastolic and mean arterial blood pressures.

Systemic vascular conductance (ml·min^{-1}·mmHg): measurement of the ease of which blood flows through the vessels in the whole-body. In the present thesis, SVC was estimated from the linear relationship with limb vascular conductance.

Systole: phase of contraction of the cardiac cycle.

Uncompensable heat stress: Conditions whereby thermal balance (i.e. core temperature) is not achieved and core temperature continues to rise in association with a mismatch between metabolic/internal heat production and heat dissipation.

Venous pressure (mmHg): the force blood exerts on the walls of the vessels as measured in the internal jugular and femoral vein.

Vertebral artery: vessel supplying oxygenated blood to the posterior part of the brain. Originating at the sub-clavian artery the vertebral artery travels to the cranium through the transverse processes of the vertebrae. The left and right vertebral arteries branch to supply the spinal cord, brainstem and latterly combine to form the basilar artery (see above).

$\dot{V}O_2$: volume of oxygen uptake per unit time. Normally expressed as absolute (l·min^{-1}) or relative to body mass (ml·kg·min^{-1}).

LIST OF ABBREVIATIONS

[A]	Plasma adrenaline concentration
ATP	Adenosine triphosphate
a-vO_2 diff	Arterio-venous oxygen difference
CCA	Common carotid artery
ctCO_2	Carbon dioxide content
ctO_2	Oxygen content
CMRO_2	Cerebral metabolic rate for oxygen
DEH	Dehydration
ECA	External carotid artery
FVP	Femoral venous pressure
gCBF	Global cerebral blood flow
HR	Heart rate
HS_{mild}	Mild heat stress
HS_{mod}	Moderate heat stress
ICA	Internal carotid artery
IJV	Internal jugular vein
JVP	Jugular venous pressure
MAP	Mean arterial pressure
MCA V_{mean}	Middle cerebral artery mean velocity
[NA]	Plasma noradrenaline concentration
OCI	Molar ratio of oxygen to carbohydrate
OGI	Molar ratio of oxygen to glucose
P_aCO_2	Partial pressure of carbon dioxide in arterial blood
P_vCO_2	Partial pressure of carbon dioxide in venous blood
Q_{10}	Arrhenius temperature coefficient
$\dot{Q}$	Cardiac output
rCBF	Regional cerebral blood flow
REH	Rehydrated
RH	Relative humidity
f_r	Respiratory frequency
SO_2	Oxygen saturation
SV	Stroke volume
SVC	Systemic vascular conductance

T_B	Blood temperature
T_c	Core temperature
T_{oes}	Oesophageal temperature
T_I	Internal temperature
$\overline{T}_{sk}$	Mean skin temperature
$\dot{V}_E$	Minute ventilation
V_T	Tidal volume
$\dot{V}CO_2$	Rate of carbon dioxide production per unit time
$\dot{V}O_2$	Rate of oxygen consumption per unit time
$\dot{V}O_{2max}$	Maximal rate of oxygen consumption per unit time
WR_{max}	Maximal work rate

CHAPTER 1 – GENERAL INTRODUCTION

1.1 Study context

Dehydration and hyperthermia induce “impressive behaviors[*sic*] and sensations” in the active man in the desert (Adolph & Dill 1938), and can severely impede athletic and maximal work performance (Rowell *et al.* 1966; Rowell *et al.* 1969b; Rowell 1974; Montain & Coyle 1992b; Rowell.1993; González-Alonso *et al.* 2008; Cheuvront & Kenefick 2014). Moreover, exogenous heat stress results in a marked circulatory strain which could impair appropriate physiological functioning; however, there remains a paucity of knowledge on the precise circulatory mechanisms underpinning an impaired aerobic exercise capacity under dehydration-induced hyperthermia and heat stressed conditions.

Numerous studies have explored the effects of body fluid losses (dehydration) on the capacity to perform strenuous exercise (Saltin 1964; Rowell *et al.* 1966; Rowell *et al.* 1969; Rowell *et al.* 1970; Rowell 1973; Rowell 1974; Sawka *et al.* 1979; Montain & Coyle 1992b; González-Alonso *et al.* 1995; González-Alonso *et al.* 1997; González-Alonso *et al.* 1998; González-Alonso 1998; González-Alonso *et al.* 2000; González-Alonso *et al.* 2008; Crandall & González-Alonso 2010). The development of dehydration during prolonged exercise in the heat attenuates skin blood flow, increases the rate of body heat storage and leads to a significant core hyperthermia and reductions in systemic and active muscle blood flow (González-Alonso *et al.* 1995; González-Alonso *et al.* 1997; González-Alonso *et al.* 1998; González-Alonso 1998; González-Alonso *et al.* 1999). The cardiovascular strain could compromise cerebral metabolism, which might play a role in the early fatigue during exercise in hot environments. However, no study to date has explored the impact of dehydration and hyperthermia on regional blood flow across the head and on cerebral metabolism during strenuous exercise.

A substantial increase in skin temperature, through the application of exogenous heat stress, degrades aerobic exercise capacity (González-Alonso & Calbet 2003; Ely *et al.* 2009; Ely *et al.* 2010; Sawka *et al.* 2012a). Despite further observations that heat stress reduces $\dot{V}O_{2max,}$ in part by altering systemic blood flow dynamics (Rowell *et al.* 1966; Rowell *et al.* 1969b; Nybo *et al.* 2001; Arngrimsson *et al.* 2004), there is surprisingly little information on the integrative processes underpinning this decline. Moreover, there is some discordance as to whether a heightened skin temperature, or the combined development of a concomitant core

hyperthermia, is the primary factor compromising aerobic capacity in hot environments (Nielsen *et al.* 1993; Nybo & Nielsen 2001a; Ely *et al.* 2009; Sawka *et al.* 2012a). To date no study has systematically addressed the role of different extents of heat stress exposure on whole-body haemodynamics during graded exercise.

Collectively, investigating the impact of heat stress on the brain, systemic and active limb metabolism during strenuous exercise will provide new insight into the circulatory limits to strenuous exercise. Additionally, information on the regulation of regional blood flow during exercise in the heat will help physiologists find strategies to negate or ameliorate the impact of stressful environments on athletic performance. To this end, the purpose of the present thesis was to further explore the regulatory factors underpinning an impaired exercise capacity in the heat stressed and dehydrated human. Specifically, the aims were; 1) to understand the effects of dehydration on cerebral blood flow and metabolism during graded exercise to volitional exhaustion (Chapter 4), 2) to explore the consequences of progressive dehydration on cerebral and extra-cranial haemodynamics and cerebral metabolism during prolonged exercise (Chapter 5), and 3) to provide insight into the brain, limb and systemic haemodynamics and metabolism in response to graded exercise with different extents of heat stress (Chapter 6). Three integrative experiments were performed at the Centre for Sports Medicine and Human Performance, Brunel University London, from June 2012 to February 2014.

CHAPTER 2 – REVIEW OF LITERATURE

2.1 Introduction

Dehydration and hyperthermia which are frequently experienced by humans exercising in hot environments are major physiological stressors that can severely hinder general physiological function, and athletic and work performance (Rowell *et al.* 1966; Rowell *et al.* 1969b; Rowell 1974; Sawka *et al.* 1985a; Sawka *et al.* 1985b; Rowell.1993; González-Alonso *et al.* 2008; Cheuvront *et al.* 2010; Cheuvront & Kenefick 2014). In strenuous environmental conditions, the strain invoked on the circulatory system by strenuous exercise challenges the maintenance of peripheral blood flow and may explain early fatigue. There is, however, limited knowledge of the precise mechanisms leading to impaired cardiovascular function and aerobic exercise capacity under dehydrated and hyperthermic conditions. In particular the challenge to the cerebral circulation is not well characterised or fully understood.

The following review first explores the literature pertinent to the effects of dehydration and concomitant hyperthermia on circulatory function during strenuous prolonged and maximal incremental exercise. Focal exploration of the cerebral haemodynamic and metabolic adjustments to strenuous exercise and the possible influences on cardiovascular capacity are discussed in section 2.3. In section 2.4 the effects of acute exposure to heat stress and the circulatory adjustments to exercise are explored. In section 2.5 the circulatory limitations to maximal aerobic power are explored. Lastly, the aims and research hypotheses of the current thesis are presented.

2.2 Dehydration and circulatory function during strenuous exercise in the heat

Performing sustained exercise in high ambient temperatures stimulates thermoregulatory sweating that can lead to the development of dehydration, when fluid intake is not proportional to fluid losses (Sawka *et al.* 1985b; Galloway & Maughan 1997; Maughan & Shirreffs 2004; Maughan & Shirreffs 2004; American College of Sports Medicine *et al.* 2007; Sawka *et al.* 2011; Cheuvront & Kenefick 2014). Depending on the type and extent of the dehydration, fluid loss can occur from the intracellular and extracellular space (Cheuvront & Kenefick 2014). Excessive sweating reduces circulating blood volume and an increase in plasma

osmolality, which is normally determined by measurements of haematocrit (Cheuvront & Kenefick 2014). Exercise delays the onset of sweating to a higher core body temperature (Charkoudian 2003; González-Alonso *et al.* 2008), and dehydration further leads to an increased rate of body heat storage by blunting the steady-state exercise sweat rate and cutaneous blood flow (Nadel *et al.* 1980; Fortney *et al.* 1981; Fortney *et al.* 1984; Fortney *et al.* 1988). This chain of events further compounds the thermoregulatory strain. Taken together, available evidence indicates that dehydration accrued during strenuous exercise in the heat poses a marked challenge to cardiovascular function (Rowell *et al.* 1966; Nadel *et al.* 1980; Fortney *et al.* 1981; Fortney *et al.* 1984; González-Alonso *et al.* 1995; González-Alonso *et al.* 1997; González-Alonso *et al.* 1998; González-Alonso 1998; Coyle & González-Alonso 2001; González-Alonso & Calbet 2003; González-Alonso *et al.* 2008; Mortensen *et al.* 2008). However, the mechanisms underpinning this phenomenon remain unresolved.

2.2.1 The effects of dehydration and hyperthermia on exercise capacity

Aerobic power and exercise capacity (i.e. time trial or time-to-exhaustion) are substantially reduced during whole-body prolonged exercise in the heat (Nielsen *et al.* 1993; Galloway & Maughan 1997; González-Alonso *et al.* 1999; González-Alonso *et al.* 2000; Cheuvront *et al.* 2010; Ely *et al.* 2010; Kenefick *et al.* 2010b; Sawka *et al.* 2012a). For example, Galloway & Maughan (1997) observed a ~45% decline in time to exhaustion when ambient air temperature was increased from 11 °C to 31 °C. When dehydration is permitted to develop, beyond a threshold purported to be equivalent to a 2% body mass deficit (American College of Sports Medicine *et al.* 2007), the capacity to continue prolonged exercise in the heat is markedly attenuated, in association with markedly suppressed circulatory function and concomitant hyperthermia (Sawka 1992; Below *et al.* 1995; González-Alonso *et al.* 1995; González-Alonso *et al.* 1997; González-Alonso *et al.* 1998; González-Alonso 1998; González-Alonso *et al.* 1999).

It has been shown that dehydration does not universally impair circulatory function among a range of exercise paradigms and environmental conditions. For example, during single-limb exercise where a relatively small muscle mass is engaged, dehydration reduces exercise duration (Montain *et al.* 1998b), but not due to a compromised active muscle blood flow (Pearson *et al.* 2013) or muscle markers of

fatigue (Montain *et al.* 1998b). During moderate whole-body exercise in a cold environment, where thermoregulatory demand for a high skin blood flow is dampened, dehydration leads to only negligible alterations in central haemodynamics (e.g. cardiac output and mean arterial pressure), when compared to a euhydrated equivalent (González-Alonso *et al.* 2000; Kenefick *et al.* 2004; Kenefick *et al.* 2010a; Cheuvront & Kenefick 2014). A similar stabilisation of central and peripheral haemodynamics, body temperature and exercise capacity is observed when participants match their sweat loss with a proportional intake of fluids (González-Alonso *et al.* 1995; González-Alonso *et al.* 1997; González-Alonso *et al.* 1998; Kenefick *et al.* 2010a). Collectively, these findings suggest that when the physiological strain on the circulatory system is low (i.e. during isolated limb exercise, exercise in cold environments, and during exercise in the heat with appropriate fluid intake), dehydration does not negatively affect exercise capacity. In contrast, dynamic whole-body exercise in the heat, particularly of a long (> 1 h) duration, would be considered a paradigm by which circulatory function is impaired with dehydration (González-Alonso *et al.* 2008).

The independent effects of dehydration on maximal aerobic power are less clear, predominantly due to the non-uniformity of protocols and variations in the levels of dehydration investigated. Studies using an exercise-induced dehydration protocol show reductions in $\dot{V}O_{2max}$ ranging from 6 to 16%, depending on the temperature of the ambient air (Buskirk *et al.* 1958; Nybo *et al.* 2001; Ganio *et al.* 2006). Nybo and colleagues observed a ~25% decline in constant power maximal exercise duration, concomitant with a 6% reduction in $\dot{V}O_{2max,}$ when participants were dehydrated (4% body mass loss) *versus* euhydrated. In this exercise bout body temperatures were maintained low (skin and peak core temperature of ~31 and 38.4 °C, respectively). Contrastingly, with simultaneous dehydration and hyperthermia (ambient temperature = 44 °C), a 16% reduction in $\dot{V}O_{2max}$ and ~53% reduction in performance time was demonstrated (Nybo *et al.* 2001). In another investigation, dehydration without noticeable hyperthermia substantially reduced the duration of constant load maximal exercise (~34% across three different dehydration protocols), but did not significantly compromise $\dot{V}O_{2max}$ (Saltin 1964). The reduced exercise time likely reflected the attainment of maximal work capacity given that peak heart rate in the dehydration trial was similar to that of the euhydration/control maximal test. Equally, in this context, it appears that relatively

small decrements in $\dot{V}O_{2max}$ are observed when body mass loss is less than 2-3%, when core hyperthermia is not endangered or if participants are well trained and experienced to performing exercise under physiologically stressful conditions (Saltin 1964; Ganio *et al.* 2006). Similarly, it has been shown that diuretic-induced moderate dehydration (~2%) does not affect cardiovascular function (evidenced by differences in heart rate, haematocrit and core temperature), nor does it impair maximal sprint and vertical jump (i.e. explosive power) performance when performing in thermoneutral environments (Watson *et al.* 2005). Nevertheless, in the heat, significant dehydration modifies the development of hyperthermia and can lead to a significant reduction on $\dot{V}O_{2max}$.

2.2.2 Mechanisms by which dehydration impairs physiological function during strenuous exercise

Fatigue during prolonged exercise is often associated with critical reductions in muscle glycogen stores (Hermansen *et al.* 1967; Coyle *et al.* 1986), however the decline in exercise capacity with exercise-induced hyperthermia appears not to be associated with earlier substrate depletion, as muscle glycogen is not typically depleted at exhaustion (Nielsen *et al.* 1990; Nielsen *et al.* 1993; Febbraio *et al.* 1996; González-Alonso *et al.* 1999; Maughan *et al.* 2007). It is also apparent that blood flow and substrate delivery to the exercising skeletal muscle is not a limiting factor, as both are maintained compared to normothermic environmental temperatures (Savard *et al.* 1988; Nielsen *et al.* 1990).

Instead, the inability to sustain exercise in hot environments has been attributed to the attainment of a high core temperature, as the development of fatigue was observed to coincide with a core temperature of ~40 °C (Nielsen *et al.* 1993; González-Alonso *et al.* 2008). This idea is supported by a number of observations made when the initial core temperature was manipulated either by heat acclimation (Nielsen *et al.* 1993), with pre-heating and pre-cooling (González-Alonso *et al.* 1999) or when the rate of rise in core temperature was manipulated (González-Alonso *et al.* 1999). The assumption of these findings was that a high 'critical' core temperature was a crucial factor limiting exercise in the heat. More specifically, because brain temperature is normally higher (+~0.2 °C) than core/arterial blood temperature (Nybo *et al.* 2002), temperature-dependent processes at the level of the central nervous system might restrict motor output

and explain early fatigue in the heat (Nielsen *et al.* 2001; Nybo & Nielsen 2001a; Nybo *et al.* 2002; Nielsen & Nybo 2003; Nybo 2003). On the other hand, the observed 'critical' temperatures were below that considered to be critical (e.g. ~43 °C) for neuronal and cell damage (White *et al.* 2012).

Dehydration compounds the circulatory challenge to exercise in the heat, altering cardiovascular function in association with reductions in systemic and active muscle blood flow and changes in metabolic and neurohumoral responses; generally termed 'cardiovascular drift' (Figure 2-1) (Coyle & González-Alonso 2001; González-Alonso *et al.* 2008). In the heat, dehydration reduces cutaneous blood flow and accentuates the rise in heart rate and core body temperature (González-Alonso *et al.* 2000). The cardiac tachycardia, coupled with the combined effect of the declining blood volume and hyperthermia, and an attenuated stroke volume are all important features underpinning the observed cardiovascular drift during prolonged exercise (González-Alonso *et al.* 1998; Fritzsche *et al.* 1999; González-Alonso *et al.* 1999; González-Alonso *et al.* 1999; Coyle & González-Alonso 2001).

A crucial observation is that the decline in systemic blood flow with dehydration during prolonged exercise in the heat is not entirely accounted for by the decline in active and non-active limb and cutaneous blood flow. It is therefore possible that blood flow to other body segments, including the cerebral circulation, is also compromised under such conditions. Because reductions in CBF normally lead to symptoms of pre-syncope (dizziness, faintness and blurred vision), reduced CBF is thought to be an important mechanism for the observed early curtailment of prolonged strenuous exercise, particularly in hot environments (Nielsen & Nybo 2003). However, the hard evidence proving this hypothesis is still lacking.

2.2.3 Impact of hyperthermia on cerebral blood flow and metabolism during strenuous exercise

Knowledge of the cerebral circulatory alterations to strenuous exercise in the heat is currently limited. It has recently been shown, however, that CBF declines with passive heat stress (Brothers *et al.* 2009a; Nelson *et al.* 2011; Bain *et al.* 2013; Ogoh *et al.* 2013b) and when exercise becomes strenuous (Jorgensen *et al.* 1992b; Hellstrom *et al.* 1996; Nybo & Nielsen 2001b; Nybo *et al.* 2002; Secher *et*

al. 2008; Sato *et al.* 2011). Cerebral perfusion, estimated by measurements of velocity in the middle cerebral artery (MCA V_{mean}), is maintained during prolonged exercise in cool conditions, but declines with uncompensable heat stress (Nybo & Nielsen 2001b; Nybo *et al.* 2002). Dehydration might further accentuate the decline in CBF, in line with its well-established effects on cardiovascular strain, although current findings are equivocal (Carter *et al.* 2006; Fan *et al.* 2008; Romero *et al.* 2011; Moralez *et al.* 2012). Nevertheless, reductions in CBF with exercise-dehydration hyperthermia could also lead to the attainment of a critically high brain temperature, which would contribute to the development of central fatigue (Nybo *et al.* 2002; Nybo *et al.* 2002; Nielsen & Nybo 2003).

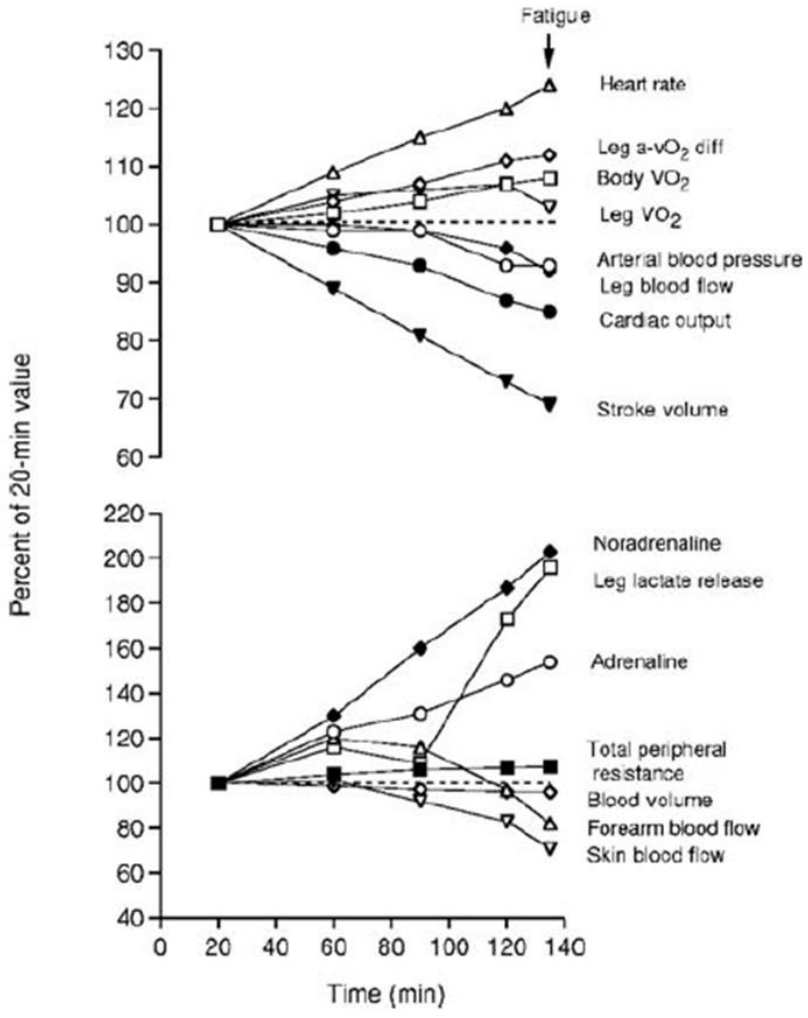


Figure 2-1. Cardiovascular consequences of dehydration during prolonged exercise in the heat. Heart rate, and systemic and leg oxygen uptake increase progressively with dehydration from values at 20 min (top panel). As dehydration develops, stroke volume, cardiac output, and blood pressure decline and non-active tissue blood flow and systemic catecholamines increase substantially (bottom panel); indicative of an increase in global sympathetic vasoconstrictor activity. From (González-Alonso *et al.* 2008).

Summary

Available evidence suggests that marked dehydration and hyperthermia induced through strenuous exercise in the heat advance circulatory strain and fatigue. The impact of a negative hydration status on maximal aerobic exercise remains equivocal. Furthermore, evidence suggests that dehydration does not necessarily affect circulatory function or exercise capacity. However, in combination with heat stress, it is capable of attenuating $\dot{V}O_{2max}$. Blood flow to the brain might also be compromised with dehydration; however, the precise regional haemodynamic alterations in response to exercise with dehydration and hyperthermia remain underexplored.

2.3 Regulation of cerebral and extra-cranial blood flow during strenuous exercise

Despite its relatively small contribution to total body weight, the brain normally has a resting blood flow of ~750 ml·min^{-1} (~53 ml·100g^{-1} ·min^{-1} based on a brain weight of 1.4 kg) (Lassen 1985), thus accounting for ~15% of the resting cardiac output (Kety & Schmidt 1948b; Lassen 1985; Madsen et al. 1993; Madsen et al. 1993; Nybo et al. 2014). Due to the high cerebral metabolic rate and the inability to store oxygen within the brain, maintaining the appropriate blood flow is of the upmost importance for the adequate supply of O_2 and removal of CO_2, as exemplified by the onset of syncope within minutes of large reductions in CBF (Zauner 1997; Van Lieshout et al. 2003; Willie et al. 2014).

Oxygenated blood is directed towards the head through two major bilateral common arteries supplying both the anterior and the posterior portions of the brain. In the anterior circulation, the right and left common carotid arteries are equally sized and provide equal volumes of blood flow, despite their differing origins. As the vessels ascend the neck, the common arteries divide at the carotid bifurcation into the external and internal carotid arteries (the ECA and ICA, respectively; Figure 2-2). The external artery is notably smaller in diameter and continues to traverse the anterior face and neck regions, branching into the superficial temporal and maxillary arteries. The larger internal carotid arteries, supplying ~75% of total CBF, continue upwards to the brain and terminate at the middle cerebral artery junction of the Circle of Willis. On the other hand, the posterior part of the brain is, to a lesser extent, also perfused by two vertebral

arteries (distributing ~25% of total CBF) (Schoning *et al.* 1994). The two bilateral vertebral arteries combine to form the basilar artery, branching at many intervals before forming the posterior portion of the Circle of Willis (Figure 2-3).

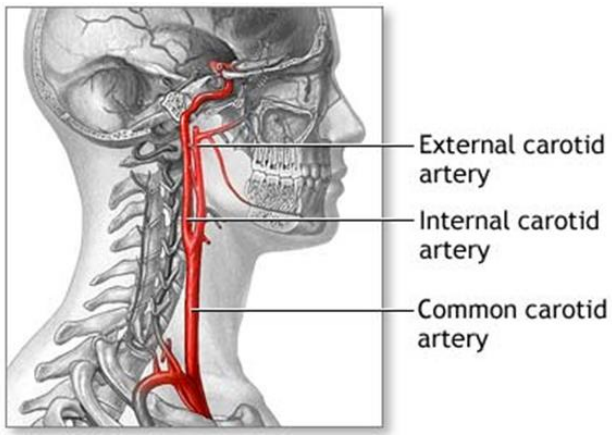


Figure 2-2. Image of the anterior neck arteries. The right common carotid artery branches from the brachiocephalic trunk and ascends the neck to the carotid sinus (location of pressure monitoring baroreceptors) before dividing into the internal and external-carotid arteries.

The arrangement of arteries within the brain constitutes a pathway to preserve CBF in the presence of a restricted/blocked artery. This is often the case at vessel bifurcations, particularly at the carotid sinus, which represents a common location for the development of plaques and stenoses (Thrush & Hartshorne 2010). Oxygenated blood traverses the deep capillary network into pial arterioles, and latterly to parenchymal arterioles which play an important role in the regulation of regional blood flow and are the location of substrate exchange between the blood and activated neurons. Oxygen and substrate exchange occurs across a unique feature of the cerebral circulation, a closed physical barrier between the capillaries and cerebral tissue (the blood-brain barrier) formed from an extension of the astrocyte end-feet between the basal lamina of the endothelium and the axons (Reese & Karnovsky 1967). This arrangement allows for the regulation of substrate exchange, preventing larger molecules (including noradrenaline) from entering the brain and allowing transfer of specific substances (including glucose and lactate) via specific transporter channels. The deoxygenated blood is drained through branches of the sagittal superior sinus, subsequently anastomosing at the transverse sinus before draining into two bilateral internal jugular veins, which parallel the common carotid arteries on their return to the central circulation (Zauner 1997).

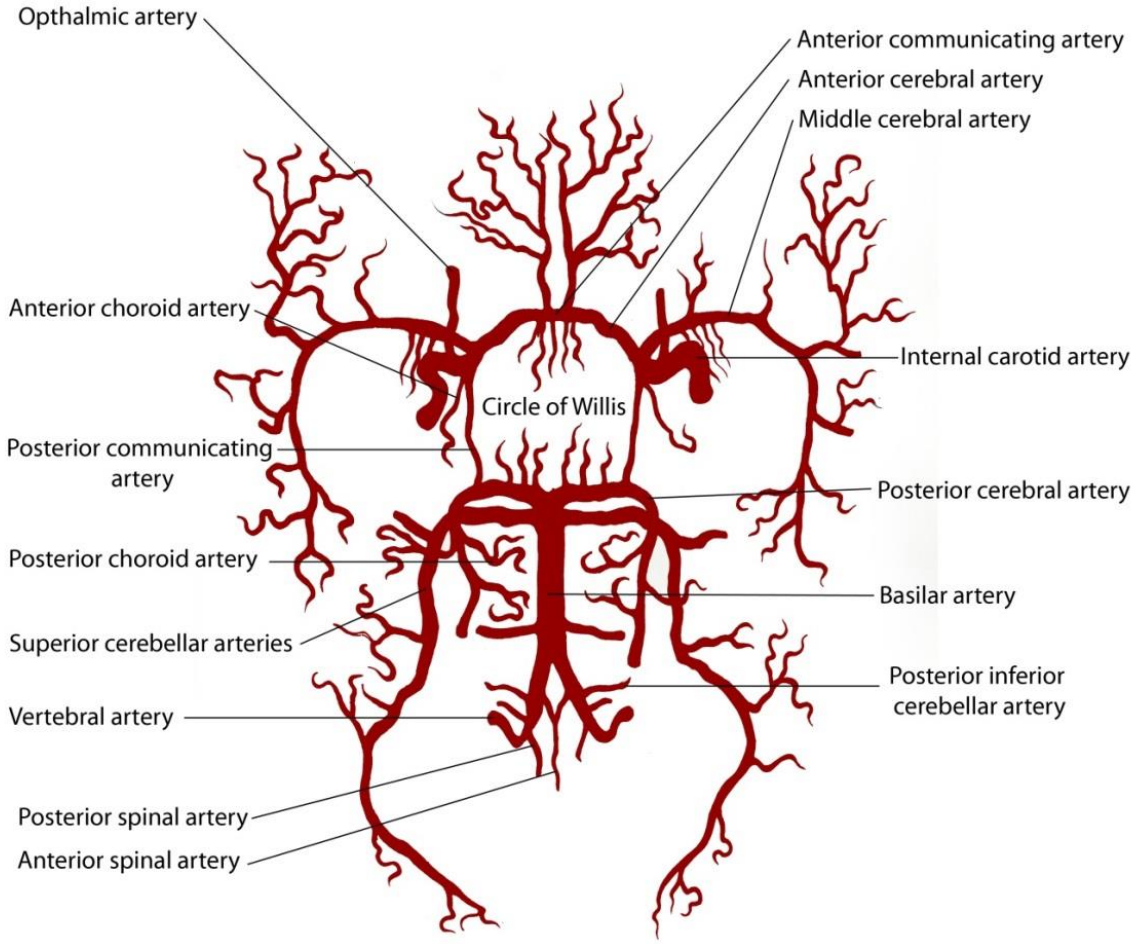


Figure 2-3. Arteries of the cerebral circulation. Blood flow to the brain is supplied through the two internal carotid and vertebral arteries, before anastomosing at the Circle of Willis. The arrangement of arteries allows for efficient redistribution of blood to metabolic active areas of the brain and provides a compensatory mechanism for flow restrictions.

2.3.1 Cerebral blood flow during exercise

Dynamic exercise requires a substantial activation of motor and cardiorespiratory neurons, and the ensuing enhanced regional metabolic demand for oxygen and glucose is generally thought to necessitate increases in regional CBF (rCBF) (Buxton & Frank 1997; Secher *et al.* 2008). However, it was previously suggested that CBF remained stable across a wide range of perfusion pressures (which generally increase with whole-body exercise), within the autoregulatory zone (see regulation of cerebral blood flow; (Lassen 1959; Ide & Secher 2000)). Recent evidence, however, opposes this early finding by showing that both rCBF and global CBF are elevated at the onset of dynamic exercise (Jorgensen *et al.* 1992c; Hellstrom *et al.* 1996; Nybo & Nielsen 2001b; González-Alonso *et al.* 2004; Sato *et al.* 2011).

The assumption of a stable CBF was initially supported by measurements made at the global level using the Kety-Schmidt method (Kety & Schmidt 1946; Kety & Schmidt 1948b; Scheinberg *et al.* 1953; Scheinberg *et al.* 1954; Hedlund *et al.* 1962; Madsen *et al.* 1993; Rowell.1993; Ide & Secher 2000; Secher *et al.* 2008; Ogoh & Ainslie 2009a). Using this method, the cerebral tissue is first saturated with an inert gas (e.g. N_2O), and CBF is determined as the ratio between the rate of N_2O uptake and the arterial to internal-jugular venous N_2O difference (Ide & Secher 2000). A number of limitations, however, undermine the accuracy of this measurement and its application to short duration dynamic exercise. First, the venous drainage of the brain is potentially heterogeneous as each of the internal jugular veins drains different proportions of the brain (Ferrier *et al.* 1993). Second, the internal jugular vein can become partially collapsed during exercise in the upright position which potentially may affect the sampling of venous blood draining the brain (Madsen *et al.* 1993; Rowell.1993; Secher *et al.* 2008). Lastly, the technique requires a steady state CBF which is likely not to be the case during dynamic exercise of high or severe intensity (Ide & Secher 2000). Measuring cerebral blood flow regionally, or with methods displaying a good temporal resolution, is therefore critical for the assessment of cerebral haemodynamics during exercise.

In contrast, modern methodological approaches show that the onset of exercise induces a significant increase in rCBF of approximately 25% (Secher *et al.* 2008), in both miniature swine (Delp *et al.* 2001) and humans (Jorgensen *et al.* 1992a; Jorgensen *et al.* 1992b; Hellstrom *et al.* 1996; Sato *et al.* 2011). Unilateral handgrip exercise instigates an increase in rCBF of the contralateral hemisphere, as measured using the initial slope index of the ^{133}Xe washout (Olesen *et al.* 1971; Olesen 1971; Herholz *et al.* 1987; Jorgensen *et al.* 1992a). Further support for the dynamic increases in rCBF, across a range of exercise and pharmacological investigations, was provided by the assessment of blood velocity in the middle cerebral artery (MCA V_{mean}; Jorgensen *et al.* 1992a; Jorgensen *et al.* 1992b), functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) (Ide *et al.* 2000). In particular, the development of trans-cranial Doppler ultrasonography (TCD) for the assessment of MCA V_{mean} has proven to be a useful tool for the assessment of regional cerebral perfusion during dynamic whole-body exercise (Aaslid *et al.* 1982; Madsen *et al.* 1993; Jorgensen 1995;

Linkis *et al.* 1995; Pott *et al.* 1997; Ide *et al.* 1998). TCD assesses the maximum envelope of the velocity through the middle cerebral artery and can detect acute changes in rCBF; ideal for measurements of cerebral perfusion during short bouts of dynamic whole-body exercise.

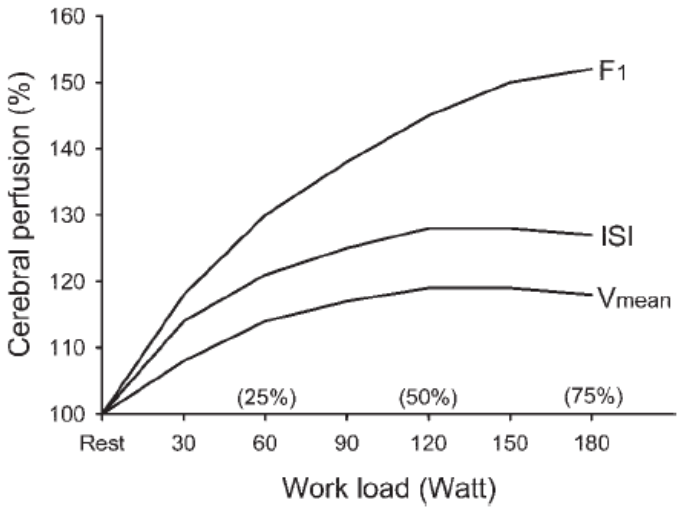


Figure 2-4. Relationship between TCD derived MCA V_{mean} and the initial slope index (ISI). Cerebral blood flow increases up to ~50% peak power before attenuating/declining towards baseline values. From (Jorgensen *et al.* 1992a) and (Secher *et al.* 2008).

Transcranial Doppler measurements of MCA V_{mean} are not without technical considerations, chiefly the fact that vessel diameter remains unknown. In accordance with Poiseuille's Law, volume flow is highly dependent on the fourth power of the radius, i.e. large changes in vessel diameter would have a substantial effect on the calculated volume flow, irrespective of the V_{mean}. Therefore, cerebral perfusion is only correctly assessed when the calibre of the vessel remains unchanged, which may not be the case across many different experimental conditions (Willie *et al.* 2011; Willie *et al.* 2012). Recently, technological developments have offset the limitations of the transcranial Doppler. Duplex ultrasonography of the internal-carotid artery (Hellstrom *et al.* 1996; Sato *et al.* 2011) allows for continuous measurement of flow velocity and vessel calibre, providing absolute blood flow of the basal cerebral arteries. Nevertheless, under most physiological exercise conditions MCA V_{mean} and duplex ultrasonography derived rCBF reproducibly show a ~25% increase in perfusion up to sub-maximal exercise intensities.

When exercise becomes strenuous there is a paradoxical decline in CBF, posing a challenge to the convective oxygen delivery to the brain. It has been shown that during graded exercise above ~60% WR_{max} (Hellstrom *et al.* 1996; Sato *et al.* 2011), global and rCBF plateau or decline to baseline values prior to volitional exhaustion (Madsen *et al.* 1993; Moraine *et al.* 1993; Hellstrom *et al.* 1996; Ide & Secher 2000; González-Alonso *et al.* 2004)(Figure 2-4). This still limited number of studies suggests that convective O_2 delivery to the brain is reduced and, in accordance with the Fick equation, brain oxygen uptake could decline unless cerebral oxygen extraction compensates for the reduced flow. In this context, it is unknown whether the severe physiological strain evoked by dehydration and hyperthermia further accentuate the decline in CBF during both graded and prolonged exercise to the extent that brain metabolism is compromised.

2.3.2 Posterior and extra-cranial blood flow during exercise

As discussed above, in addition to the two anterior arteries (two ICAs, latterly forming the two MCAs), the posterior portion of the brain is perfused by two vertebral arteries located within the spinal column, anastomosing to form the basilar artery. Whilst these arteries supply a smaller proportion of the total CBF, recently it has been observed that blood flow in the vertebral arteries (VA) increases with exercise intensity (Sato *et al.* 2011). Furthermore, hypoxia induces larger relative increases in VA flow compared to that in the ICA (Ogoh *et al.* 2013a; Lewis *et al.* 2014b), suggesting that the regulation of blood flow to the posterior portion of the brain might be prioritised at high exercise intensities. These findings are consistent with observations of enhanced rCBF to the posterior circulation (Spinal cord, Cerebellar Ventral Vermis and Medulla) in miniature swine during increasing exercise intensity (Delp *et al.* 2001).

The external-carotid artery is formed at the branch of the carotid bulb and ascends the neck with a similar, but superficial, trajectory when compared to the internal carotid artery. Subsequent branches perfuse the outer cranium (superficial temporal, posterior auricular and occipital artery) and the face and neck (facial, superior thyroid, lingual and maxillary arteries). Resting blood flow to the regions supplied by the ECA equate to ~250 to 300 $ml \cdot min^{-1}$, raising the total head blood flow under normal resting conditions to 1 $l \cdot min^{-1}$. Whilst a decline in cerebral perfusion is evident at high exercise intensities, blood flow to the extra-cranial

circulation increases linearly from rest to intense exercise (Hellstrom *et al.* 1996; Sato *et al.* 2011). The study of Sato *et al.* shows a doubling in ECA blood flow from rest to 80% WR_{max} during semi-recumbent cycling, supported by an elevation in blood flow through the common carotid artery. A 2-3 fold increase in ECA blood flow is also observed with passive heat stress (Bain *et al.* 2013), which may be indicative of a role for extra-cranial blood flow in local thermoregulation.

Summary

Although once considered to remain constant during exercise, rCBF responds dynamically to increasing work rate. This response may be important for the maintenance of oxygen and substrate delivery to active regions of the brain. Beyond moderate exercise, global CBF declines towards baseline values which could potentially challenge cerebral metabolism and contribute to impaired physiological function unless compensatory adjustments occur. In contrast, extra-cranial flow appears to increase linearly with increasing exercise intensity to near maximal levels. The physiological explanations for the apparent differential regional responses remain as yet unclear. No study to date has explored the cerebral and extra-cranial circulatory dynamics during exhaustive prolonged and maximal exercise in the heat.

2.3.3 Cerebral metabolism during exercise

Accentuating the decline in cerebral and limb blood flow could compromise local aerobic metabolism because, unlike the skeletal muscle, the brain has a negligible oxygen storage capacity. Transient reduction in blood flow to the brain with head-up tilt and upon standing, with cardiac insufficiency, cause symptoms of syncope and a risk of total loss of consciousness (Ide *et al.* 1999a; Van Lieshout *et al.* 2003).

At rest the brain consumes approximately 3.5 ml $O_2 \cdot min^{-}1 \cdot 100g^{-1}$, equating to ~20% of whole-body oxygen uptake for a normal adult brain size of 1.4 kg (Kety & Schmidt 1946). The nomenclature for the rate of oxygen uptake at the level of the brain is the cerebral metabolic rate for oxygen ($CMRO_2$). As with the traditional measures of CBF, the change (or lack thereof) in the $CMRO_2$ is largely dependent on the method of measurement used. Regional and global increases in the $CMRO_2$ are observed with positron-emission tomography (PET) during intense

visualisation tasks (Fox & Raichle 1986; Roland *et al.* 1987), hand movement (Raichle *et al.* 1976), and with prolonged exercise with hyperthermia (Nybo *et al.* 2002). On the other hand, others report a relatively stable aerobic metabolism (Scheinberg *et al.* 1954; Madsen *et al.* 1993; Ide & Secher 2000) during light exercise, which presumably engages higher level of neuronal activity compared to rest (Secher *et al.* 2008). To this end it is plausible that the increased CBF is proportionally matched by a lower oxygen extraction (González-Alonso *et al.* 2004; Fisher *et al.* 2013). It remains unclear as to whether the $CMRO_2$ would be enhanced, remain stable or be compromised with a significant reduction in CBF when dehydration and hyperthermia are superimposed on strenuous exercise in heat stress conditions.

It has been shown that the brain takes up large amounts of carbohydrate during strenuous exercise (Dalsgaard *et al.* 2004a; Dalsgaard *et al.* 2004b; González-Alonso *et al.* 2004; Dalsgaard 2006; van Hall *et al.* 2009; Volianitis & Secher 2009). At rest and during intense exercise the brain displays an RQ of ~1 (Dalsgaard et al. 2004), indicating that carbohydrates, mainly blood glucose, are the primary fuel of the astrocytes and neurons (Mintun *et al*. 2001; Dienel 2012a; Dienel 2012b). On the other hand, lactate is considered to be an important metabolic substrate within the brain. This is demonstrated by astrocytes in culture which display an affinity for lactate as a primary fuel (Bouzier-Sore *et al.* 2003), and the observation that the cerebral uptake of lactate is substantially enhanced during strenuous exercise in humans (Quistorff *et al.* 2008; van Hall *et al.* 2009). Specifically, at rest, the ratio of oxygen to glucose uptake (oxygen-glucose index) is 6:1 (that is, 6 O_2 + 1 $C_6H_{12}O_6$ produces 6 H_2O + 6 CO_2), more commonly observed to be ~5.7 with some “anaerobic contribution” (Dalsgaard et al. 2004; Dalsgaard *et al*. 2004). However, during intense cerebral activation, such as very strenuous exercise engaging a large muscle mass, the ratio of oxygen-to-carbohydrate uptake can decline to very low levels (Dalsgaard *et al.* 2004; Secher *et al.* 2008; Volianitis & Secher 2009). This indicates that the uptake of carbohydrate, be it glucose and/or lactate, is in ‘excess’ of the oxidative rate (Dalsgaard *et al.* 2002; Dalsgaard *et al.* 2004a; Dalsgaard 2006). Uncertainty remains on the precise function of the imbalance in oxidative metabolism during strenuous exercise; however, its extensive decline prior to fatigue during exercise is suggested to be associated with a ‘central’ metabolic fatigue (Dalsgaard 2006).

It is unclear whether dehydration and hyperthermia constitute a metabolic challenge to the brain, similar to that observed during very intense whole-body exercise.

In addition to the potential role of a reduction in the CMR in so-called “central” fatigue, reductions in oxygen delivery to the brain might constitute a scenario whereby a reduced cerebral function precipitates the attainment of fatigue during intense exercise (Rasmussen *et al.*, 2007*b*; Nybo & Rasmussen, 2007; Subudhi *et al.*, 2008, 2009). Strenuous exercise enhances cerebral metabolic demand (Delp *et al.*, 2001; Secher *et al.*, 2008) and, coupled with a reduction in cerebral perfusion, might lower the cerebral mitochondrial oxygen tension ($PmitoO_2$) to levels (e.g. > that 5mmHg reduction) that are commensurate to a cerebral oxygen deficit that compromises cerebral function (Rasmussen *et al.*, 2007*a*). However, this is unlikely during maximal exercise at sea level where the $PmitoO_2$ is not lowered to such “critical levels” (Nybo & Rasmussen, 2007; Fisher *et al.*, 2013).

Hypoxia impairs exercise performance, perhaps through central cerebral mechanisms as the administration of supplementary oxygen during maximal exercise in hypoxia restores cerebral oxygenation and restores exercise capacity (Subudhi *et al.*, 2008, 2009); however it is unclear as to whether this in itself reflects a salient mechanism by which hypoxia leads to fatigue as oxygen interventions are not localised solely to the cerebral tissue (Olin *et al.*, 2011; Subudhi *et al.*, 2011). Interpretation of these findings may also be hindered by the employment of near-infrared spectroscopy (NIRS) of the frontal cortex as an indicator of cerebral oxygenation (Subudhi *et al.*, 2008, 2009), as measure one local region may not reflect the metabolic conditions of the brain as a whole. Additionally, it might not be that reduced perfusion of this region of the brain is necessarily a precursor to fatigue. In this light it has been shown that this region is normally associated with a reduced perfusion (Delp *et al.*, 2001), which may be interpreted to mean that activity of the frontal cortex is not overtly required for exercise performance (whereas activity and blood flow to areas associated with cardiovascular control may yet be; see (Delp *et al.*, 2001). Lastly, manipulation of CO_2 within the blood, through carbon dioxide inhalation, normalises cerebral oxygen delivery but leads to similar (if not slightly attenuated) exercise capacity (Subudhi *et al.*, 2011). It seems more likely that attenuated systemic and

locomotor muscle O_2 delivery are more important factors preceding fatigue during maximal exercise (González-Alonso *et al.*, 2004; Nybo & Rasmussen, 2007; Subudhi *et al.*, 2011).

2.3.4 Regulation of cerebral blood flow

For an organ that is not capable of storing large amounts of oxygen, the brain is fully dependent on the tight regulation of blood flow to maintain O_2 supply. A number of mechanisms have been implicated in the control of rCBF at rest and during dynamic exercise including, but not limited to, autoregulation, blood gasses and sympathetic activity (Faraci & Heistad 1998; Querido & Sheel 2007; Secher *et al.* 2008; Ogoh & Ainslie 2009a; Ogoh & Ainslie 2009b; Ainslie & Ogoh 2010; Willie *et al.* 2014). Dehydration and hyperthermia could lead to significant alterations in blood flow and thus these mechanisms may play a role in adjusting vascular tone to ensure adequate perfusion during stressful environmental conditions.

Blood flow, perfusion pressure and vascular resistance. The supply of blood to a given region of the circulation is dependent on the balance between the "driving force" of blood from the central circulation (determined by the perfusion pressure gradient), and local resistance to flow. The interplay between blood flow, pressure, and resistance is derived from Ohm's Law (Volts = Current x resistance) applied to the circulation and at the local level is;

Blood flow = Perfusion pressure/Vascular resistance

The perfusion pressure gradient is determined from the subtraction of intracranial pressure (ICP) at the level of the brain (Zauner 1997), and femoral venous pressure at the level of the leg, from mean arterial blood pressure. With respect to the brain it is often not possible to obtain direct measures of intra-cranial pressure as this is reserved for clinical practice and surgery related to the brain. Although not ideal, jugular venous pressure can be used as a substitute, if it is deemed to be greater than, or similar to, the expected ICP.

The aforementioned determination can be considered simplistic as it does not consider the possible changes in blood viscosity, and the complex arrangements

of blood vessels in some regions (e.g. the brain). Hagen-Poiseuille's Law more comprehensively considers these factors, where P is pressure, μ is viscosity, l is length and r is the radius;

$$CBF = P / [8 - \mu l) / (r^4)]$$

Central processes contribute to increased cerebral perfusion pressure and support the rise in blood flow; however, given the importance of vessel radius, factors altering local vascular tone can have a more significant bearing on the regulation of blood flow, particularly during exercise and environmental stress.

Cerebral blood flow, autoregulation and central haemodynamics

The first observations that CBF remained stable during exercise gave rise to the concept of 'cerebral autoregulation' (CA). In the work of Lassen (1959), existing data from a range of physiological perturbations were used to assess the relationship between CBF and arterial blood pressure (Figure 2-5). From this work the principle that CBF was maintained stable across a wide range of cerebral perfusion pressures was postulated, eventually becoming the prevailing theory to explain the apparent stability of CBF (Lassen 1959; Lassen 1974; Paulson *et al.* 1990). This classic dogma, however, has recently been challenged because these findings were from multiple subsets of participants and often did not control for the confounding effects of CO_2 and drug administration (on the cerebrovascular responses to changes in perfusion pressure) (Secher *et al.* 2008; Lucas *et al.* 2010; Willie *et al.* 2014). A further confounding factor is that changes in MCA vessel diameter can obscure the accurate determination of CA (Lucas *et al.* 2010; Willie *et al.* 2014).

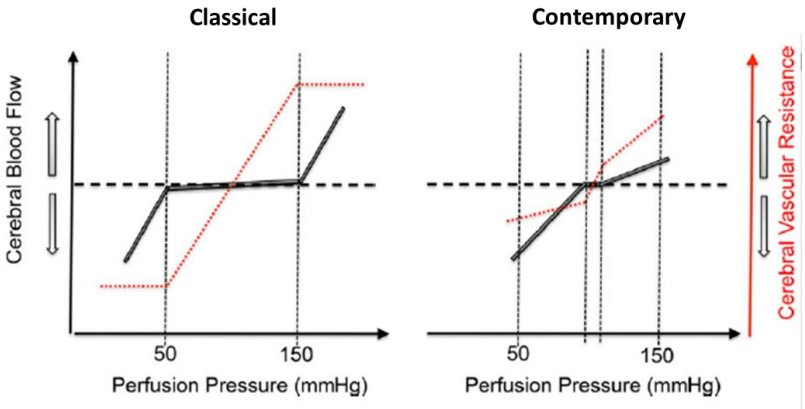


Figure 2-5. Cerebral autoregulatory curves based on classical and current observations. The traditional autoregulatory curve depicted in the left panel displays the consistency of CBF over a wide range of perfusion pressures. On the right panel, the more recent depiction of a much smaller autoregulatory range. Furthermore the slope of the response is now considered to be unequal with hypotension and hypertension, respectively. (From Willie *et al.* 2014).

Alterations in mean arterial blood pressure (MAP) do not appear to influence changes in rCBF. This is highlighted by observations that cerebral perfusion declines back to resting baseline levels despite the maintenance of a high MAP during post-exercise muscle ischemia (Aaslid *et al.* 1989; Jorgensen *et al.* 1992b). On the other hand, the increase in cardiac output seems to be very important for the rise in CBF. This is supported by observations that the magnitude of rise in MCA V_{mean} is blunted with pharmacological β_1-adrenergic block and in patients with atrial fibrillation (Ide *et al.* 1998; Ide *et al.* 1999a; Ide *et al.* 2000). In addition, manipulating $\dot{Q}$ and central venous pressure (CVP) with serum albumin infusion and lower body negative pressure (LBNP) increases and reduces cerebral perfusion, respectively (Ogoh *et al.* 2005a). Techniques which preserve central blood volume (e.g. muscle tensing) attenuates the decline in cerebral perfusion when assuming a standing position (van Lieshout *et al.* 2001). Moreover, heat stress reduces central blood volume and induces a significant hyperventilation which in combination can lead to reductions in CBF, symptoms of pre-syncope and orthostatic intolerance (Wilson *et al.* 2006). In the context of the present thesis it is not known whether reductions in MAP and $\dot{Q}$ during exercise with dehydration and hyperthermia are important for the effective regulation of CBF.

Local regulation of cerebrovascular tone during exercise

Local processes dictating vasoactive tone, either by acting directly or externally on the endothelium to regulate vessel calibre, are of fundamental importance to the appropriate regulation of CBF (Faraci & Heistad 1998; Ogoh & Ainslie 2009b). Perhaps the most influential factor causing alterations in cerebrovascular tone is respiratory-induced changes in the partial pressure of carbon dioxide in arterial blood (P_aCO_2). It has long been known that raising P_aCO_2 through CO_2 administration induces cerebral 'expansion[*sic*]' in dogs (Roy & Sherrington 1890), a finding that was subsequently confirmed in healthy humans (Kety & Schmidt 1948a). It is now established that increasing P_aCO_2 (hypercapnia) induces cerebral vasodilation, whereas reducing P_aCO_2 (hypocapnia) causes cerebral vasoconstriction (Ainslie *et al.* 2005; Querido & Sheel 2007; Secher *et al.* 2008; Ogoh & Ainslie 2009b; Willie *et al.* 2012; Willie *et al.* 2014). The significant role of changes in arterial blood gas tensions was recently addressed in the meritorious study of Willie and colleagues who, through independent manipulations of inhaled CO_2, demonstrated that wide ranging elevations and reductions in P_aCO_2 induce

corresponding cerebral vasodilation and constriction, respectively (Figure 2-6) (Willie *et al.* 2012). The latter study also refutes the dogma that only downstream pial vessels participate in cerebrovascular tone (Fog 1938), by showing an ~8% change in internal carotid artery diameter across the hypocapnic/hypercapnic range, and in doing so support similar findings from animal models (Faraci *et al.* 1987). Under resting conditions the change in CBF is shown to be ~3-4%/mmHg change in $P\text{a}CO_2$ (the 'CO_2 reactivity')(Linkis *et al.* 1995; Willie *et al.* 2012) and the slope of the relationship increases with exercise-hyperthermia (Rasmussen *et al.* 2006). As is apparent in Figure 2-6, the reactivity to CO_2 is lower in the posterior circulation compared to the anterior (i.e. ICA) circulation (~2% *vs.* 3-4% per mmHg change in $P\text{a}CO_2$). No relationship between CO_2 and vascular tone is observed in the external-carotid circulation or in other peripheral arteries, and thus the role of CO_2 in blood flow regulation is therefore confined to the cerebral vasculature (Ainslie *et al.* 2005; Sato *et al.* 2012).

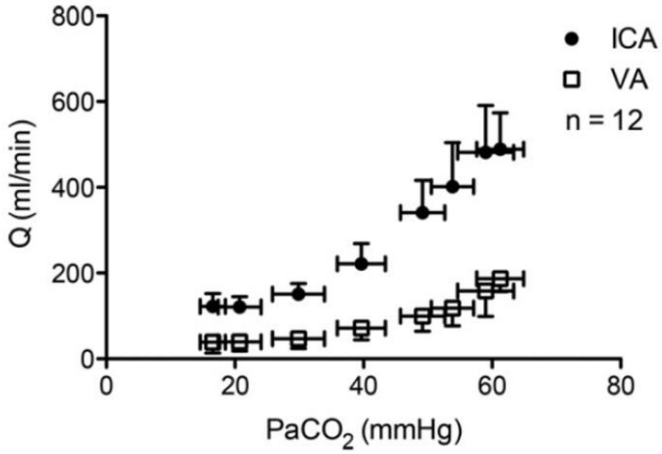


Figure 2-6**. Anterior (ICA) and posterior (VA) CBF during progressive hypocapnia and hypercapnia.** Baseline $P\text{a}CO_2$ is ~40 mmHg. Note that the reactivity to CO_2 (i.e. slope of the CO_2/flow relationship) is steeper in the hypercapnic *vs.* hypocapnic range. From Willie *et al*. 2012, 2014).

From rest to sub-maximal exercise, a slight rise in P_aCO_2 in response to cerebral metabolic CO_2 production contributes to the increase in rCBF (Kontos *et al.* 1978; Faraci & Heistad 1998; Secher *et al.* 2008). As exercise progresses to levels beyond the respiratory compensation point, a substantial decline in P_aCO_2, induced in response to thermal and non-metabolic stimulated hyperventilation, are thought to be the primary mechanism underpinning the cerebral vasoconstriction and the decline in CBF (White & Cabanac 1996; Nielsen *et al.* 2002; Secher *et al.*

2008; Willie *et al.* 2012). CO_2 can interact intravascularly in conjunction with endothelium derived vasoactive substances (NO, EDHF, PGI_2) or, due to its ability to readily cross the blood-brain-barrier, can act extravascularly or stimulate reflex control of smooth muscle tone via alterations in cerebral spinal fluid pH (Sokoloff 1960; Yoon *et al.* 2012).

Other than CO_2, control of cerebrovascular tone has been ascribed to sympathetic-mediated pathways (Lee *et al.* 1976; Mitchell *et al.* 2009; Ogoh & Ainslie 2009a; Seifert & Secher 2011). This observation is supported by the findings of enhanced noradrenaline spillover from the cerebral vasculature with pharmacological manipulations of sympathetic activity (Mitchell *et al.* 2009). Sympathetic activity might be important to modulate potentially large surges in perfusion pressure (Ogoh *et al.* 2008; Ainslie 2009; Tzeng & Ainslie 2013); however, the direct physiological role during exercise remains controversial (Strandgaard & Sigurdsson 2008b; van Lieshout & Secher 2008b).

Summary

The balance between appropriate CBF and metabolic substrate demand is precisely regulated by a number of important mechanisms; most notably P_aCO_2. These mechanisms may be of particular importance during exercise with dehydration and heat stress because of the expected large alterations to the central circulation and respiratory pattern. This is not to say that other mechanisms (e.g. sympathetic activity, PO_2, neurovascular coupling and peripheral reflexes) are not influential on cerebrovascular tone.

2.4 Heat stress and circulatory function during strenuous exercise

It is well established that high intensity exercise in uncompensable heat stress conditions induces a marked physiological strain that includes high levels of hyperthermia (Rowell *et al.* 1969a; Rowell *et al.* 1970; Detry *et al.* 1972; Rowell 1993). Importantly, exercise heat stress can lead to similar severe hyperthermia and physiological instability in the exercising human, as described above for exercise-induced dehydration. The following section outlines the cardiovascular responses to heat stress and its impact on maximal aerobic power.

2.4.1 Circulatory adjustments to heat stress at rest

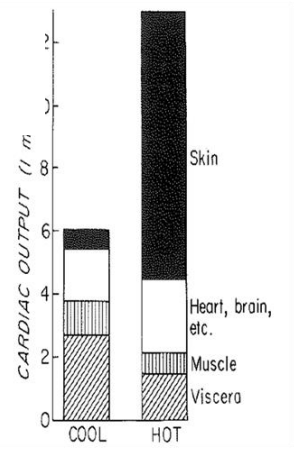
Under resting conditions a substantial increase in environmental temperature leads to numerous cardiovascular adjustments, in response to enhanced thermoregulatory demand. Heat exposure sufficient to increase skin and core temperature induces a marked cutaneous vasodilation, estimated indirectly to increase skin blood flow from resting values of ~300 ml·min^{-1} to a peak of 7-8 l·min^{-1} (Rowell *et al.* 1970; Rowell 1974; Brengelmann *et al.* 1977; Nielsen *et al.* 1984; Rowell 1984; Rowell 1993; Crandall & González-Alonso 2010; Johnson & Kellogg 2010). The rise in skin blood flow in response to elevations in local temperature is mediated by both the withdrawal of vasoconstrictor tone and cutaneous active vasodilation (Johnson & Kellogg 2010). Specifically, cutaneous vascular tone is altered through an initial axon-reflex and subsequently through a slower acting nitric-oxide (NO) dependent vasodilation (Kellogg *et al.* 1998; Charkoudian 2010; Johnson & Kellogg 2010).

The increase in skin capacitance requires a large increase in cardiac output, brought forth predominantly by increases in heart rate and to a lesser extent the sympathetically-mediated redistribution of central blood volume (Rowell *et al.* 1969a; Rowell *et al.* 1971; Niimi *et al.* 1997; Minson *et al.* 1998; Wilson *et al.* 2007). Cardiac output doubles from resting normothermic conditions, with the majority of the increase hypothesised to be directed to the skin (Figure 2-7)(Roddie *et al.* 1956; Rowell *et al.* 1970; Niimi *et al.* 1997; Crandall *et al.* 2008; Crandall & González-Alonso 2010). Blood flow requirements are further met by visceral and splanchnic vasoconstriction (Figure 2-7) (Rowell *et al.* 1965; Rowell *et al.* 1968; Rowell *et al.* 1970; Crandall *et al.* 2008). It is currently unclear whether a brief exposure to heat stress, sufficient to raise skin temperature but not core temperature, augments cardiac output at rest and if so whether cardiac output remains elevated during incremental exercise.

The redistribution of blood to the skin and evaporative heat loss through sweating serves as the primary (if not only) mechanism of substantial heat liberation from the body during heat stress (Rowell.1993). At rest and during sub-maximal exercise, blood flow redistribution (contributing ~1 l·min^{-1}) (Minson *et al.* 1998) and an elevation in cardiac output (~2-4) l·min^{-1}, proportional to the invoked heat stress, is capable of balancing the simultaneous demands for thermoregulation

and active muscle substrate supply, whilst maintaining arterial blood pressure (Kenney *et al.* 2014). However, when exercise becomes strenuous, the blood flow requirements of the active muscles are prioritised and the rise in skin blood flow is attenuated (González-Alonso *et al.* 2000; González-Alonso *et al.* 2008).

Rowell, 1986



Crandall *et al.* 2008

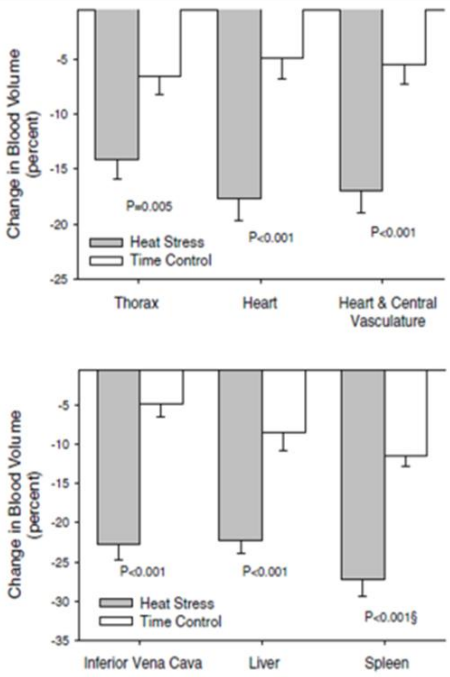


Figure 2-7. Elevations in cardiac output and reductions in central blood volume during severe heat stress inducing marked increases in both core and skin temperatures. Note that the blood flow values in the top graph are theoretical values**.** From Rowell 1986, Crandall *et al.* 2008.

2.4.2 Impact of external heat stress on exercise capacity

It has generally been observed that $\dot{V}O_{2max}$ is suppressed in the heat, but previous findings have been equivocal. Initially it was shown that $\dot{V}O_{2max}$ was not discernibly lower, despite a markedly reduced $\dot{Q}_{max}$, in hot ambient conditions (Rowell *et al.*

1965; Rowell *et al.* 1966). In these studies it is possible that the prolonged period of data collection (5 months), and changes in the participant characteristics, might have mitigated the impact of the heat exposure. Subsequently, Rowell *et al.* observed a small but significant decline in $\dot{V}O_{2max}$ of 1.6 $ml \cdot kg^{-1} \cdot min^{-1}$ (-3%) in participants who performed graded treadmill running to volitional exhaustion in hot (43.3 °C) and cool (25.6 °C) ambient environments (Rowell *et al.* 1969b). Small declines of 4–7% have also been shown across a range of environmental temperatures (35–49 °C) (Klausen *et al.* 1967; Pirnay *et al.* 1970; Sawka *et al.* 1985a; Arngrimsson *et al.* 2004). These findings were generally observed in non-acclimated men and began without any pre-heating induced elevations in core temperature. When the trials were preceded with exercise or passively induced pre-heating, the decline in $\dot{V}O_{2max}$ is shown to be far more substantial (~20-25%) (Pirnay *et al.* 1970; Nybo *et al.* 2001; Arngrimsson *et al.* 2004; Lafrenz *et al.* 2008). Only Arngrimsson and colleagues (2004) explored the reduction in $\dot{V}O_{2max}$ across a range of experimental temperatures during graded exercise using a within-subject design, though a consistent finding from these studies was that brief periods of heat exposure during short-duration graded exercise are unlikely to suppress $\dot{V}O_{2max}$ to any great extent.

On the other hand, evidence has suggested that high skin temperature, independent of core temperature, is an important mechanism by which exercise capacity is reduced in hot environments (Ely *et al.* 2010; Kenefick *et al.* 2010a; Sawka *et al.* 2011; Sawka *et al.* 2012a; Nybo *et al.* 2014). High skin blood temperatures narrow the core-to-skin temperature gradient and thus the maintenance of convective heat loss requires a larger increase in cutaneous blood flow (Sawka *et al.* 2012a). Ely and colleagues found that a high skin temperature is associated with degraded aerobic performance during a 15 min time trial, with only a modest increase in core temperature (Ely *et al.* 2010). This interpretation is in contrast to previous findings of a minimal effect of transient elevations in skin temperature on $\dot{V}O_{2max}$ (Arngrimsson *et al.* 2004), which is an important indicator of aerobic performance. Nevertheless, in the self-paced paradigm, increasing skin temperature induces behavioural thermoregulatory changes (pacing, reducing cadence, gear changes, etc.) that might contribute to the performance decrement with skin hyperthermia. No study to date has explored the haemodynamic

alterations underpinning maximal incremental exercise, with different extents of heat stress exposure.

2.4.3 Mechanisms by which heat stress impairs physiological function during maximal incremental exercise

Despite the observations that maximal aerobic power is reduced in the heat in the majority of the studies, there is limited understanding of the primary mechanisms explaining such a decline. It has been theorised that the inability to achieve a $\dot{V}O_{2max}$ equivalent to cool ambient conditions is a consequence of reductions in peripheral blood flow due to an attenuated cardiac output at high exercise intensities, secondary to a reduced stroke volume (Figure 2-8; (Rowell.1993). Stroke volume and cardiac output, however, are not reduced in trained individuals, exercising at maximal intensities in the heat (González-Alonso & Calbet 2003). This latter finding argues against the premise that high skin blood flow requirements and a decline in central blood volume, as postulated by Rowell, are the primary factor reducing maximal aerobic power in the heat. Instead, subsequent evidence suggests that heat stress and dehydration perpetuate reductions in cardiac filling and end-diastolic volume, and the increasing heart rate further restricts end-diastolic filling through a shortened cardiac cycle (Nadel 1980; González-Alonso & Calbet 2003; Stöhr *et al.* 2011b; Trinity *et al.* 2012). Collectively, it appears that heat stress advances the attainment of the cardiovascular regulatory limit which may explain the attenuated exercise capacity in the maximally active human (González-Alonso *et al.* 2008).

Attenuated systemic blood flow in the heat may reflect an inadequate brain and active muscle perfusion and thus oxygen and substrate supply. Whilst no studies have directly manipulated temperature to investigate brain and active limb haemodynamics during graded exercise, evidence from constant-load maximal exercise supports the premise that systemic, limb blood flow (González-Alonso & Calbet 2003) and cerebral blood flow velocity (González-Alonso *et al.* 2004) decline at a faster rate in the heat stress conditions. It remains unknown whether the decline in $\dot{V}O_{2max}$ is attributed to a similar attenuation in blood flow during graded exercise to volitional exhaustion.

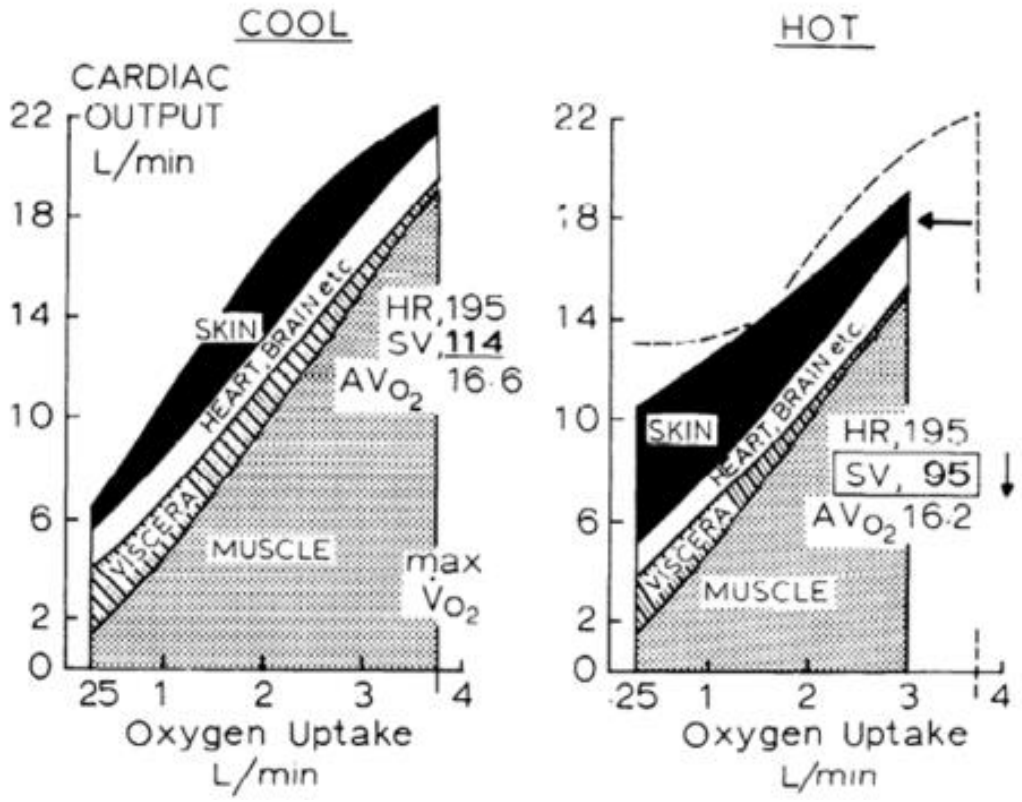


Figure 2-8. Distribution of cardiac output in untrained individuals during graded exercise in cool (25.6 °C) and hot (43.3 °C) conditions. Note that these regional blood flow data are theoretical estimations from untrained participants. $\dot{V}O_{2max}$ is proposed to be reduced concomitantly with a lower peripheral blood flow. From Rowell, 1974.

Passive heating augments limb blood flow, due predominantly to enhanced cutaneous thermoregulatory demand (Roddie *et al.* 1956; Rowell *et al.* 1969a). There is evidence that muscle vasodilation also contributes to the elevations in limb blood flow (Heinonen *et al.* 2011; Pearson *et al.* 2011). The superimposition of heat stress during prolonged exercise appears not to alter limb blood flow during isolated limb (Savard *et al.* 1988) and dynamic exercise (Nielsen *et al.* 1990). During constant intensity maximal exercise, however, limb blood flow declines at a faster rate in the heat, and is an important factor in the compromised exercise duration when limb oxygen extraction can no longer increase (González-Alonso & Calbet 2003). Surprisingly, however, there remains a paucity of data on the cerebral, systemic and active muscle haemodynamic responses to graded exercise to volitional exhaustion under heat-stressed conditions.

2.5 Circulatory adjustments to incremental aerobic exercise

Dynamic whole-body exercise poses a marked challenge to physiological regulation and requires a vast integrative circulatory response to meet the metabolic requirements of the active musculature (Krogh & Lindhard 1913; Rowell.1993; Wagner 1996; Hughson & Tschakovsky 1999). This regulation can be compromised in conditions of a high exogenous heat stress; however, the precise circulatory adjustments are not well characterised or understood. The following section outlines the known circulatory adjustments to exercise and proposed limitations to maximal oxygen uptake; with possible implications for exercise heat stress.

2.5.1 Circulatory adjustments to exercise

At the onset of graded exercise neural control of the cardiovascular system ('central command' and peripheral reflexes) acts to meet the metabolic demands of the active skeletal musculature (Rowell 1992; Raven *et al.* 2006; Boushel 2010; Raven 2012; Fadel 2013). Central command and reflex feedback are responsible for the rapid elevations in minute ventilation ($\dot{V}_E$), of importance for the maintenance of resting blood gas tensions, and for maintaining the pressure gradient for O_2 to support oxygen transport from the lung to the pulmonary circulation (Romer & Polkey 2008; Forster *et al.* 2012).

At the central circulatory level vagal withdrawal (up to ~100 beats·min^{-1}) and sympathetically-mediated activation of β_1-adrenergic receptors (Rowell.1993) act to increase heart rate in a linear fashion until exhaustion. Increased ventricular filling pressure raises stroke volume up to submaximal exercise intensities (Hill & Lupton 1923; Astrand *et al.* 1964; Higginbotham *et al.* 1986; Rowell.1993) and, together with the rising heart rate, serve to increase cardiac output at a rate of ~6 l·min^{-1} for every 1 l·min^{-1} increase in oxygen uptake (Andersen & Saltin 1985; Rowell *et al.* 1986; Delp & Laughlin 1998; González-Alonso *et al.* 2002; Mortensen *et al.* 2005; Mortensen *et al.* 2008). Systemic and limb vascular conductance increase to permit the large increase in the required blood flow and oxygen delivery (Hughson & Tschakovsky 1999; Mortensen *et al.* 2008), with the concomitant elevations in blood pressure proposed to be mediated by baroreflex 'resetting' to a heightened level in line with the exercise hyperaemia (Raven *et al.*

2006). A widening of the systemic arterio-venous oxygen difference accessing the resting 'oxygen reserve', coupled with enhanced central output, permits the rise in systemic oxygen uptake ($\dot{V}O_2$), reflecting an increased aerobic metabolism (Hill & Lupton 1923; Rowell *et al.* 1969b; Rowell *et al.* 1969; Rowell 1974; Rowell 1993).

These acute circulatory alterations to exercise are elegantly described by the Fick equation where oxygen consumption ($\dot{V}O_2$) is the product of the cardiac output ($\dot{Q}$) and systemic oxygen extraction (a-vO_{2diff});

$$\dot{V}O_2 = \dot{Q} \text{ x a-v}O_{2diff}$$

Overall, these well-defined responses to the onset of exercise sufficiently support skeletal muscle $\dot{V}O_2$ during exercise, with and without heat stress and dehydration, with a small muscle mass, or during exercise up to sub-maximal intensities (Andersen & Saltin 1985; Richardson *et al.* 1993; Mortensen *et al.* 2005; Mortensen *et al.* 2008). Beyond sub-maximal intensities, this regulation is challenged by the functional limitations of the cardiovascular system; it may yet be that the circulatory challenge of superimposed heat stress is of significant consequence and explains the compromised incremental exercise capacity in the heat.

2.5.2 Circulatory determinants of maximal aerobic exercise

Given the vast integrative physiological response to exercise, identification of a single factor underpinning maximal aerobic power is a clear oversimplification of the myriad of regulatory networks underpinning whole systems regulation and has therefore been strongly debated (Richardson *et al.* 1993; Rowell.1993; Wagner 1996; Bassett & Howley 2000; Richardson *et al.* 2000; Calbet *et al.* 2004; Saltin & Calbet 2006). The primary role of the circulation in the exercising human is to supply oxygen to the active muscles at a rate congruent to local metabolic demand. Limitations to oxygen transport can occur throughout the oxygen transport cascade (Wagner 1996) and are broadly described as either convective or diffusive limitations (Figure 2-9).

Whilst traditionally viewed as being overbuilt for exercise (Dempsey 1986; Rowell.1993), mechanisms across the respiratory system have the potential to

limit to O_2 transport (Richardson *et al.* 1993; Wagner 1996; Wagner 2011). The primary role of the respiratory system is to elevate minute ventilation ($\dot{V}_E$), which increases with exercise intensity (Casaburi *et al.* 1989), in order to elevate alveolar ventilation at a rate concurrent to metabolic demand. In some elite athletes, however, alveolar ventilation may limit $\dot{V}O_{2max}$ when a marked arterial hypoxemia develops, characterised by a decline in alveolar PO_2 congruent to a lack of a fall in PCO_2; thereby endangering the O_2 diffusive gradient (Step 1; Figure 2-9) (Dempsey & Wagner 1999). This is exemplified when reducing the inspired O_2 (FiO_2), and PO_2 with hypoxia (Amann & Calbet 2008; Lundby *et al.* 2008; Vogiatzis *et al.* 2008; Calbet *et al.* 2009). The capacity of the muscle mitochondria to extract and utilise O_2 would also be affected by the reduced PO_2 gradient; however, mitochondrial respiratory rate is not thought to be an important factor (Andersen & Saltin 1985) as it has been shown to exceed pulmonary $\dot{V}O_2$ at maximal work rates (Boushel *et al.* 2011).

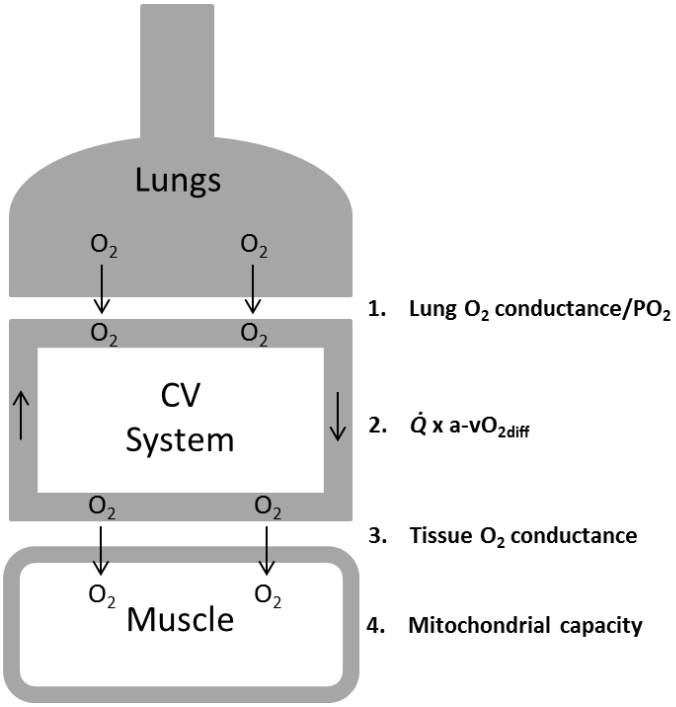


Figure 2-9. Simplified depiction of the functional limitations to oxygen transport. The transport of oxygen from the air to the muscle mitochondrion can be limited at the level of the lung (1), central circulation (2), capillary diffusion (3) and by mitochondrial capacity (4). Adapted from (Rowell.1993; Richardson *et al.* 2000).

The observation that neither alveolar-to-arterial and capillary-to-mitochondria O_2 diffusion are factors normally contributing to maximal oxygen uptake in humans at

sea level (Rowell.1993; Saltin & Calbet 2006; Boushel *et al.* 2011), lead the following review to focus on the components of the Fick principle as a major factor limiting circulatory function during maximal exercise.

Central circulatory limitations to $\dot{V}O_{2max}$

From a central circulatory standpoint (Step 2; Figure 2-9), the maximum oxygen uptake ($\dot{V}O_{2max}$) is bound by the maximally attainable heart rate, stroke volume and systemic arterial-venous O_2 difference (Rowell.1993);

$$\dot{V}O_{2max} = \dot{Q}_{max} \times a\text{-}vO_{2diff\ max}$$

Assuming that pulmonary and oxygen extraction capacity is upheld during maximal exercise, maximal cardiac output and reductions in active muscle perfusion are considered to be a primary factor restricting maximal aerobic power in normally active individuals at sea level (Amann & Calbet 2008; Calbet *et al.* 2009). This assertion is supported by the findings that the recruitment of greater muscle mass during strenuous exercise does not raise cardiac output further (Secher *et al.* 1977; Calbet *et al.* 2007), and active muscle blood flow can attain substantially greater levels during isolated limb exercise when the systemic circulatory strain is minimal (Andersen & Saltin 1985; Calbet *et al.* 2004; Mortensen *et al.* 2005; Mortensen *et al.* 2008). It has been shown that the linear increase in cardiac output, supporting whole-body metabolism during light exercise, is lost prior to volitional exhaustion during whole-body incremental cycling exercise in normothermic conditions (Mortensen *et al.* 2005; Mortensen *et al.* 2008; Stöhr *et al.* 2011a; Trinity *et al.* 2012).

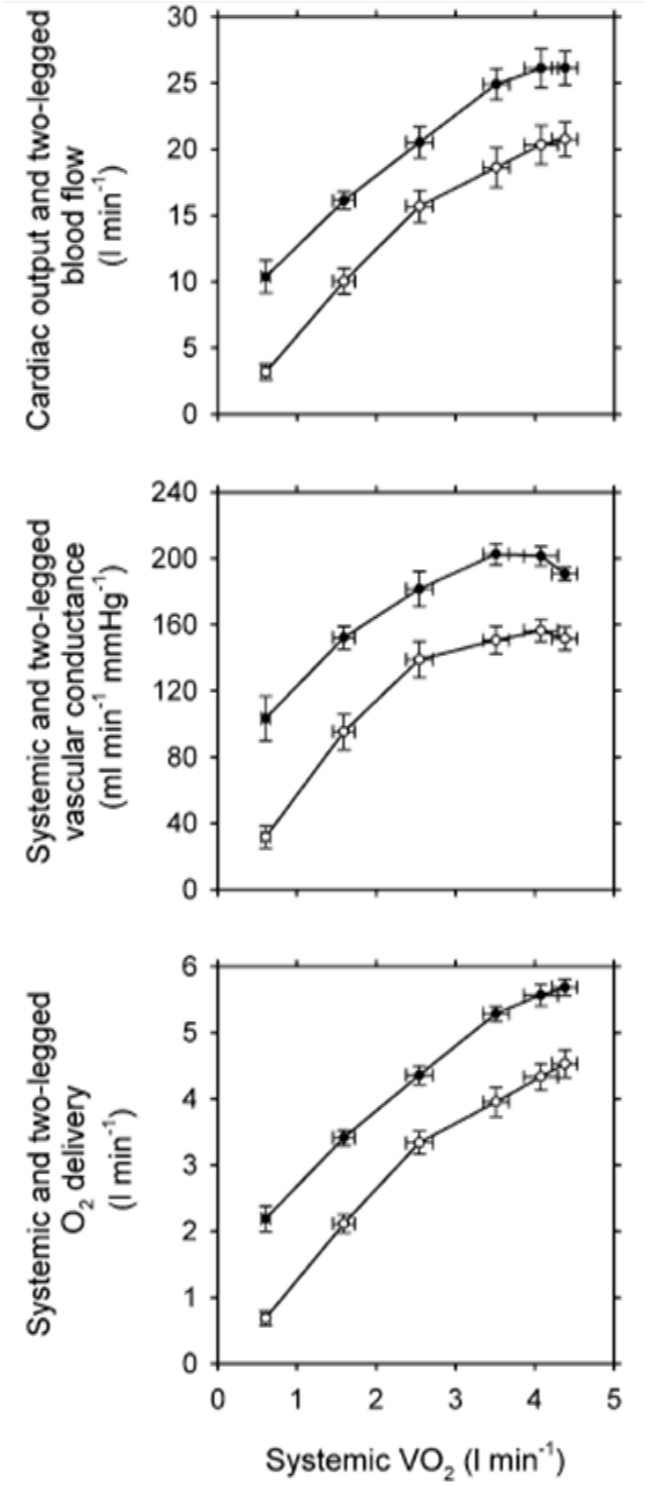


Figure 2-10. Systemic and limb blood flow, vascular conductances and O_2 delivery during incremental exercise. Note that the rate of rise in $\dot{Q}$ (filled circles) and LBF (open circles) is attenuated, concomitant to a declining systemic and limb vascular conductance. The rise in both systemic and leg O_2 delivery is attenuated above ~50% of maximal oxygen uptake. From (Mortensen *et al.* 2005).

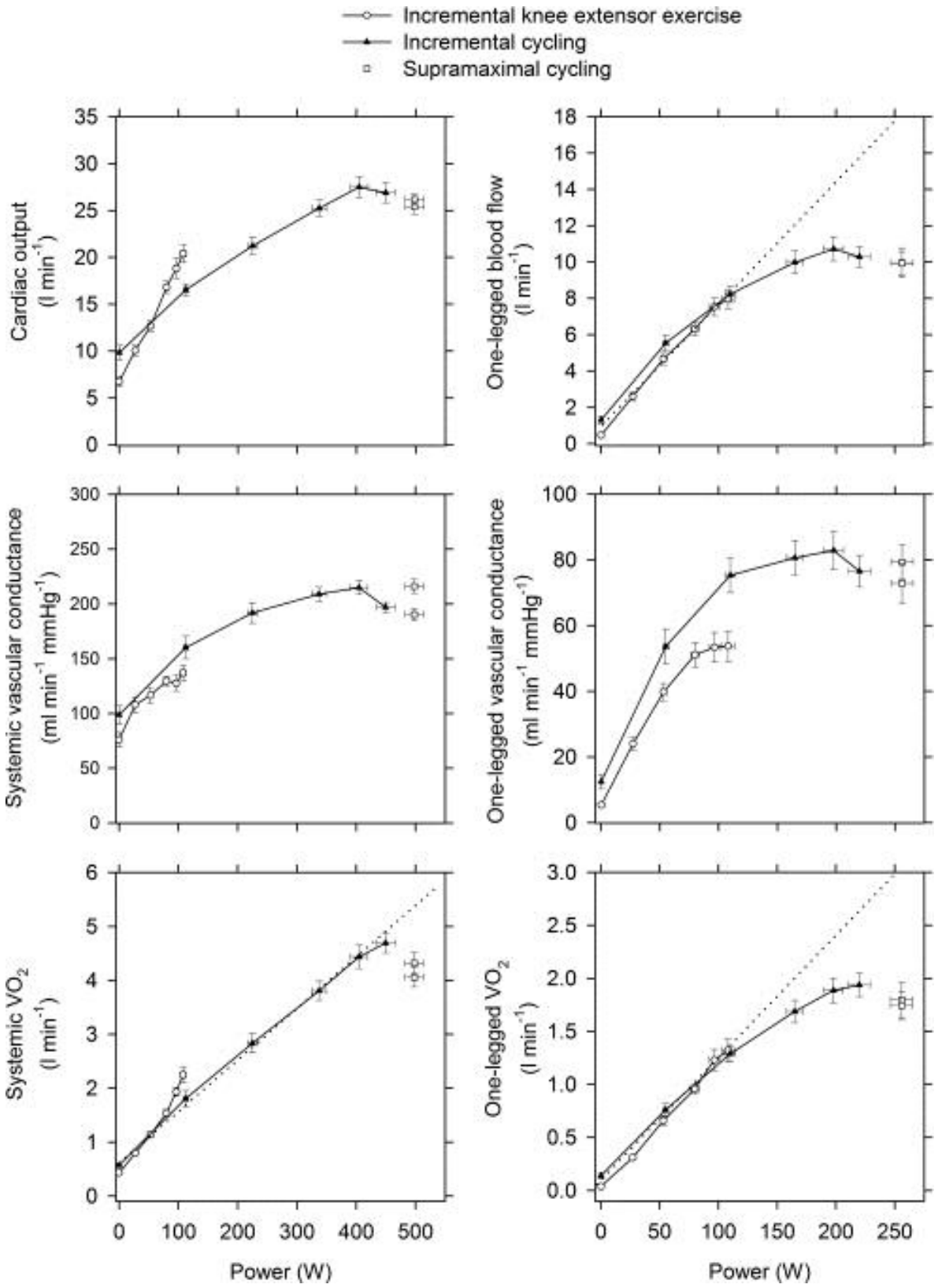


Figure 2-11. Systemic haemodynamics and metabolism (left panels) and the relationship between active muscle O_2 demand and supply (right panels). During small muscle mass exercise (open circles), cardiac output increases linearly with increasing work rate and one-legged oxygen uptake increases linearly with demand. However, when greater muscle mass is employed during dynamic whole-body exercise (filled triangles), limb blood flow is curtailed with an attenuated local vascular conductance, causing an attenuation in limb muscle oxygen uptake compared to the metabolic demand (or power output). From (Mortensen *et al.* 2008).

The circulatory challenge of performing dynamic whole body exercise is exemplified by the observation that systemic and active limb O_2 delivery is markedly attenuated at high intensities (Figure 2-10) (Mortensen *et al.* 2005). That is, the relationship between limb $\dot{V}O_2$ and exercise intensity is blunted (from 12 $ml \cdot W^{-1} \cdot min^{-1}$ to 9 $ml \cdot W^{-1} \cdot min^{-1}$ beyond ~50% WR_{max}), concomitant to a plateau in systemic and limb O_2 delivery. The disparate O_2 supply was confirmed in a follow up study that included constant maximal exercise at a substantially higher (≈110% WR_{max}) work rate (Figure 2-11) (Mortensen *et al.* 2008). An interesting observation in the same study was that limb vascular conductance was attenuated (above ~50% peak power) during both single-leg knee extensor and whole body exercise (Figure 2-11; middle right graph), but blood flow was still matched to demand with single limb exercise (Figure 2-11; lower right graph). Critically, despite augmenting metabolic demand, leg and systemic blood flow and O_2 uptake were not elevated above that observed during incremental exercise; indicating a circulatory limit to convective oxygen supply during strenuous exercise eliciting maximal aerobic power.

On further investigation of the factors underpinning $\dot{V}O_{2max}$, a commendable study utilised atrial heart pacing to investigate whether heart rate instilled a limit on raising cardiac output and systemic/limb oxygen supply during incremental exercise (Munch *et al.* 2014). It was shown that elevating heart rate above levels normally observed during incremental exercise did not influence systemic and limb perfusion (Figure 2-12), through concomitant reductions in stroke volume. Taken together, mechanisms restricting stroke volume appear to be a major determinant of maximal aerobic power by compromising convective O_2 transport (Higginbotham *et al.* 1986; Rowell, 1993; Calbet *et al.* 2007; Stöhr *et al.* 2011b; Bada *et al.* 2012; Munch *et al.* 2013). Notwithstanding, O_2 demand and the vast increase in peripheral (active limb) blood flow, are considered to be highly influential in the decline in venous return and stroke volume (Mortensen *et al.* 2007; González-Alonso *et al.* 2008; Bada *et al.* 2012; Munch *et al.* 2014). There is currently no information on the circulatory adjustments to incremental exercise to volitional exhaustion under heat stress conditions.

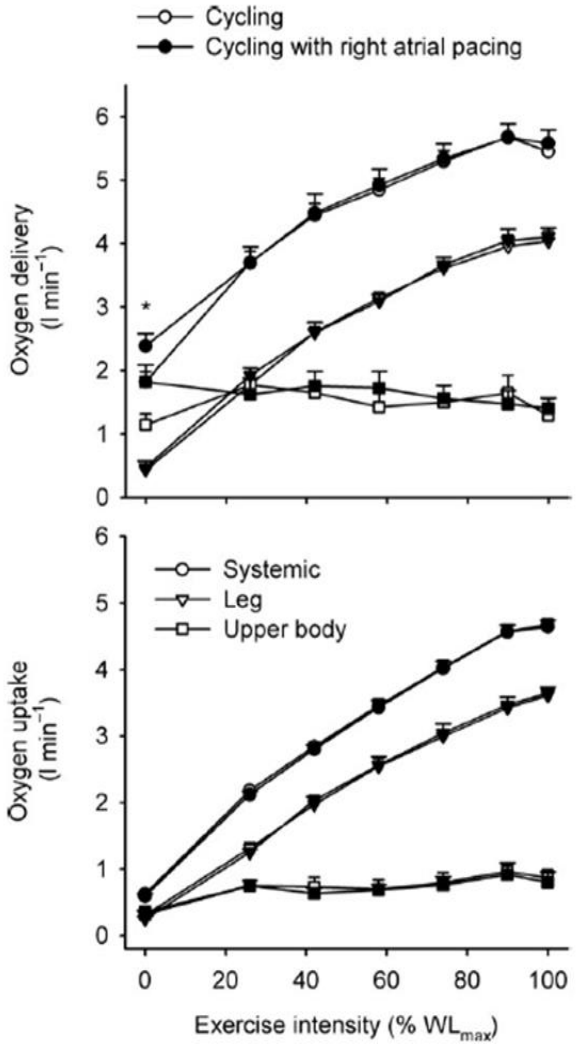


Figure 2-12. Systemic, leg and upper body O_2 delivery and O_2 uptake during incremental exercise, with and without heart pacing. Note that haemodynamics and metabolism were unaffected by increasing heart rate by 20 beats·min^{-1} with atrial pacing. From (Munch *et al.* 2014).

Summary

There is still much debate on the primary factor/s underpinning maximal aerobic power. Restrictions at each level of the oxygen transport chain could play a role in limiting $\dot{V}O_{2max}$. It is, however, evident that a compromised limb blood flow prior to exhaustion, attenuates active muscle $\dot{V}O_2$ and thus appears to be a major factor in the chain of events leading to fatigue during maximal incremental exercise. Because heat stress and dehydration further challenge circulatory function, specifically cardiac output, investigating the haemodynamic and metabolic alterations to incremental exercise in the heat will provide further understanding of the processes limiting maximal aerobic power.

2.6 Overall summary

Exercise induced dehydration and exercise heat stress can both lead to core hyperthermia that can impair physiological function and reduce exercise capacity. Dehydration and concomitant hyperthermia accelerates the decline in active limb blood flow during prolonged exercise in the heat. It could be that reductions in cerebral blood flow, and a compromised cerebral metabolism, contribute to the reduced exercise capacity and early fatigue in the heat; however, no study to date has investigated the circulatory responses across the head during prolonged exercise with heat stress and dehydration. An attenuated active muscle blood flow, secondary to a reduced stroke volume, appears to underpin the decline in maximal aerobic power. However, the precise circulatory and metabolic alterations remain to be fully elucidated. Investigating the cerebral and active muscle circulatory adjustments to strenuous exercise in the heat, with and without dehydration, will provide new knowledge and understanding of the mechanisms restricting exercise capacity under environmentally stressful conditions.

2.7 Thesis aims and hypotheses

No study has systematically examined the impact of dehydration-induced hyperthermia and heat stress on brain, muscle and systemic haemodynamic and metabolic dynamics during strenuous incremental and prolonged exercise. The subsequent chapters address the primary aims of the thesis that are outlined below;

Chapter 4 aims;

1. To determine whether dehydration accelerates the attenuation in cerebral blood flow normally occurring during graded incremental exercise to exhaustion and whether subsequent rehydration reverses these effects.
2. To identify whether cerebral metabolism is compromised prior to volitional exhaustion during graded incremental exercise.

Chapter 4 research hypotheses;

1. Dehydration will reduce maximal aerobic power in the heat concomitant to early reductions in cerebral blood flow during graded incremental exercise to exhaustion.
2. The cerebral metabolism will be maintained through compensatory increases in substrate metabolism.

Chapter 5 aims;

1. To determine whether dehydration reduces brain and extra-cranial blood flow during prolonged submaximal exercise in the heat and whether rehydration reverses these responses.
2. To determine whether alterations in regional haemodynamics are associated with impaired cerebral metabolism.

Chapter 5 research hypotheses;

1. Dehydration will accentuate the increase in internal temperature and lead to early exhaustion with concomitant reductions in cerebral and extra-cranial blood flow.
2. Maintaining hydration status will blunt the rise in internal temperature, prolong submaximal exercise capacity and attenuate or prevent the decline in regional blood flow across the head.

Chapter 6 aims;

1. To determine whether a combined elevation in internal and skin hyperthermia with moderate heat stress is associated with compromised maximal aerobic capacity and attenuated brain, muscle and systemic blood flow during maximal incremental exercise.
3. To determine whether high skin hyperthermia alone or conversely combined skin and internal hyperthermia, impair brain, muscle and systemic haemodynamics during maximal incremental exercise in trained humans.

Chapter 6 research hypotheses;

1. Combined elevations in internal and skin hyperthermia with moderate heat stress exposure will reduce maximal aerobic capacity with an associated attenuation of brain, muscle and systemic blood flow.
2. Elevations in skin hyperthermia alone will not be a sufficient stimulus to alter circulatory dynamics or compromise maximal aerobic capacity during maximal incremental exercise.

CHAPTER 3 –GENERAL METHODOLOGY

3.1 Introduction

The following chapter describes the general methodology utilised in studies 1 (Chapters 4 and 5) and 2 (Chapter 6), respectively. The pre-experimental methods are first outlined, followed by detailed descriptions of the methods used. Finally the general statistical analysis procedures are outlined. Specific methods and protocols used in the individual studies are presented in Chapters 4 to 6.

3.2 Pre-experimental methods

3.2.1 Ethics

For the studies presented in this thesis, ethical approval was obtained from the School of Sport and Education Research Ethics Committee and the Brunel University Research Ethics Committee (Appendix I - Ethical approval). All research procedures adhered to the ethical principles for medical research using human participants, in accordance with the guidelines presented by the World Medical Association (Declaration of Helsinki).

3.2.2 Participant recruitment

The participants who enrolled in each of the studies chose to do so of their own free will. Recruitment posters, blog and website postings and direct contact with cycling and triathlon clubs were the principal means for recruitment. After initial contact was made, descriptive information sheets (Appendix I - Ethical approval) were provided to the participants. All participants who took part were non-smokers and free from cardio-respiratory, metabolic and neurological disease as established by the completion of a pre-participation health questionnaire consent form (Appendix III – Health Questionnaire). After a reflective period and an opportunity to ask questions related to the studies, participants provided their written consent (Appendix IV - Consent form) to participate. Owing to the invasive nature and time commitments of the studies, appropriate remuneration was provided.

3.2.3 Anthropometry

In all studies, participant's height and mass were assessed using a combined stadiometer and scales (Seca 798, Gmbh & Co, Germany). The scales were regularly calibrated in accordance with manufacturer recommendations.

Descriptive measurements are located within the methods section of each study and are presented in metric units (kg, cm, m, etc.).

3.2.4 Assessment of maximal work rate

Chapters 4 & 5 – for the assessment of each individual's maximal work rate (WR_{max}), participants performed an incremental step test to the limit of tolerance in a semi-recumbent cycling position (Figure 3-1). Tests were conducted one week prior to the first familiarisation session and were performed on an electronically braked cycle ergometer (Lode Angio, Groningen, Netherlands) with a backrest inclination of ~45 °. The test began with an initial work rate of 20 W for 3 min followed by step increments of 60 W/3 min until the limit of tolerance was reached.

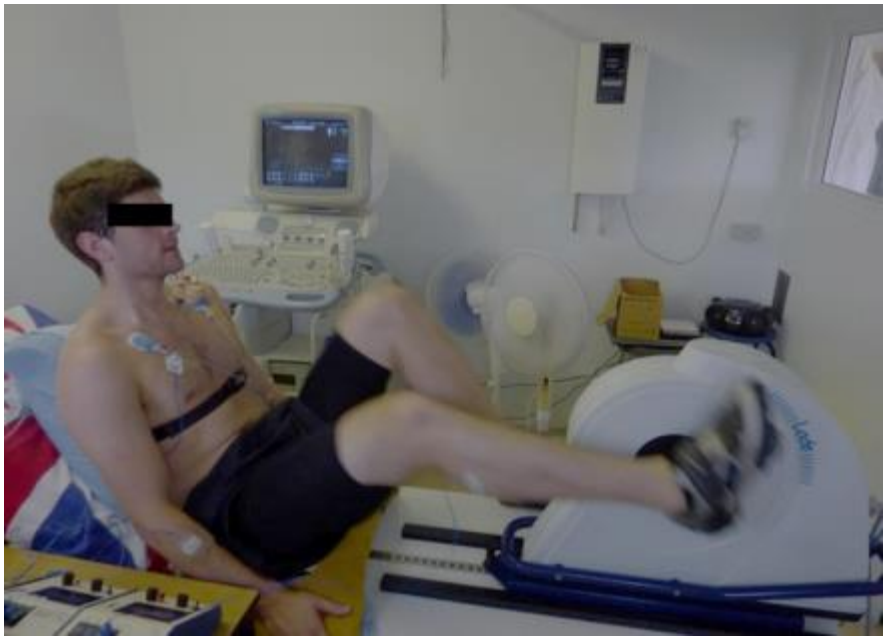


Figure 3-1. Semi-recumbent cycling position employed in chapters 4 & 5.

Chapter 6 – for the assessment of WR_{max}, participants performed an incremental step test to the limit of tolerance on an electronically braked cycle ergometer in the upright position (Lode Excalibur, Groningen, Netherlands). The test began with an initial work rate at 50% of individual's predicted maximal aerobic power (estimated using Hansen's rule), for 2.5 min, followed by step increments of 10% predicted/2.5 min until the limit of tolerance was reached.

In both studies, WR_{max} was established by the addition of the power output at the final fully completed stage, to the fraction of work performed in any subsequent partially completed stage. Participants maintained a constant cadence throughout the incremental test within a range of between 70-90 r.p.m. and the test was terminated when cadence declined to <60 r.p.m. for more than 3 s, despite strong verbal encouragement to continue.

Figure 3-2. Experimental arrangement for Chapter 6.

3.3 Testing methodology

3.3.1 Fundamentals of ultrasound and B-mode imaging

The use of ultrasonography of the carotid arteries as a means of investigating atherosclerotic plaques and stenosis is a common procedure in the clinical setting (Grant *et al.* 2003). It is a relatively cost-effective method of investigating medical conditions non-invasively and, in recent years, its application to physiological research has expanded and been validated at rest and during a variety of exercise conditions, for cervical arteries (Schoning *et al.* 1994; Hellstrom *et al.* 1996; Schoning & Scheel 1996; Sato & Sadamoto 2010; Sato *et al.* 2011) and peripheral arteries (Rådegran 1997; Shoemaker *et al.* 1997). The following sections describe the fundamental principles of both ultrasound imaging and Doppler ultrasound,

each with a description of how these techniques were used for the measurement of blood flow.

Basics of ultrasound

Ultrasonography uses high frequency sound waves to measure the displacement of particles through a given medium, which in this application refers to the displacement of sound through muscle, blood and blood vessels (Thrush & Hartshorne 2010). Sound is defined by its frequency (*f*) which is the number of displacement cycles (sampling rate) passing through a point in a given media in 1 s. It is also described by its wavelength (λ) which is the distance between two consecutive points of a waveform, of identical magnitude and direction. The time taken for the sound wave to move through a given medium by one wavelength is known as the period (Ƭ). The amplitude of a sound wave is the observed maximal positive deflection point and velocity is a measure of the rate and change of an object's position.

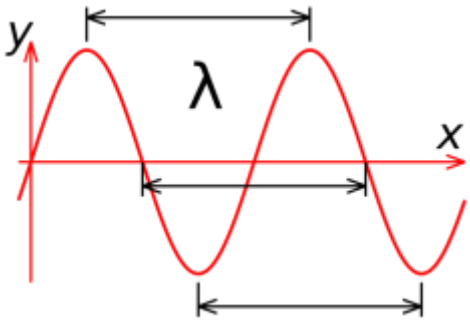


Figure 3-3. Graphical representation of the physics of wavelength and frequency. In reference to ultrasound, the displacement of particles (y) and the depth of a given media (x) and the wavelength (λ).

The speed of the emitted sound waves is required to distinguish the depth of different media located in the scan area (e.g. bone, muscle, blood, etc.). The speed of a sound wave (*c*; $m \cdot s^{-1}$) is a product of its wavelength and frequency; $c = \lambda f$. Most ultrasound systems consider *c* to be an average of all soft tissues (i.e. ~1540 $m \cdot s^{-1}$), that is despite the observable differences in the speed of sound through the different tissues (Table 3-1. Speed of sound in media within the body).

Table 3-1. Speed of sound in media within the body.

Media	Speed of sound
Air	330
Water	1480
Fat	1450
Blood	1570
Muscle	1580
Bone	3500

The speed of sound is dependent on the density of the tissues it travels through. Adapted from Thrush & Hartshorne 2010).

In the present thesis, a linear array transducer (10L; GE Healthcare, Horton, Norway) was used to generate ultrasound waveforms. The transducer converts electrical energy into ultrasound via the mechanical vibration of 128 piezoelectric crystals when a given voltage is passed through them. The converted energy, emitted as sound waves, travels through the tissues of the body towards the region of interest (artery) and, depending on the characteristics of the medium, are reflected, scattered or absorbed. Reflected sound waves are detected by the transducer and processed. The label provided to the transducer (10L) refers to the output frequency (i.e. 10 MHz). The choice of operating frequency depends on the depth of the area of interest. Higher frequency probes provide a high-resolution image at shallow depths, whereas low frequency probes have greater penetration but a compromised image resolution, with most medical ultrasound scanners operating between 2 and 20 MHz (Thrush & Hartshorne 2010). To image a region of interest, the ultrasound waves are released in pulses. This is important as it allows time for the reflected sound to return back for processing within the system. Furthermore, the use of pulsed ultrasound provides a clear measure of the depth of the target/region of interest using the equation; $d = tc/2$ where the distance (d) is determined if the time (t) between the transmission and reception of a signal and the velocity (c) of the sound wave are known. The pulsed ultrasound signal is made up of several sine waves operating at different frequencies, with the final signal a summation of multiple waves having different periods and amplitudes.

For optimal 2-D imaging of the intended artery or vein, the transducer is maintained perpendicular to the vessel to ensure that a high proportion of the transmitted sound waves are reflected back to the transducer for processing. Any deviation from a perpendicular angle, such as an oblique angle in Figure 3-5, would result in either a lack of sound returning to the transducer (reflection proportional to the angle of incidence) or refraction (sound travelling along a different path to the emitted sound), depending on a difference in the speed of sound across a tissue boundary, where the ultrasound beam is bent beyond the boundary (Thrush & Hartshorne 2010). The imaging of arteries relies on specular reflection of the ultrasound emitted from the transducers. A physical boundary such as the vessel wall and lumen of an artery provide low acoustic impedance and generate strong echoes returning to the transducers, providing a clear image. The clarity of the image is also dependent on the potential barriers between the probe and the region of interest. The extent of this barrier is termed acoustic impedance, and can be affected by skin (subcutaneous fat) and muscle thickness. Blood cells absorb less sound and specular reflection occurs in all direction due to their rough edges, resulting in fewer returning signals at the transducer and explaining the non-visible vessel lumen. All of the aforementioned considerations (specular and scattering reflection, refraction, and acoustic impedance) can reduce the intensity of the returning signals (attenuation) (Thrush & Hartshorne 2010).

The ultrasound transducer operates in a multi-array function which allows for the formation of many scan lines; that is, the 128 piezoelectric crystals can be grouped to produce separate outputs. This arrangement results in the easy analysis of multiple vessels of interest for example at the bifurcation of the carotid artery where the different branches appear clear, despite differing positions and depths. Given the aforementioned attenuation of ultrasound through a variety of media, two possible methods help to improve the amplitude of returning signals and thus improve image resolution. Firstly, output power of the transducer can be enhanced (i.e. apply a greater voltage). Prolonged exposure to high power outputs may lead to thermal (TI) or mechanical (MI) injury, both of which can cause structural damage of the capillaries and bleeding. In regards to output power, the general rule of “as low as reasonably achievable (ALARA)” was adhered to. An alternative method of enhancing the received signals is to amplify the returning waves. Often

this is useful for the identification of similar tissue boundaries at different depths, where the deeper structures pose a greater attenuation than shallow boundaries due to the longer time required for the transmission and reflection of the signal. Time gain compensation (TGC) dials can be used to amplify signals across the depth of the image to enhance signals from deeper structures. This can also be used to improve the contrast between tissue boundaries, such as artery vessel walls and the lumen (Thrush & Hartshorne 2010). Again, caution must be taken using this approach as enhancing the gain to high levels may cause the reflected signal to be indistinguishable from noise. Signal (and thus image) quality can also be enhanced through the focussing of the ultrasound beam to a specific region, or depth of interest. By varying/delaying the excitation of the aforementioned groups of crystals across the transducer, transmitted scan lines interfere to focus on a given position. This electrical delay is also utilised to steer beams originating from different groupings across the transducer, which subsequently overlap to enhance the image (compound imaging). The principles of beam forming and the ability to produce groups along the transducer are also crucial for the generation of an appropriate Doppler angle for velocity measurements (Thrush & Hartshorne 2010).

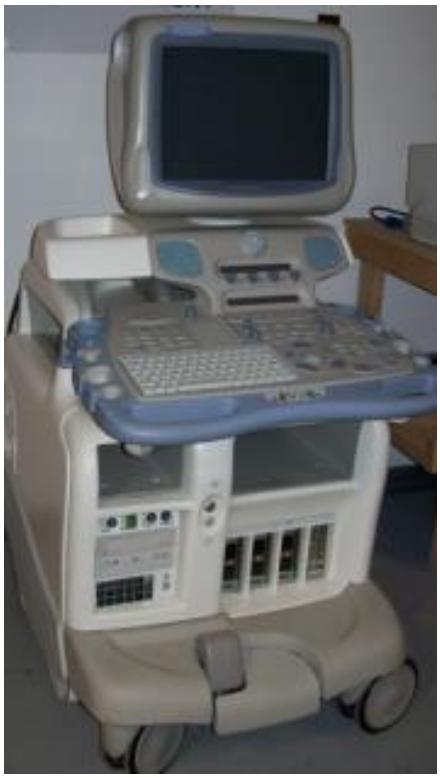


Figure 3-4. Vivid 7 ultrasound system used in the present thesis.

2D B-mode measurements of CCA, ICA and ECA diameter

Prior to the experimental day, initial measurements of the right common, internal and external carotid arteries for vessel diameter were made in brightness-mode (B-mode) imaging using a ultrasound unit (Figure 3-4; Vivid 7 Dimension, GE Healthcare, Horton, Norway) and a linear array transducer (10L, GE). Carotid artery imaging was made on the right lateral cervical region. An assumption is made forthwith that the right and left cervical arteries are homogenous, in spite of their differing anatomical origins, and this is substantiated by research indicating that there appears to be no side-to-side differences in vessel diameters (Krejza *et al*., 2006). An example b-mode image is presented in Figure 3-5 and with colour imaging to indicate blood flow (Figure 3-6).

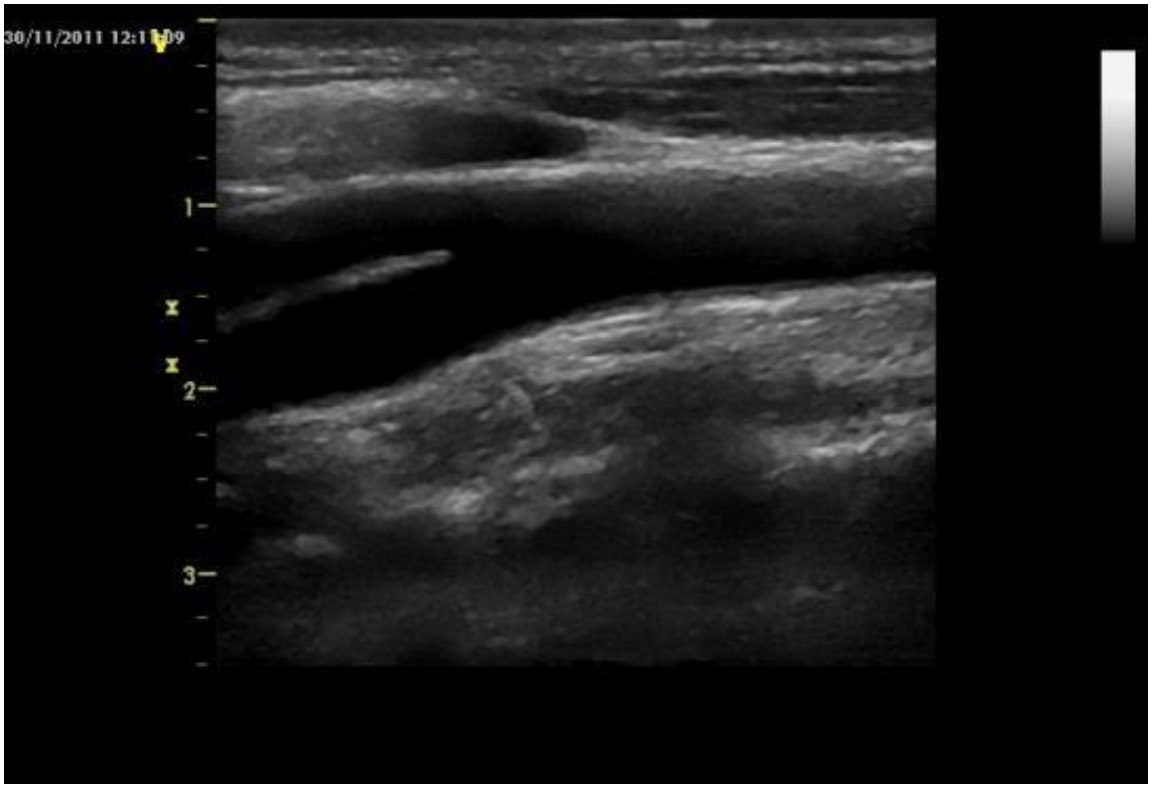


Figure 3-5. Example image of the carotid bifurcation. The common carotid artery splits into the internal (lower vessel) and external (upper vessel) carotid arteries. Note that the internal carotid artery is more often located superior to the external carotid.

In the majority of cases it was not possible to view the bifurcation as clear as in the presented image, and in the majority of participants measurements of vessel diameter were made in each vessel sequentially. For diameter measurements the vessel was maintained at a perpendicular angle to the transducer to ensure maximal reflection of sound waves for the production of clear and accurate images

of the vessel walls (clear visualisation of the *intimae, media and adventitia*). To ensure a reliable measurement of vessel diameters, an experienced sonographer used callipers to identify the leading edge of the near wall and the leading edge of the far wall (Hellstrom *et al*., 1996).

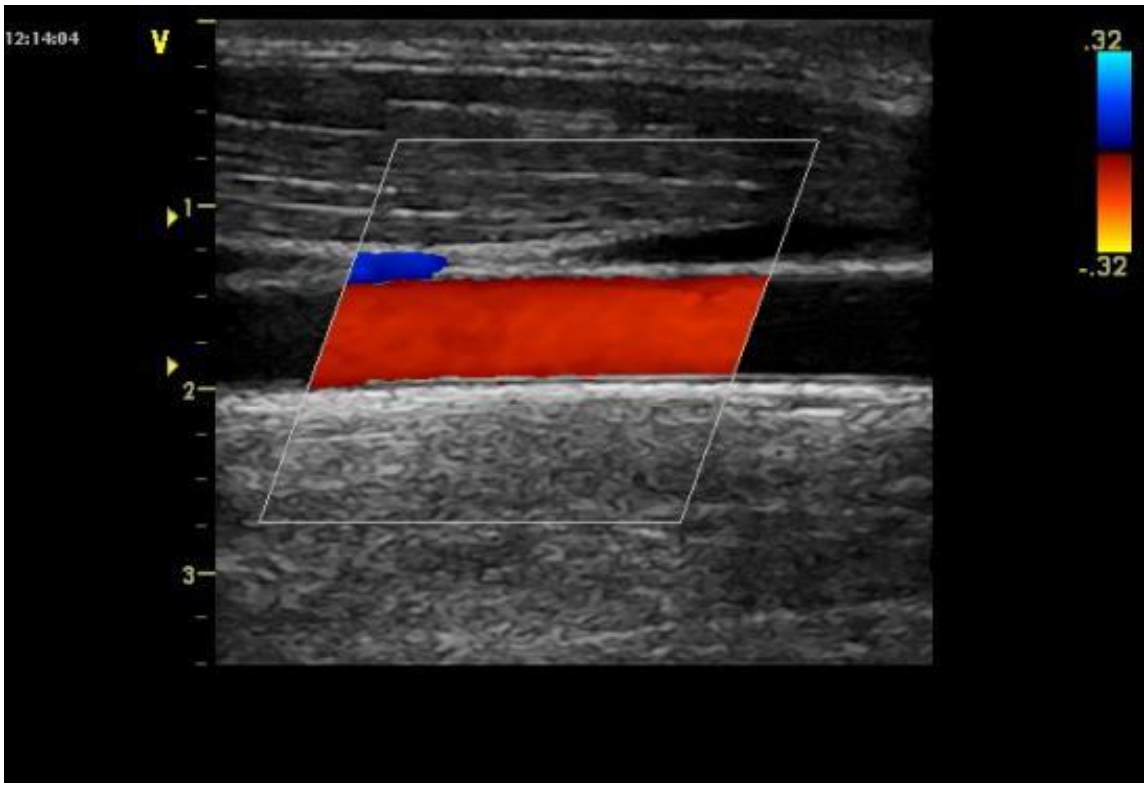


Figure 3-6. 2D image with colour overlay. Red colouring indicates blood arterial blood flow from right to left. Image also shows clearly defined vessel walls including the distal intima (white line at 2cm depth), media (thin dark band below intima) and adventitia.

Vessel diameter was calculated from the B-mode images taken at rest and during each exercise condition and were weighted more to diastole (2/3) than systole (1/3) as previously described (Hellstrom *et al.* 1996; Rådegran 1997; Sato *et al.* 2011).

$$CSA = \pi\ (mean\ diameter/2)^2$$

3.3.2 Fundamentals of Doppler ultrasonography

The Doppler Effect (Christian Doppler, 1842) is described as the change in the observed frequency of sound due to the relative motion of an observer, object or both. Common examples of the Doppler Effect in a real world setting are the observed change in pitch of a siren or the sound of a bee in flight as it is travels towards and beyond you. If the source of the sound and the observer remain

stationary, the observed sound has the same frequency as the transmitted sound. If the observer moves closer to the sound, the observer will cross the emitted waves faster than when stationary and thus a higher frequency is interpreted than is emitted. The opposite is true when the observer moves away from the sound source. If the source of sound moves towards a stationary observer, as often is with the siren example, the wavelength will shorten and the observer will hear a higher frequency. Again, the opposite case is true when the source moves away the wavelength becomes longer resulting in a lower observed frequency of sound. This change in frequency is defined as the Doppler shift and is proportional to the speed of the source and observer.

Basics of Doppler

In its application to vascular ultrasound, the Doppler Effect is used to assess the velocity of blood through a given vessel. The transducer first acts as the stationary sound source whereas the blood cells are the moving receivers of the sound. Upon reflection of the sound, the blood becomes the moving source of sound and the transducer is the stationary observer. The Doppler shift observed is dependent on the velocity of the moving blood and the frequency of the sound waves initially transmitted from the transducer. The accurate interpretation of the observed frequency of the moving blood is intimately related to the angle of the observer (i.e. the angle of the ultrasound beam/transducer). The Doppler shift frequency (f_d) is then calculated as; $f_d = 2vf_t \cos\theta/c$ where; v is blood velocity, f_t = transmitted frequency and cosθ is the cosine of the angle between the ultrasound beam and the direction of blood flow. The latter is known as the angle of insonation and, when used incorrectly, is a main source of error in blood velocity measurements (Figure 3-7). Preferably the cosθ would be 0° (i.e. the ultrasound beam is directly behind the flow of the blood) as this would provide the maximal detectable Doppler shift frequency. Conversely, if the cosθ were at 90°, no Doppler shift would be detectable. The Doppler shift is extracted from the returning signal, usually through a process of demodulation which involves calculation of the product of the transmitted signal by the received signal, which is then filtered to reveal the “true” Doppler signal (Thrush & Hartshorne, 2010).

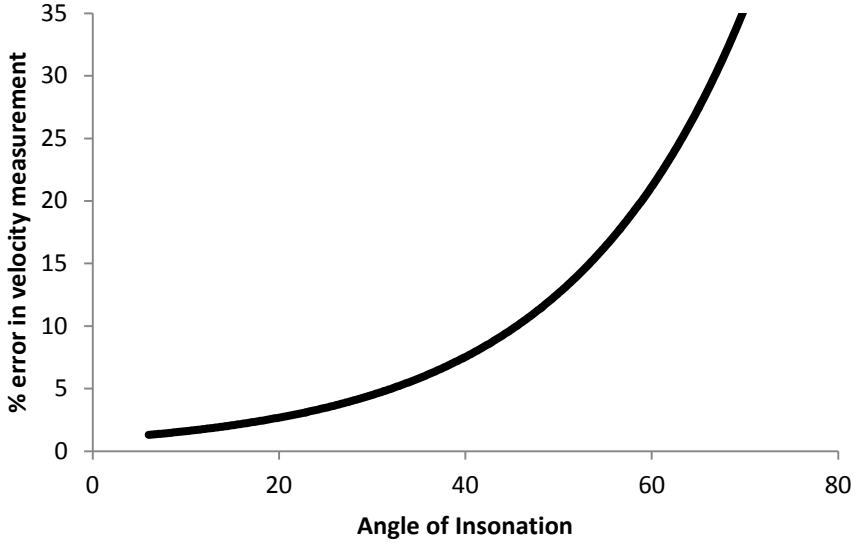


Figure 3-7. Graphical representation of the error associated with altering the angle of insonation.

Once the signal is produced, blood velocity can be extracted by spectral analysis. That is, the Doppler signal is broken down into its component frequencies (which will vary with the differing velocities of blood across the luminal space over time) and presented as a frequency spectra (Figure 3-8). The spectra can then be analysed and a velocity is then attributed to the obtained frequencies. Signals in the present study were obtained in pulse-wave (PW) mode which operates by; 1) emitting a pulse, 2) waiting for a given time period, 3) receiving the returning pulse and 4) waiting before emitting the next pulse. The length of time taken for this process to occur depends on the depth and sample volume of the region of interest. To measure the frequencies of the blood flow in a vessel, thousands of pulses at a given frequency are propagated along the beam path every second (Pulse repetition frequency, PRF). Given that the PW mode requires time to emit and receive the Doppler signal, there is an upper limit of the PRF as the system must wait until the emitted pulses return before a new pulse is deployed.

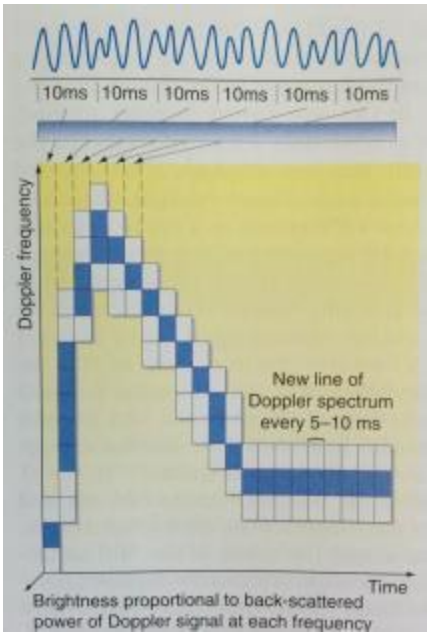


Figure 3-8. Representative distribution of Doppler derived frequencies over time. From (Thrush & Hartshorne 2010).

The constantly changing blood velocity and a good range resolution (information of the depth of the returning signals) make PW mode the most appropriate for blood flow measurements. However, there is an upper limit in PW mode that can lead to error in the measured frequencies (and thus blood flow velocity errors). This is the Nyquist limit which states that the PRF must be more than twice the maximum Doppler shift frequency (or blood velocity) to be measured. A PRF that is less than twice the Doppler shift frequency will cause aliasing, leading to an underestimation of the measured signal (Figure 3-9). It is possible to make small adjustments to the PRF to avoid aliasing, but there remains an upper limit. The physical depth of the region of interest is the most likely cause of aliasing in a signal when measurements are made in PW mode. For most measurements of blood flow velocity in human studies, appropriate use of the PRF and adjustments to the ultrasound system can often negate these problems.

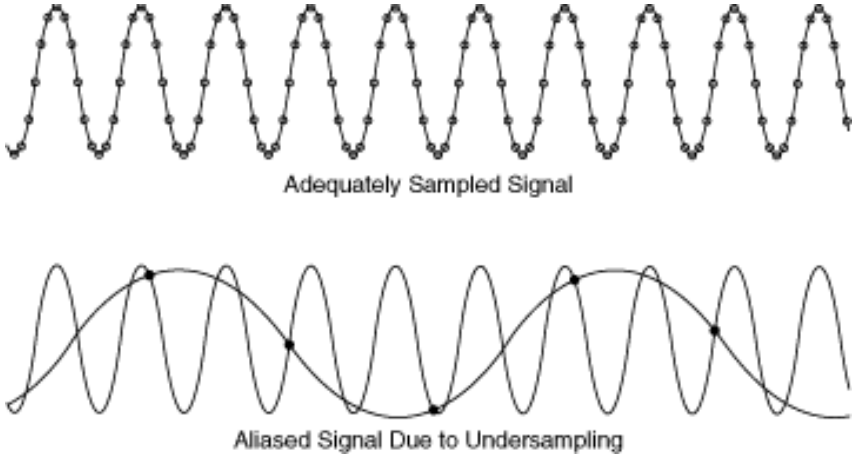


Figure 3-9. Impact of aliasing of derived Doppler signal. Note that use of a low PRF (lower panel) underestimates the Doppler shift.

Pulse-wave Doppler velocity measurements

PW (4.5 MHz) velocity measurements were made in duplex mode; allowing for both B-mode and PW mode images to be obtained simultaneously. This helps to ensure the correct placement of the sample volume, angle of insonation and vessel alignment through continuous visual inspection during blood flow velocity measurements. Blood velocity was calculated using the rearranged Doppler shift frequency equation;

$$V = f_d c / 2 f_t \cos\theta$$

Velocity waveforms from 10-20 cardiac cycles were obtained in pulse-wave (PW) mode for a measure of time averaged mean blood flow velocity (TAM V; $cm \cdot s^{-1}$). This average was taken to eliminate any irregular artefacts associated with dynamic changes in breathing pattern during intense exercise. Care was taken to ensure that the transducer was in a stable position on the skin and that the angle of insonation was as low as possible and always maintained below 60°. In all measurements it was ensured that the sample volume was placed in the centre of the vessel of interest, in line with the direction of blood flow, and adjusted to cover the entirety of the lumen. This was to account for the different velocities of the centre of the lumen compared to the velocity at the vessel walls.

Calculation of blood flow

Blood flow was calculated as the product of vessel cross sectional area, as described previously, and time averaged mean velocity (multiplied by 60 for conversion to $ml \cdot min^{-1}$), where;

$$\text{Blood Flow} = \text{TAM}V \times \text{CSA} \times 60$$

Measurements of both diameter and velocity were made ~1.0-2.0 cm proximal to the carotid bifurcation for the CCA and, ~1.0-1.5 cm distal to the carotid bifurcation in the ICA to avoid the turbulent flow apparent in this area (Sato *et al*., 2011).

Prior to the first study, a series of coefficient of variation experiments were performed to ensure the intra-observer variability of the researchers was in accordance with accepted values. Eight participants visited the laboratory on two occasions, the first for resting measurements and the second for exercise measurements. Three resting measurements of the CCA, ECA and the ICA were made non-consecutively on the same day. Average variation during exercise was calculated from measurements made at three exercise intensities. The Coefficient of Variation (CV) values for diameter and flow for each vessel are presented in Table 3-2.

3.3.3 Cerebral blood velocity

As a surrogate estimation of alterations in cerebral blood flow, mean blood velocity of the middle cerebral artery (MCA V_{mean}) was obtained continuously and non-invasively using a transcranial Doppler ultrasound system (DWL Doppler, Singen, Germany). Due to the impedance of the skull, the intra-cerebral arteries cannot be imaged effectively using a higher resolution system and thus 2D B-mode imaging of the vessel are not possible. Any changes in velocity through the insonated vessel were assumed to be reflective of changes in blood flow, as vessel diameter is thought to be constant in the majority of cases (Bishop *et al.* 1986; Serrador *et al.* 2000; Peebles *et al.* 2008), but less so in others (Wilson *et al.* 2011). This technique is validated and reliably used to quantify blood velocities in the cerebral arteries.

Table 3-2. Coefficient of variations for CCA, ICA and ECA.

Measurement	Condition	CV (%)
CCA Diameter	Rest	1.2 ± 0.4
	Exercise	0.6 ± 0.3
CCA Flow	Rest	4.3 ± 1.0
	Exercise	5.3 ± 1.6
ICA Diameter	Rest	1.4 ± 0.6
	Exercise	0.6 ± 0.4
ICA Flow	Rest	2.8 ± 0.9
	Exercise	5.0 ± 1.6
ECA Diameter	Rest	1.8 ± 1.0
	Exercise	4.0 ± 1.5
ECA Flow	Rest	2.1 ± 1.1
	Exercise	5.1 ± 1.4

Values are mean ± SEM.

A 2 MHz frequency transducer was used to assess blood velocity in the MCA, as high frequencies do not penetrate the skull bones effectively (Aaslid *et al.* 1982). In initial investigations of the participants, a hand-held transducer was used to identify the MCA. The transducer was placed on the transtemporal window (Figure 3-10; Aaslid *et al*., 1986b) and was adjusted with minute movements until a clear Doppler signal and best signal-to-noise ratio were achieved (Aaslid *et al.* 1982). With a transtemporal approach, the MCA is best viewed in the anterior plane, as the angle of insonation is close to zero, allowing absolute velocity values to be obtained (Willie *et al.* 2011).

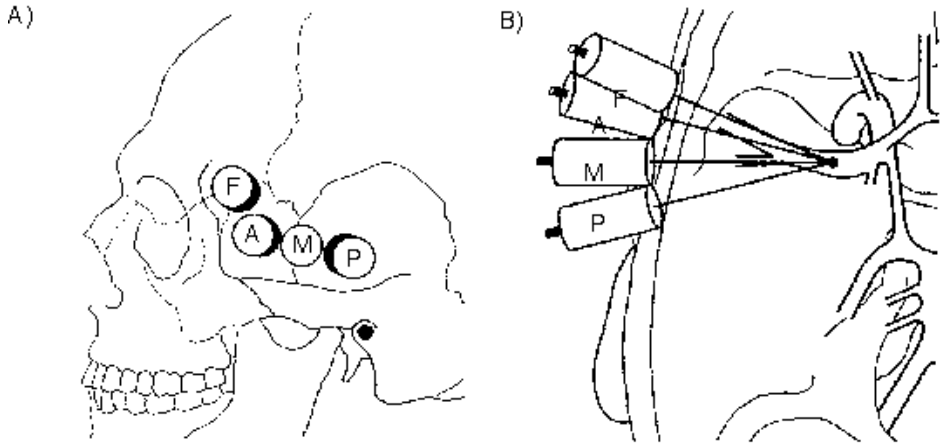


Figure 3-10. Positioning of TCD transducer for the adequate measurements of MCA V_{mean}. F = frontal, A = anterior, M = medial and P = Posterior. Adapted from; Fujioka & Douville, Anatomy and freehand examination. In Newell, D. W. and Aaslid, R: Trancranial Doppler, Raven Press, New York, 1992.

The depth setting was maintained between 45 and 60 mm, maximising the potential of identifying the MCA, with the MCA often observed at a depth of ~50 mm (Willie *et al.* 2011). A custom-made TCD headset (DiaMon, DWL, Compumedics, Singen, Germany) was then attached to the participants, housing a similar transducer, and was fixed in place for the entirety of the exercise conditions. To ensure reproducibility of the placement of the TCD probe and a similar angle of insonation, photographs of the participants were obtained in the initial visit and used to guide placement on subsequent visits. MCA V_{mean} was measured continuously using an appropriate software package (QL 2.6.1, Compumedics DWL, Singen, Germany).

3.3.4 Leg blood flow (Chapter 6)

Blood flow during incremental exercise was determined using the constant-infusion thermodilution method (Ganz *et al.* 1964; Andersen & Saltin 1985; González-Alonso *et al.* 1998; González-Alonso *et al.* 2000). Briefly, a quad lumen catheter, with three side ports to aid with saline/blood mixing during the infusions, was inserted anterograde into the right common femoral vein. Infusate and venous blood temperatures were measured continuously during the infusion of cold saline (~20 s, 120-160 ml·min^{-1}; Harvard pump, Harvard Apparatus, Millis, MA, USA). Blood temperature was measured using a thermister (T204a, PhysiTemp, Clifton, New Jersey, USA) inserted through the catheter, ~10 cm beyond the tip whereas, saline infusate temperature was measured using a flow-through housing unit

positioned at the entrance of the catheter. Infusate temperature was corrected across the range of infusion rates used (1.0 °C at rest at 120 ml·min^{-1} and 0.6 °C during maximal exercise at 160 ml·min^{-1}) accounting for the elevation in infusate temperature as it travels through the catheter. Leg blood flow was then calculated using the following heat balance equation;

$$LBF = V_I \times [S_I C_I / S_B C_B] \times [(T_B - T_I / T_B - T_M) - 1]$$

where, V_I is the rate of saline infusion (ml·min^{-1}), T_B is blood temperature prior to saline infusion, T_I is the temperature of the saline and T_M is the mixed blood and saline temperature at steady state. S_I and S_B (1.005 and 1.045 g·cm^{-3}) and, C_I and C_B (4.173 and 3.600 J·g^{-1}°·C^{-1}) are the specific gravities and specific heat of the blood and infusate, respectively (producing a constant of 1.115 for LBF calculations).

3.3.5 Tissue oxygenation

Cerebral oxygenation of the pre-frontal cortex was measured using near-infrared spectroscopy (NIRS, INVOS, Somanetics, Troy, MI, USA) (Madsen & Secher 1999; Rasmussen *et al.* 2007). A pair of NIRS optodes, containing an infrared light emitter and two receiving detectors, spaced 30 mm and 40 mm from the light source respectively, were placed on the skin of the forehead covering the frontal cortex. Near-infrared light (730-810 nm) is intermittently passed through the tissues and either absorbed, by oxy- and de-oxyhaemoglobin, or scattered back to the detector, whereby the total concentrations of oxyhaemoglobin can be determined. Care was taken to avoid placing the NIRS pads too close to the superior sagittal sinus which prevents the infrared light from entering the cranial tissue.

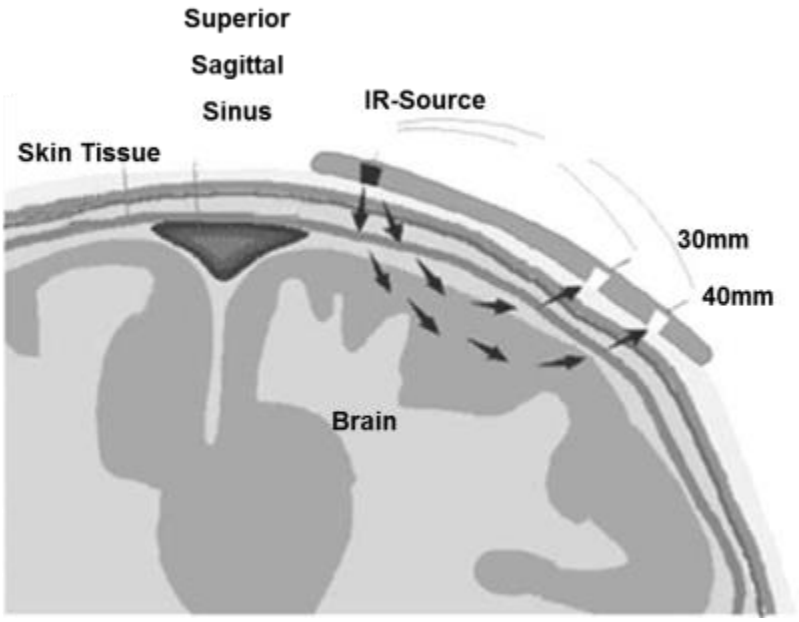


Figure 3-11. Graphic depicting the use of near-infrared spectroscopy to determine oxygenation of the frontal lobe. (Courtesy of Somanetics Inc.).

The optodes were securely fastened to the forehead using adhesive tape and were held in place using a modified headset, which also housed the TCD transducer. Care was also taken to ensure no external light was allowed to enter the optode which can interfere with the data collected.

3.3.6 Ventilatory and metabolic parameters

Pulmonary and gas exchange and ventilation were measured breath-by-breath at the level of the mouth using an online, portable metabolic cart (Quark b^2, Cosmed, Italy). With their nose occluded, participants breathed into a mouthpiece containing a digital turbine transducer for the measurement of inspired and expired volumes. O_2 and CO_2 concentrations of the sampled air were obtained from analyser cells within the metabolic cart. The analyser was calibrated prior to each exercise test using certified gas concentrations (CO_2 = 5%, O_2 = 15%; balanced with N_2). Breath-by-breath data were obtained from each test and pulmonary gas exchange indices included; minute ventilation ($\dot{V}_E$), tidal volume ($\dot{V}_T$), oxygen uptake ($\dot{V}O_2$) and carbon dioxide output ($\dot{V}CO_2$).

3.3.7 Catheterisation

Arterial and venous blood samples were withdrawn simultaneously from catheters inserted percutaneously under local anaesthesia. All catheter insertions were

performed by a medically trained anaesthetist, with many years of experience. All insertions were made under sterile conditions with appropriate dressings used to maintain catheter position and participant comfort throughout the protocols.

Arterial catheterisation

For arterial blood samples and pressure measurements in studies one and two respectively, a catheter (1.1 mm internal diameter, 20 gauge) was inserted percutaneously into the brachial artery of the non-dominant arm.

Venous catheterisation

Chapters 4 & 5 – for venous blood samples and pressure measurements a central venous catheter (16 gauge, 2.3 mm ID; Multi-Med M2716HE, Edwards Lifesciences, USA) was inserted retrograde, using the Seldinger technique, into the left internal jugular vein and subsequently advanced ~15 cm to its bulb at the base of the skull to ensure no contamination of blood from extra-cerebral sources (Jakobsen & Enevoldsen 1989). Participants lay supine in a slight Trendelenburg position to maintain an open and full jugular vein. Successful placement was indicated by a resting jugular venous pressure of <~10 mmHg, a steady flow of blood and easy flushing of saline through the catheter.

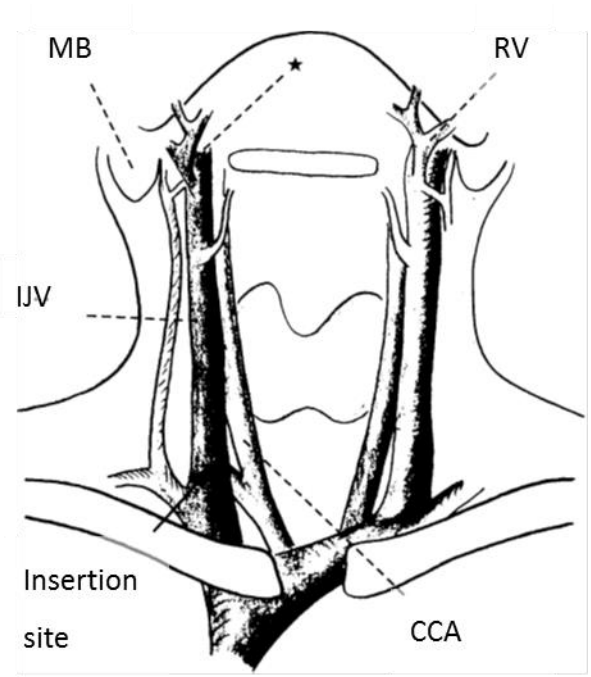


Figure 3-12. Depiction of IJV catheterisation. Catheter was inserted and subsequently advanced to the base of the skull (asterix). IJV = internal jugular vein, MB = mastoid bone, RV = retromandibular vein and CCA = common carotid. From (Jakobsen & Enevoldsen, 1989).

Chapter 6 – for venous blood samples, pressure measurements and saline infusion a central venous catheter (Logicath Quad lumen, 18 gauge, 2.3 mm ID; MXA234X16X85, Smiths Medical International LTD) was inserted anterograde, using the Seldinger technique, into the right common femoral vein. Catheterisations were made under local anaesthetic (1% lidocaine) and were guided by real-time ultrasound imaging to ensure correct placement as advised by the National Institute for Clinical Excellence (National Institute for Clinical Excellence 2002).

Arterial and jugular venous pressure waveforms were recorded using transducers (Pressure Monitoring Kit, TruWave, Edwards Lifesciences, Germany) zeroed at the level of the right atrium in the midaxillary line (arterial) and at the level of the tip of the catheter (jugular and femoral venous). Pressure waveforms were sampled at 1000 Hz, amplified (BP amp, ADInstruments, Oxfordshire, UK) and connected to a data acquisition unit (Powerlab 16/30, ADInstruments, Bella Vista, NSW, Australia) for offline analysis.

Non-invasive blood pressure

Arterial catheterisations were not possible in the control trials for studies 1 and 2. Blood pressure waveforms and subsequent derived variables (see model flow method below) were obtained from the finger using a non-invasive blood pressure system (Finometer® Pro, Finapress Medical Systems, The Netherlands). This system uses an inflatable finger cuff with inbuilt photo-electric plethysmography to detect finger pulse pressure waveforms. The shape of pulse waves and pressure levels obtained at the finger are inherently different to that of the brachial artery due to wave distortion and changes in pressure gradients. To ensure comparison to the invasive measurements, corrections were made to ensure a reliable and valid estimate of brachial artery pulse pressure and derived values. Firstly, the finger cuff was corrected for its distance below the heart using a height sensor that was nulled at the finger and subsequently positioned at the level of the heart. Following this, a return-to-flow (RTF) correction was used to correct the measured finger pulse pressures to reflect brachial artery pulse pressures. This process involved a protocol of stepwise arm cuff occlusion, to suprasystolic levels, followed by gradual deflation. The first pulsation measured at the finger during cuff deflation

is referred to as RTF; at which point a simultaneous measure of arm cuff pressure allows for the RTF correction, and thus a reconstructed brachial artery pressure pulse wave, to be applied. Waveform corrections resulted in derived systolic, mean and diastolic pressures falling in line with the Association for the Advancement of Medical Instrumentation (AAMI) recommendations.

Prior to calibration and the commencement of measurements, individuals age, height, weight and gender were input. After the height and RTF corrections, finger pulse-pressure measurements began after a manufacturer determined calibration period, where the finger cuff obtained pulse waves were zeroed repeatedly over 10, 20, and 30 s time windows, respectively. After this initial calibration, the system calibrated every 60 s, allowing for the collection of representative pulse waves over a prolonged duration, whilst maintaining proper functioning. Beat-to-beat reconstructed BAP waveforms, heart rate, stroke volume and cardiac output were recorded (Powerlab 16/30, ADinstruments, Bella Vista, NSW, Australia) and stored for offline analysis.

Model flow method

Estimates of stroke volume were obtained from direct blood pressure (catheter derived) and reconstructed brachial artery pressure waveforms (rBAP; Finometer), and waveforms were analysed using appropriate software (Beatscope 1.1a, FMS BV, Amsterdam, The Netherlands). A three-element model (Wessling *et al*., 1993) was used to compute aortic flow using principle haemodynamic properties of the arterial system: 1) non-linear pressure dependent aortic compliance (how effective the aorta and arterial system are able to store the elastic energy derived from the left ventricle upon contraction); 2) characteristic impedance of the aorta (extent to which the aorta impedes pulsatile flow); 3) time-dependent systemic vascular resistance (sum total of the resistance of all vascular beds). Using the aforementioned characteristics, the model flow method calculates aortic flow over time (thus estimating stroke volume). Cardiac output derived from the Modelflow calculation is comparable to other methods during exercise (Sugawara *et al.* 2003).

3.3.8 Heart rate

Heart rate was obtained by telemetry using a chest strap and recordable monitor (Team 2, Polar Electro, Kempele, Finland). Files were downloaded and analysed offline.

3.3.9 Blood parameters

Blood samples were withdrawn simultaneously from the arterial and venous catheters into pre-heparinised syringes (Pico 50, Radiometer, Copenhagen, Denmark). Samples were purged of atmospheric content and immediately analysed for a variety of haemodynamic variables (pH, Sa/vO_2, $a/v\ ctO_2$, PO_2, PCO_2, [Hb] and [La^-]). Analyses were made using a blood gas analyser (ABL 800 Flex, Radiometer, Copenhagen, Denmark).

Prior to sample analysis, the system was calibrated for accuracy. Firstly, four quality control AutoCheck (Radiometer, Copenhagen, Denmark) ampoules were run to assess the drift and range of the system. Following this, two calibration programs were systematically run to ensure reliability of results. A 1-point calibration was processed, whereby each parameter is assessed against a solution/gas of known composition. If the obtained drift (how much the known solution differs from that which is analysed) is within an acceptable range the calibration is passed. A second 2-point calibration, run more infrequently, is similar to the 1-point calibration, but instead uses two known solutions/gases to assess the reliability of the measured values. 1-point (every 30 min) and 2-point (every 4 h) were run regularly to maintain the system.

Additional samples were collected directly into stop solution containing S-(4-nitrobenzyl)-6-thioinosine (NBTI; 5 nM), 3-isobutyl-1-methylxanthine (IBMX; 100 µM), forskolin (10 µM), EDTA (4.15 mM), NaCl (118 mM), KCl (5 mM), and tricine buffer (40 mM), to prevent further metabolism of ATP within the sample, prior to being placed into plastic tubes and centrifuged for 3 min at 4000 *g* to separate the supernatant (Gorman *et al.* 2003). ATP levels in the supernatant were assessed using an ATP kit (BioThema AB, Dalarö, Sweden). Plasma ATP was determined in duplicate at room temperature (20-22 °C) by the luciferin-luciferase technique using a luminometer with three automatic injectors (Orion Microplate Luminometer,

Berthold Detection System, GmbH, Pforzheim, Germany). The following equation describes the bioluminescence analysis;

$$\text{ATP + D-luciferin + } O_2 \xrightarrow{\textit{luciferase}} \text{AMP + PPi + oxyluciferin + } CO_2 \text{ + light}$$

Luciferin reacts with ATP, which subsequently reacts with luciferase with the addition of O_2. This compound readily disassociates to release light and it is the extent to which light emitted is proportional to the ATP contained in the sample, when compared against a pre-determined calibration curve of standards contained in the ATP kit. Detection thresholds provided by the manufacturer are 10^{-12} mol·l^{-1} (minimum) and 10^{-6} mol·l^{-1} (maximum). Haemoglobin concentration was obtained to assess the degree of haemolysis in the sample which, if of a high level, can influence the final derived values.

Plasma catecholamines

Arterial and venous blood samples were collected in 2 ml syringes and transferred to EDTA tubes, centrifuged at 3000 r.p.m. and 4 °C for 10 min. After separation, plasma was extracted and immediately placed into Eppendorf tubes and stored in liquid nitrogen before transfer to a -80 °C freezer for storage and later analysis. Plasma adrenaline and noradrenaline was determined using an enzyme immunoassay kit (DEE6500 2-CAT, Demeditec Diagnostics GmbH, Germany). Briefly, adrenaline and noradrenaline were extracted using a cis-diol-specific affinity gel, acylated and then enzymatically derived. In the present study, the microtiter plate format was used for the competitive enzyme-linked immunosorbent assay (ELISA). Plasma samples, standards and controls were first acylated and acid extracted before they were then added to microtiter 96 well plate coated with antibodies for adrenaline and noradrenaline. Anti-rabbit IgG conjugated with peroxidase was added and after periods of mixing and washing, a colourless substrate (Tetramethylbenzidine; TMB) was added to the wells and incubated with the samples. The enzyme conjugates which are bound to the antigens cause a colour change to blue, a process which is then prevented by the addition of stop solution causing a further colour change to yellow. The extent of colour change (and thus concentration determined from the calibration curve) is dependent on the number of enzyme conjugates that are bound to the antigens on the base of

the well. That is, if there is a high concentration of a given protein (or adrenaline/noradrenaline in this thesis) in the plasma sample, more will bind to the antigens than that of the enzyme conjugated protein, which will produce less colour change and *vice versa*. The absorbance of the wells was then assessed using a microplate reader set at a 450 nm. Controls and standards were used for the determination of calibration curves which define the final concentration of adrenaline and noradrenaline within the samples. Derived adrenaline and noradrenaline values are comparable to UK control values using HPLC (r = 0.96, 0.99, respectively).

Regional noradrenaline (NE) spillover was estimated and assessed in line with published methods (Hardebo & Owman 1980; Esler *et al.* 1984; Eisenhofer *et al.* 1988; Esler *et al.* 1988; Esler *et al.* 1990; Mitchell *et al.* 2009). Regional net spillover of noradrenaline from a given organ is calculated using the Fick principle, i.e. the product of arteriovenous difference in plasma NE concentration, and plasma flow. Across most organs noradrenaline flux is bi-directional and there is a required knowledge of the regional fractional extraction of NE from arterial blood. However due to the presence of a blood-brain-barrier (BBB), NE uptake is unidirectional and thus spill-over detected in the internal jugular vein is thought to originate primarily from the sympathetic nervous activity of the cerebral vasculature (Hardebo & Owman 1980; Esler *et al.* 1988; Mitchell *et al.* 2009). Thus, estimated increases in sympathetic nerve activity through measurements of the spillover of noradrenaline into plasma were made using the Fick principle.

3.3.10 Calculated variables

Vascular conductance for the leg, brain and systemic circulations was calculated as flow divided by perfusion pressure (MAP-jugular/femoral venous blood pressure). Arterial and venous oxygen content was obtained and used to calculate a-vO_2 difference and oxygen extraction (a-vO_2 diff/C_aCO_2) across the leg and brain. The cerebral metabolic rate for oxygen ($CMRO_2$), leg $\dot{V}O_2$, and glucose and lactate uptake were calculated as the product of flow and substrate a-v difference. Further calculations relative to each study can be found in the respective chapters.

3.3.11 Core, skin and blood temperature

Core temperature was assessed using different methods in the two studies. Intestinal temperature (Chapters 4 & 5) was obtained using an ingestible telemetry pill (CorTemp, HQInc, Palmetto, Florida, USA) and data recorder, linked to an analogue-to-digital converter and data acquisition hardware/software (Powerlab). Each telemetry pill was individually calibrated prior to ingestion. Briefly each pill was activated and immersed, for at least 6 min, in water baths set at varying temperatures (36, 38, 40 and 42 °C) in the region of normal core body temperatures. Comparisons were made between the sensor and a calibrated mercury thermometer with individual regression plots were created for each pill, to obtain a correction factor for observed temperatures (Byrne & Lim 2007; Hunt & Stewart 2008). The sensors were consumed by the participants in the late evening (~21:00), preferably with a meal, to ensure correct placement in the gastrointestinal tract in accordance with manufacturer and published recommendations (Goodman *et al.* 2009). The telemetry pill was used due to its more rapid response time when compared with a standard rectal thermister (Byrne & Lim 2007). Oesophageal temperature (Chapter 6) was obtained using a thermistor (PhysiTemp, NJ) inserted through the nasal passage and into the oesophagus at ¼ standing height. A small volume (<0.8 ml) of anaesthetic gel (Lidocaine Hydrochloride, Instillagel, CliniMed, UK) was self-applied to the nasal passage to reduce discomfort associated with the insertion procedure.

Skin temperature was obtained using cabled thermistors (Chapters 4 & 5; Type T thermocouple, PhysiTemp, NJ) and wireless data loggers (Chapter 6; iButton®, Maxim Integrated, California, USA; Figure 3-13), located at four sites (forearm, chest, thigh and calf (Ramanathan 1964; Mitchell & Wyndham 1969). Weighted skin temperature was then calculated using the following equation;

$$0.3\ (T_{chest} + T_{forearm}) + 0.2\ (T_{thigh} + T_{calf})$$

This method of calculating mean skin temperature has strong agreement with other methods and was used in the present study due to the logistical limitations of the numerous other measurements taken (Mitchell & Wyndham 1969).

Figure 3-13. Thermochron iButton®.

Blood temperature in the internal jugular vein (Chapters 4 & 5) and common femoral vein (Chapter 6) was obtained by the insertion of a thermistor (T-204D, PhysiTemp, Clifton, NJ) through the catheter and beyond its tip. All cabled thermistors were connected to a thermocouple meter (TC-2000, Sable Systems, Las Vegas, NV, USA) and converted to an online acquisition unit (Powerlab 16/30, ADinstruments, Bella Vista, NSW, Australia).

3.3.12 Statistical analysis

Data presented in the present thesis were examined using a statistical software package (SPSS Version 20, IBM Corporation, Armonk, NY, USA). The alpha level was set at $P < 0.05$ for the rejection of the null hypothesis. Coefficient of determination (R^2) was used to assess the relationship between given variables. The specific tests used to analyse the data are presented in the individual chapters.

CHAPTER 4 – Dehydration affects cerebral blood flow but not its metabolic rate for oxygen during maximal exercise in trained humans

4.1 Summary

Intense exercise is associated with a reduction in cerebral blood flow (CBF), but regulation of CBF during strenuous exercise in the heat with dehydration is unclear. We assessed internal (ICA) and common-carotid artery (CCA) haemodynamics (indicative of CBF and extra-cranial blood flow), middle cerebral artery velocity (MCA V_{mean}), a-v differences, and blood temperature in 10 trained males during incremental cycling to exhaustion in the heat (35 °C) in control, dehydrated and rehydrated states. Dehydration reduced body mass (75.8 ± 3 *vs.* 78.2 ± 3 kg), increased internal temperature (38.3 ± 0.1 *vs.* 36.8 ± 0.1 °C), impaired exercise capacity (269 ± 11 *vs.* 336 ± 14 W), and lowered ICA and MCA V_{mean} by 12-23% without compromising CCA blood flow. During euhydrated incremental exercise on a separate day, however, exercise capacity and ICA, MCA V_{mean} and CCA dynamics were preserved. The fast decline in cerebral perfusion with dehydration was accompanied by increased O_2 extraction ($P < 0.05$), resulting in a maintained cerebral metabolic rate for oxygen ($CMRO_2$). In all conditions, reductions in ICA and MCA V_{mean} were associated with declining cerebral vascular conductance, increasing jugular venous noradrenaline, and falling $PaCO_2$ ($R^2 \geq 0.41$, $P \leq 0.01$) whereas CCA flow and conductance were related to elevated blood temperature. In conclusion, dehydration accelerated the decline in CBF by decreasing $PaCO_2$ and enhancing vasoconstrictor activity. However, the circulatory strain on the human brain during maximal exercise does not compromise $CMRO_2$ because of compensatory increases in O_2 extraction.

4.2 Introduction

Heat-stress, with or without dehydration, compromises blood flow to active muscles and skin during strenuous exercise as the systemic circulation becomes compromised (González-Alonso & Calbet 2003; González-Alonso *et al.* 2008; Crandall & González-Alonso 2010). Intense exercise in the heat is also associated with a marked decline in middle cerebral artery blood velocity (MCA V_{mean}), suggesting attenuated cerebral perfusion (Nybo & Nielsen 2001a; 2001b; González-Alonso *et al.* 2004). Changes in MCA V_{mean}, however, may not reflect alterations in cerebral blood flow (CBF) as the vessel cross sectional area remains unknown (Madsen *et al.* 1993; Jorgensen 1995; Wilson *et al.* 2011; Willie *et al.* 2012). Additionally, dehydration intensifies the effect of heat stress on active muscle blood flow and increases the rate of heat storage in part by attenuating skin perfusion (Sawka *et al.* 1985b; González-Alonso *et al.* 1995; González-Alonso *et al.* 1998; Montain *et al.* 1998a; Cheuvront *et al.* 2010). It remains, however, unknown whether dehydration affects CBF during maximal incremental exercise in the heat.

On the transition from rest to moderate exercise, regional and global CBF increase to support neuronal activity (Ide & Secher 2000; Secher *et al.* 2008; Ogoh & Ainslie 2009a). However, CBF reaches a plateau or declines to baseline values prior to the attainment of maximal work rate (Madsen *et al.* 1993; Moraine *et al.* 1993; Hellstrom *et al.* 1996; Ide & Secher 2000; Sato *et al.* 2011). During intense exercise, restricted cerebral perfusion could challenge the cerebral metabolic rate for oxygen ($CMRO_2$) (Nybo & Rasmussen 2007; Rasmussen *et al.* 2010) and in part explain the orthostatic intolerance and reduced motor output with heat stress (Van Lieshout *et al.* 2003; Wilson *et al.* 2006; Brothers *et al.* 2009c; Nelson *et al.* 2011; Ross *et al.* 2012; Bain *et al.* 2013). Alternatively, reduced CBF can be compensated by increased oxygen extraction such that $CMRO_2$ is maintained or increased (Nybo *et al.* 2002; González-Alonso *et al.* 2004). Whether the $CMRO_2$ remains adequate during strenuous exercise in the heat with concomitant dehydration is yet unknown.

Understanding the mechanisms restricting CBF in intensely exercising humans is important for devising strategies that could ameliorate or delay its potential deleterious effects. During exercise, attenuation of CBF is in part due to cerebral

vessel vasoconstriction, concomitantly with an increased systemic and regional cerebral sympathetic activity, increasing body temperature, and reduced arterial carbon dioxide tension (P_aCO_2) (Wilson *et al.* 2002; Querido & Sheel 2007; Fan *et al.* 2008; Secher *et al.* 2008; Seifert & Secher 2011). The cerebral vasculature is highly sensitive to changes in P_aCO_2, with elevations resulting in vasodilation and reductions leading to vasoconstriction (Kety & Schmidt 1948a; Ogoh & Ainslie 2009b; Willie *et al.* 2012). At rest, these responses are of importance for maintenance of a stable pH across the brain and reflect the sensitivity of the brainstem to acute changes in CO_2. However, P_aCO_2 only accounts for ~7% of the CO_2 transported from the cerebral tissue whereas the majority of CO_2 is bound to haemoglobin (23%) or buffered as bicarbonate (70%). If local tissue pH balance is important for regulation of CBF, blood CO_2 content ($ctCO_2$) could account for the alterations in cerebrovascular tone. It is also evident that changes in CO_2 are not associated with changes in conduit artery and extra-cranial (i.e. common (CCA) and external carotid (ECA)) tone and perfusion, as blood flow in these vessels increases progressively with exercise intensity (Hellstrom *et al.* 1996; Sato *et al.* 2011). Extra-cranial blood flow is likely to be controlled by thermoregulatory, rather than pH regulatory mechanisms (Fan *et al.* 2008; Sato *et al.* 2011; Sato *et al.* 2012; Bain *et al.* 2013; Ogoh *et al.* 2013b); yet direct evidence for a relationship between flow and blood temperature is lacking. While evidence indicates differences in blood flow responses to exercise at the vascular beds perfusing the head, the impact of dehydration on graded exercise in the heat and the potential role of $ctCO_2$, P_aCO_2 and blood temperature on these responses, have not been investigated.

The purpose of this study was to investigate cerebral and extra-cranial blood flow and $CMRO_2$ during incremental exercise to exhaustion in the heat, with and without dehydration, and to provide insights into the vascular mechanisms underpinning these responses. CBF was measured using Doppler ultrasonography, and arterial to internal jugular venous differences for oxygen, CO_2 and noradrenaline were measured for assessment of the exchange of these substances across the brain. We hypothesised that dehydration would accelerate the attainment of maximal CCA blood flow but also accentuate the reduction in CBF during exercise in association with the lowering of P_aCO_2 and $ctCO_2$ and the

increase in sympathetic activity, and yet increased O_2 extraction would maintain or enhance $CMRO_2$.

4.3 Methods

4.3.1 Ethical approval

Fully informed, written consent was obtained from the participants prior to the study. All procedures were approved by the Brunel University Research Ethics Committee (RE07-11) and conformed to the guidelines of the declaration of Helsinki.

4.3.2 Participants

Ten healthy experienced cyclists (mean ± SD; age 29 ± 5 years, stature 183 ± 5 cm, mass 78 ± 9 kg and $\dot{V}O_{2peak}$ 59 ± 6 ml·kg^{-1}·min^{-1}) participated in the study. All participants were non-smokers and free from cardio-respiratory, metabolic and neurological disease. Participants arrived at the laboratory postprandial with a normal hydration status and were required to have abstained from strenuous exercise and alcohol intake for 24 h and caffeine consumption for 12 h.

4.3.3 Experimental design

The participants visited the laboratory for 3 preliminary sessions followed by 2 experimental sessions, each separated by at least one week. On the first session the participants were introduced to the experimental set-up and familiarised with the methodology. Investigation of the extra-cranial arteries and MCA V_{mean} Doppler spectra determined the reliability of images and identified the temporal ultrasound window and the position for the best signal-to-noise ratio. Participants performed incremental exercise on a semi-recumbent cycle ergometer (Lode Angio, Groningen, Netherlands) with a backrest inclination of 45^{o}, to establish the maximal work rate (WR_{max}), maximal heart rate, and $\dot{V}O_{2peak}$. The initial work rate was 20 W for 3 min, followed by step increments of 60 W every 3 min until the limit of tolerance. Pedal cadence was maintained between 70 and 90 r.p.m. and the test was terminated when it dropped below 60 r.p.m., for more than 3 s, despite strong verbal encouragement to continue. On the second and third visits, participants cycled in an environmental chamber set at 35 °C (relative humidity

50%) in the semi-recumbent position for 2 h at 55% WR_{max} with heart rate and intestinal temperature recorded. No fluid consumption was permitted during exercise and body mass was recorded before and immediately post exercise.

The experimental days (visits 4 and 5) included three semi-recumbent incremental cycling exercise tests consisting of five, 3 min stages of increasing intensities to WR_{max} (Figure 4-1). On the first experimental trial, incremental cycling was completed in the following conditions: 1) in a '*control'* hydrated state, 2) '*dehydrated'* (DEH) ~5 min after 2 h of sub-maximal cycling without fluid ingestion and 3) 'rehydrated' REH after 1 h recovery with full fluid replacement. Work rates for control and REH were the same (67 ± 3, 134 ± 5, 202 ± 8, 269 ± 11 and 336 ± 14 W, corresponding to 20, 40, 60, 80, and 100% of WR_{max}) but in anticipation of a reduced exercise capacity when dehydrated, WR in DEH was reduced by 20% to maintain the same number of exercise stages and test duration with work rates set at 54 ± 2, 108 ± 4, 161 ± 7, 215 ± 9 and 269 ± 11 W, respectively. On the second experimental trial (i.e., euhydration trial), carried out on a separate day, participants completed the same incremental and prolonged exercise protocols, but hydration was maintained through fluid ingestion according to the body mass loss. Fluid was provided in aliquots of ~160 ml every 10 min during the 2 h of sub-maximal exercise and also pre- and post-incremental exercise at the same work rates. The euhydration trial was used to isolate the effect of dehydration on the observed haemodynamic responses to incremental exercise and to control for the effect of repeated exercise (Figure 4-5). In both trials, incremental exercise was performed in the heat (35 ^{o}C, RH 50%) with pedal cadence maintained at 70-90 r.p.m. Participants were exposed to the environmental conditions for 1 h prior to commencement of the protocol.

In the dehydration trial, cerebral haemodynamics and blood samples from the brachial artery and left internal jugular vein were obtained simultaneously in the final minute of each exercise stage (Figure 4-2). Intestinal, skin, and jugular venous temperatures and arterial and jugular venous pressures were recorded. The same measures were collected in the euhydration trial, except for the arterio-venous (a-v) blood sampling and jugular venous temperatures and pressures.

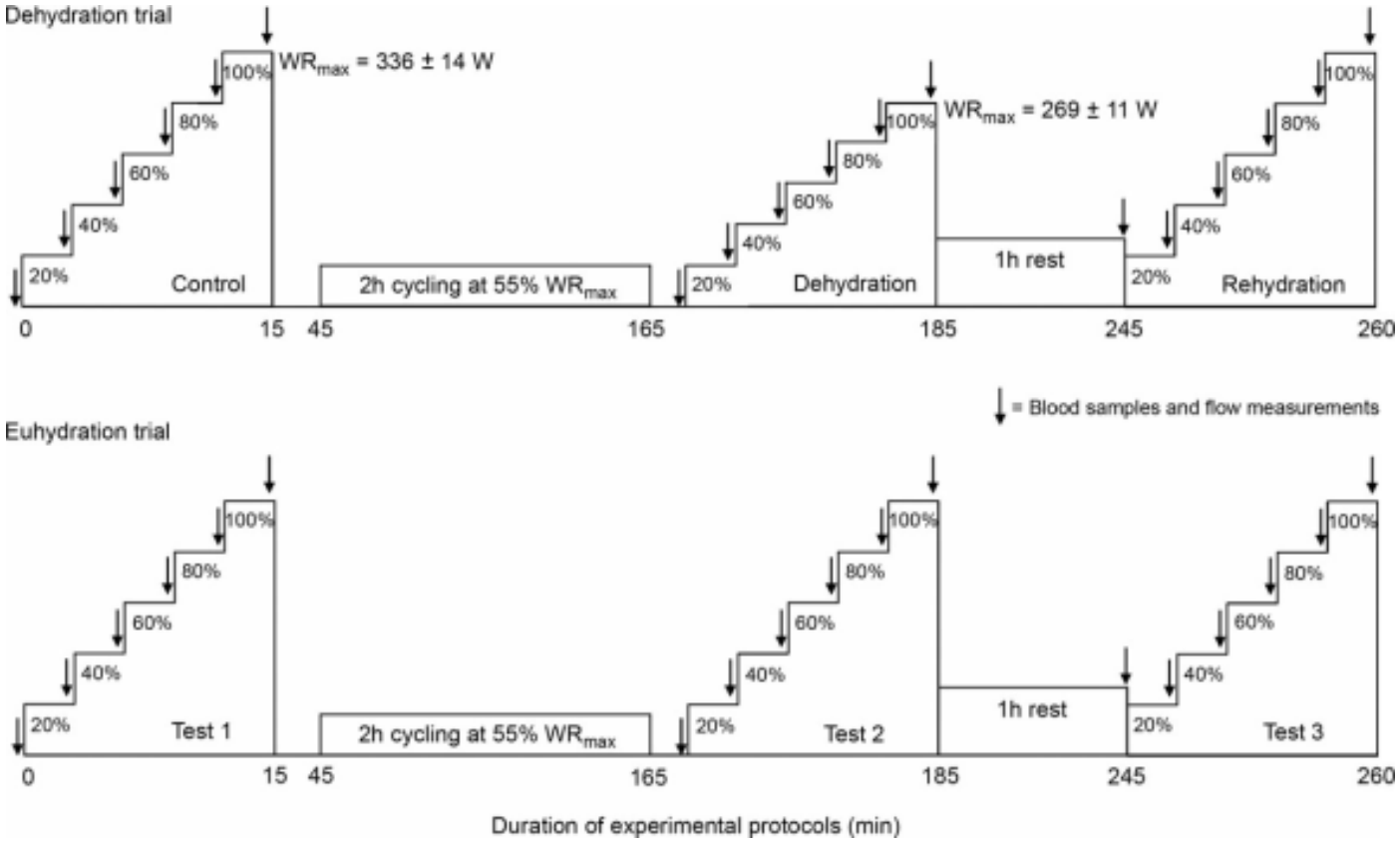


Figure 4-1. Experimental design of the present study. Participants completed 2 trials (i.e. dehydration and euhydration trials) separated by at least one week. Each trial consisted of 3 incremental cycle ergometer exercise tests until volitional exhaustion. The incremental exercise consisted of five, 3 min stages at 20, 40, 60, 80 and 100% of WRmax. In the dehydration trial, WRmax was approximately 20% lower when participants were dehydrated compared to when they were euhydrated or rehydrated (269 ± 11 *vs.* 336 ± 14 W). In the euhydration trial, however, WRmax was the same in the 3 incremental exercise tests.

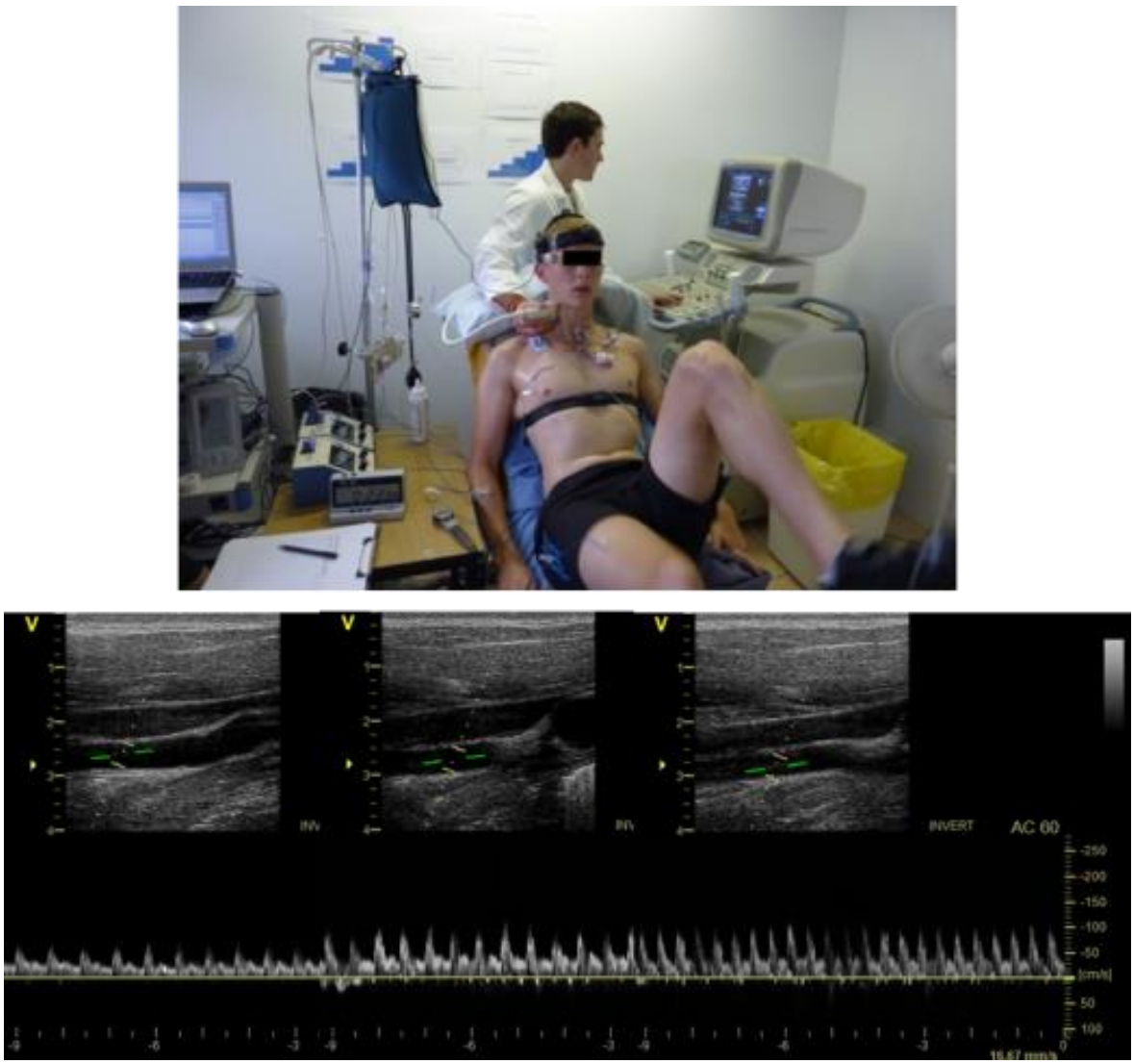


Figure 4-2. Experimental set up and ultrasound recording. The photo shows one of the participants in the study performing an incremental cycling test on a semi-recumbent cycle ergometer (Lode Angio, Groningen, Netherlands) with a backrest inclination of 45^{o}, while measurements of ICA and CCA blood flow were obtained at each stage. Representative images of real time ICA blood velocity recordings at rest, submaximal and peak exercise are shown.

4.3.4 Cerebral haemodynamics

Blood flow was obtained sequentially from the right CCA and internal carotid arteries (ICA) at rest and in the final minute of each work rate using an ultrasound system (Vivid 7 Dimension, GE Healthcare, UK) equipped with a 10 MHz linear array transducer. Measurements were performed by an experienced sonographer with care taken to maintain sampling site and vessel insonation angle. Participants were seated on the cycle ergometer and encouraged to maintain a consistent head position for optimal ultrasound scanning. ICA and CCA measurements were typically taken ~1.0-1.5 cm above and ~1.5 cm below the carotid bifurcation,

respectively (Sato *et al.* 2011; Willie *et al.* 2012) with settings maintained across the protocol. Test-retest reliability was assessed during pilot studies and the coefficient of variation for CCA and ICA volume flow measurements at rest were 2.8 ± 0.9% and 4.3 ± 1.0%, and during exercise were 5.3 ± 1.6% and 5.0 ± 1.6%, respectively. For calculation of blood flow, two-dimensional brightness mode images for CCA and ICA diameter were taken, followed by pulse-wave measurements for the assessment of time-averaged mean velocity. Systolic and diastolic diameters were measured with the mean diameter calculated as systolic diameter X 1/3 + diastolic diameter X 2/3.

Time-averaged mean flow velocity (TAM V_{mean}; $cm \cdot s^{-1}$) was measured in pulse-wave mode, taken as the average of three continuous 12 s periods. Average diameter and flow velocity profiles were made from ≥15 cardiac cycles to attenuate respiration artefacts. The sample volume was maintained at the centre of the vessel lumen and adjusted to cover its width. Care was taken to ensure a consistent insonation angle below 60°. Mean flow velocity profiles were traced automatically and analysed offline for determination of TAM *V* (EchoPAC BT12, Version: 112 GE Healthcare, Norway). Blood flow ($ml \cdot min^{-1}$) was then calculated by mean flow velocity times cross sectional area (CSA: π x (mean diameter/2)^2);
Blood flow = TAM *V* x CSA x 60. Due to technical limitations, blood flow measurements were made in all work rates except the 100% stage in control and rehydration conditions. Blood flow in these stages was estimated using the individual % decline in MCA V_{mean} from 80-100%. MCA V_{mean} was measured using 2 MHz pulsed trans-cranial Doppler ultrasound (Doppler-Box, Compumedics DWL, Singen, Germany). The right MCA was insonated through the temporal ultrasound window at a depth of 45-60 mm. Signal quality was optimised according to Aaslid *et al.* (1982).

4.3.5 Catheter placement and blood sampling

While resting with a slight head-down tilt; catheters for blood sampling, blood pressure (MAP), internal jugular venous pressure and blood temperature were inserted into the brachial artery of the non-dominant arm and after local anaesthesia (2% lidocaine) in the left internal jugular vein (Double Lumen Catheter, 16 gauge, 2.3 mm; Multi-Med M2716HE, Edwards Lifesciences, USA),

using the Seldinger technique, and advanced to the jugular bulb. For measurement of jugular venous blood temperature, a thermistor (T204D, PhysiTemp, Clifton, New Jersey, USA) was inserted through the catheter and connected to a thermocouple meter (TC-2000, Sable Systems, NV, USA). The internal jugular catheter was inserted under ultrasound guidance and catheters were regularly flushed with 0.9% saline to maintain patency. The time from catheterisation to the commencement of resting measurements was ~1 h.

4.3.6 Blood variables

Arterial and jugular venous blood samples were drawn into pre-heparinised syringes and analysed immediately for blood gas variables (ABL 800 FLEX, Radiometer, Copenhagen, Denmark). Internal jugular venous blood temperature was input for samples to be autocorrected temperature. The analyser was calibrated at regular intervals in accordance with manufacturer guidelines. Additional arterial and jugular venous blood was collected in 2 ml syringes and transferred to EDTA tubes, centrifuged and separated. Plasma adrenaline and noradrenaline was subsequently determined using an enzyme immunoassay kit (DEE6500 2-CAT, Demeditec Diagnostics GmbH, Germany). Blood samples were also collected directly in stop solution (Gorman *et al.* 2003; Kalsi & González-Alonso 2012). Plasma ATP was then determined using the luciferin-luciferase technique by a luminometer with three automatic injectors (Orion Microplate Luminometer, Bethold Detection System GmbH, Pforzheim, Germany).

4.3.7 Heart rate, blood pressure and temperatures

Heart rate was obtained from a chest strap (Polar Electro, Kempele, Finland). Arterial and internal jugular venous pressure waveforms were recorded using transducers (Pressure Monitoring Kit, TruWave, Edwards Lifesciences, Germany) zeroed at the level of the right atrium in the midaxillary line (arterial) and at the level of the tip of the catheter (jugular venous). Arterial pressure waveforms were sampled at 1000 Hz, amplified (BP amp, ADInstruments, Oxfordshire, UK) and connected to a data acquisition unit (Powerlab 16/30, ADInstruments) for offline analysis. Intestinal temperature was measured using an ingestible telemetry pill (HQInc, Palmetto, Florida, USA) and mean skin temperature from four sites (standard weightings of chest, abdomen, thigh and calf (Ramanathan 1964)) was

obtained using a wired thermocouple system (TC-2000, Sable Systems, NV, USA).

4.3.8 Calculations

Cerebral vascular conductance (CVC) indices were calculated by dividing blood flow in the ICA and CCA, and MCA V_{mean} by cerebral perfusion pressure (difference between MAP and jugular venous pressure). Arterial oxygen content was used to quantify O_2 delivery through the MCA and ICA, respectively. $CMRO_2$ and CO_2 production indexes were calculated as 2 x ICA flow multiplied by the a-v O_2 difference and/or, v-aCO_2 difference. Whole blood CO_2 content ($ctCO_2$) was also calculated (Douglas *et al.* 1988).

4.3.9 Data analysis

A one-way repeated-measures ANOVA was used for the assessment of changes over time (i.e. rest and increasing exercise intensities). Where significant differences were found, appropriate *post hoc* analysis were made using the Dunn-Sidak correction. Where applicable, measured variables between conditions were analysed using a two-way repeated-measures ANOVA in which condition (Control, DEH and REH) and exercise phase (rest, 20, 40, 60, 80 and 100%) were the main factors. Multiple regressions for within-subject repeated measures were used for the analysis of the relationship between blood flow and blood gas variables and temperatures (Bland & Altman, 1995; Slinker & Glantz, 2008). Statistical significance was set at $P < 0.05$ and all analyses were made using IBM SPSS Statistics (Version 20, IBM Corporation, Armonk, NY, USA).

4.4 Results

4.4.1 Hydration and temperature

In the dehydration trial (Figure 4-1), body mass in DEH was lower compared to control (75.8 ± 2.7 *vs.* 78.2 ± 2.7 kg, corresponding to a 3.1 ± 0.3% body mass loss, $P < 0.01$), and was restored in REH (77.7 ± 2.9 kg). DEH was accompanied by an increased arterial and venous [Hb] ($P < 0.01$; Table 4-2), indicative of a reduction in blood volume, whereas REH reversed these responses. Prior to exercise, intestinal and internal jugular venous temperatures were higher in DEH

compared to control (38.3 ± 0.1 *vs.* 36.8 ± 0.1 and 37.7 ± 0.1 *vs.* 36.5 ± 0.1 °C, respectively, both $P < 0.001$; Figure 4-7 C), but were restored to control values in REH (36.5-36.8 °C). In DEH, both intestinal and blood temperature remained elevated and increased with work rate to a peak of 38.2 ± 0.1 °C ($P < 0.01$; Figure 4-7 C). In control, intestinal and internal jugular venous temperature increased progressively to 37.4 ± 0.1 and 37.9 ± 0.1 °C, with similar responses observed during REH. Mean skin temperature ($\overline{T}_{sk}$) was unchanged across exercise intensities and between incremental conditions (33.8 ± 0.3, 32.6 ± 0.4 and 33.1 ± 0.3 °C in control, DEH and REH, respectively) (Table 4-1). Heart rate followed the same pattern with peak values being similar in all three conditions (179 ± 4, 184 ± 2 and 179 ± 3 beats·min^{-1} in control, DEH and REH, respectively).

In the euhydration trial, body mass was the same at the start of each of the three incremental cycling tests. Prior to exercise intestinal temperature was higher in the second and third test, compared to the first control test (37.8 ± 0.2 and 37.2 ± 0.1 *vs.* 37.0 ± 0.1 °C; $P < 0.05$). During exercise, intestinal temperature increased with exercise intensity and reached 37.8 ± 0.1, 37.5 ± 0.1 and 37.4 ± 0.1 °C, at exhaustion. Similarly to the dehydration trial, mean $\overline{T}_{sk}$ was unchanged across exercise intensities and between incremental tests (33.3 ± 0.2, 32.7 ± 0.3 and 33.3 ± 0.2 °C respectively). Heart rate was elevated prior to the second test compared to first, but peak heart rate was not different (176 ± 2, 176 ± 3 and 177 ± 3 beats·min^{-1}, in the first, second and third tests, respectively).

4.4.2 Brain haemodynamics and metabolism

During control exercise on the dehydration trial, ICA blood flow and MCA V_{mean} increased by ~17 ± 2% from rest to submaximal exercise and thereafter declined to resting values (both $P < 0.05$; Figure 4-3 A & D). Conversely during DEH, ICA blood flow did not increase from rest to moderate exercise, but declined to below resting values at WR_{max} (-11% *vs.* Rest, $P < 0.05$). ICA blood flow responses to REH were similar to control. In all conditions, the decline in blood flow at high exercise intensities was associated with reductions in vessel diameter and blood velocity. In contrast to ICA blood flow, CCA blood flow did not change during low intensity exercise in control, but increased progressively with further increases in exercise intensity (Rest = 0.47 ± 0.02 *vs.* 0.60 ± 0.02 l·min^{-1}, $P < 0.01$) (Figure 4-3

C). During DEH, CCA blood flow was elevated ($P < 0.05$) at the start of exercise and did not change throughout incremental exercise. CCA blood flow responses to REH incremental exercise were similar to control. The increases in CCA blood flow in control and REH were associated with increases in blood velocity ($P < 0.05$). In the euhydration trial, ICA and CCA blood flow, and MCA V_{mean} were similar at rest and during incremental exercise (Figure 4-5).

At rest, ICA O_2 delivery, a-vO_2 and v-aCO_2 difference, and $CMRO_2$ and brain rate of CO_2 production ($\dot{V}CO_2$) indices were not significantly different across the three experimental conditions of the dehydration trial. From rest to sub-maximal exercise (40% WR_{max}) in control, ICA O_2 delivery increased, v-aCO_2 difference decreased, while the a-vO_2 difference was unchanged (Figure 4-3 B,E & F). When exercise intensity became strenuous (≥ 60%), ICA O_2 delivery declined to baseline values, as with ICA blood flow, and v-aCO_2 and a-vO_2 difference increased progressively to exhaustion (~32% increase *vs.* rest, $P < 0.05$). Additionally, there was a progressive increase in brain $\dot{V}CO_2$ index up to WR_{max} (Figure 4-3 G). During DEH, ICA O_2 delivery remained constant up to 60% WR_{max}, before declining to below resting values. Moreover, v-aCO_2 difference, a-vO_2 difference and brain $\dot{V}CO_2$ index were elevated at WR_{max} ($P < 0.05$). ICA O_2 delivery was somewhat restored in REH whereas v-aCO_2 and a-vO_2 difference, and brain $\dot{V}CO_2$ index were similar to CON. Overall, these responses resulted in a maintained $CMRO_2$ index at rest and throughout exercise to exhaustion (Figure 4-3 H). Brain glucose uptake was also maintained across all exercise intensities and hydration conditions, whereas brain lactate uptake increased at high exercise intensities (Figure 4-4).

4.4.3 Blood pressure and vascular conductance

At rest and during incremental exercise in the dehydration trial, MAP was lower in DEH compared to control whereas jugular venous pressure was not different across incremental exercise conditions ($P < 0.01$; Figure 4-6). Brain perfusion pressure was therefore lower in DEH compared to control ($P < 0.01$). Concurrently, ICA, CCA and MCA vascular conductance were higher in DEH, compared to control and REH, at rest ($P < 0.01$; Figure 4-6). However, in all incremental exercise conditions, ICA and MCA vascular conductances were not different at sub-maximal exercise intensities before declining at WR_{max} ($P < 0.05$).

During control, CCA vascular conductance declined from rest to sub-maximal exercise intensities before recovering to baseline values at WR_{max}, whereas in DEH CCA vascular conductance continued to decline. In contrast to the haemodynamic alterations seen in the dehydration trial, in the euhydration trial MAP and ICA, CCA, and MCA vascular conductance were similar at rest and throughout the three exercise tests.

Table 4-1. Temperature responses to incremental exercise in different hydration states.

		Incremental cycling exercise (% WR_{max} in Control)					
		Rest	20%	40%	60%	80%	100%
Intestinal temperature (°C)	Control	36.8±0.1	36.8±0.1	36.9±0.1*	37.0±0.1*	37.2±0.1*	37.4±0.1*†
	Dehydration	38.3±0.1	38.0±0.1*	38.0±0.1*	38.1±0.1*	38.2±0.1*†	-
	Rehydration	36.8±0.2	36.8±0.2	36.9±0.2*	37.1±0.2*	37.3±0.2*†	-
Blood temperature (°C)	Control	36.5±0.1	36.5±0.2	36.8±0.2*	37.1±0.2*	37.5±0.1*	37.9±0.1*
	Dehydration	37.7±0.1	37.8±0.1	37.9±0.1	38.1±0.1*	38.2±0.1*	-
	Rehydration	36.4±0.2	36.4±0.2	36.7±0.2*	37.0±0.2*	37.3±0.2*	-
Mean skin temperature (°C)	Control	34.1±0.3	33.7±0.3	33.9±0.3	33.9±0.3	33.5±0.3	33.3±0.3
	Dehydration	32.9±0.3	32.6±0.4	32.6±0.5	32.6±0.3	32.6±0.3	-
	Rehydration	33.3±0.2	33.3±0.3	33.2±0.3	33.0±0.3	32.7±0.3	-

Values are mean ± SEM for 10 participants. * different from rest $P < 0.05$, † different from previous intensity.

Table 4-2. Blood variable responses to incremental exercise in different hydration states (i.e., control, dehydration and rehydration).

			Incremental cycling exercise (% WR_{max} in Control)					
			Rest	20%	40%	60%	80%	100%
Hb ($g \cdot l^{-1}$)	Control	*a*	141 ± 5	145 ± 4*	147 ± 4*	149 ± 4*	154 ± 4*	158 ± 4*
		v	140 ± 5	144 ± 4*	146 ± 4*	148 ± 4*	152 ± 4*	156 ± 4*
	Dehydration	*a*	152 ± 4	151 ± 4	152 ± 4	154 ± 4*	156 ± 4*	-
		v	152 ± 4	148 ± 3	149 ± 3	149 ± 4	152 ± 3†	-
	Rehydration	*a*	140 ± 3	141 ± 3	142 ± 3	146 ± 3*	149 ± 2*	148 ± 4*
		v	140 ± 4	139 ± 3	142 ± 3	147 ± 3*	147 ± 3*	148 ± 4*
SO_2 (%)	Control	*a*	98.5 ± 0.2	97.7 ± 0.1*	97.8 ± 0.2*	97.5 ± 0.3*	97.3 ± 0.4*	96.6 ± 0.4*
		v	64.7 ± 1.0	66.0 ± 1.5	68.7 ± 1.0*	67.0 ± 1.1	64.9 ± 1.5†	61.0 ± 2.1†
	Dehydration	*a*	98.1 ± 0.4	97.4 ± 0.1	97.4 ± 0.1	97.5 ± 0.4	97.9 ± 0.2	-
		v	65.7 ± 0.8	63.2 ± 1.2*	64.0 ± 0.9	63.9 ± 1.3	63.4 ± 2.0	-
	Rehydration	*a*	98.5 ± 0.1	97.2 ± 0.5*	97.4 ± 0.2*	97.2 ± 0.1*	97.2 ± 0.3*	97.0 ± 0.7*
		v	65.9 ± 1.4	65.0 ± 1.5	65.4 ± 2.2	65.7 ± 1.9	65.6 ± 3.2	65.9 ± 6.3
PO_2 (mmHg)	Control	*a*	99 ± 3	90 ± 2*	94 ± 3	94 ± 4	96 ± 4	97 ± 4
		v	36 ± 1	36 ± 1	38 ± 1*	38 ± 1*	39 ± 1*	40 ± 1*
	Dehydration	*a*	101 ± 4	91 ± 2	90 ± 2	94 ± 4	96 ± 2	-
		v	40 ± 1	37 ± 1*	37 ± 1*	39 ± 2*	38 ± 1*	-
	Rehydration	*a*	105 ± 2	93 ± 3*	89 ± 2*	89 ± 2*	91 ± 3*	96 ± 8*
		v	37 ± 1	36 ± 1	36 ± 1	38 ± 1	38 ± 2	38 ± 2
ctO_2 ($ml \cdot l^{-1}$)	Control	*a*	192 ± 6	195 ± 6	199 ± 5*	201 ± 5*	206 ± 5*	211 ± 6*
		v	127 ± 4	131 ± 5	138 ± 5†	137 ± 4	136 ± 5	131 ± 5
	Dehydration	*a*	206 ± 5	203 ± 5	203 ± 5	207 ± 6	210 ± 5*	-
		v	140 ± 2	134 ± 6	131 ± 3	132 ± 3	133 ± 4	-
	Rehydration	*a*	191 ± 4	189 ± 4	191 ± 4	195 ± 4*	200 ± 3*	203 ± 5*
		v	127 ± 2	124 ± 2	127 ± 3	132 ± 3	132 ± 6	124 ± 4
pH	Control	*a*	7.39 ± 0.01	7.38 ± 0.01*	7.36 ± 0.01*	7.36 ± 0.01*	7.36 ± 0.01	7.31 ± 0.01*
		v	7.33 ± 0.01	7.32 ± 0.02	7.32 ± 0.01	7.32 ± 0.01	7.32 ± 0.01	7.26 ± 0.01*
	Dehydration	*a*	7.40 ± 0.01	7.38 ± 0.01	7.38 ± 0.01	7.38 ± 0.03	7.41 ± 0.02	-
		v	7.34 ± 0.01	7.32 ± 0.01	7.30 ± 0.03	7.33 ± 0.02	7.38 ± 0.01*	-
	Rehydration	*a*	7.38 ± 0.01	7.37 ± 0.01	7.37 ± 0.01	7.37 ± 0.01	7.37 ± 0.01	7.34 ± 0.03
		v	7.33 ± 0.01	7.32 ± 0.02	7.32 ± 0.01	7.32 ± 0.01	7.33 ± 0.01	7.32 ± 0.02

Values are mean ± SEM for 10 participants. Haemoglobin (Hb), oxygen saturation (SO_2, %), partial pressures of oxygen (PO_2) and oxygen content (ctO_2) for arterial (a) and internal jugular venous (v) blood. Rehydration values at 100% are n=5. * different from rest $P < 0.05$, † different from previous intensity.

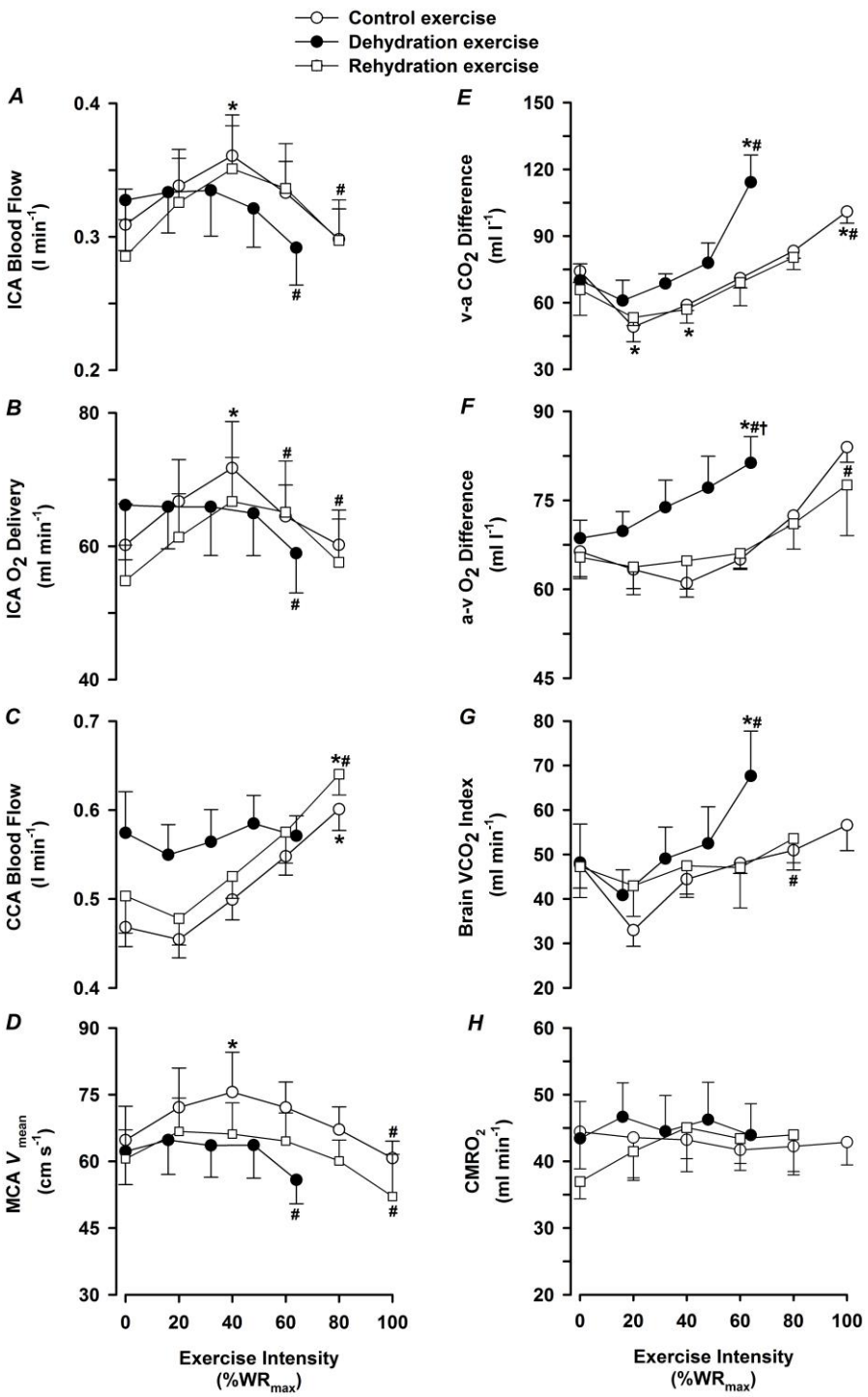


Figure 4-3. Cerebral haemodynamics and oxygen parameters during incremental exercise in different hydration states. Left panel; Internal carotid artery blood flow (A), ICA oxygen delivery (B), common carotid artery blood flow (C) and middle cerebral artery velocity (D). Right panel; jugular venous to arterial CO_2 difference (v-aCO_2; E), arterial to jugular venous oxygen difference (a-vO_2; F), brain CO_2 release (G), and brain oxygen uptake ($CMRO_2$; H) for control (open circles), dehydration (closed circles) and rehydration (open squares) conditions. Values are mean ± SEM. P values represent ANOVA results. * is $P < 0.05$ *vs.* rest, # is $P < 0.05$ *vs.* sub-maximal exercise (i.e. ~40% WRmax).

Table 4-3. Blood gases and metabolite responses to incremental exercise in different hydration states.

			Incremental cycling exercise (% WR_{max} in Control)					
			Rest	20%	40%	60%	80%	100%
PCO_2 (mmHg)	Control	*a*	39 ± 1	42 ± 1*	43 ± 1*	42 ± 1*	40 ± 1*	36 ± 1*†
		v	50 ± 1	52 ± 1	52 ± 1	53 ± 1*	52 ± 1	50 ± 1†
	Dehydration	*a*	37 ± 2	39 ± 1	39 ± 1	40 ± 2	37 ± 1†	-
		v	49 ± 1	50 ± 1	48 ± 2	47 ± 3	48 ± 2	-
	Rehydration	*a*	38 ± 1	39 ± 1	39 ± 1	39 ± 1	36 ± 1†	33 ± 1†
		v	49 ± 1	49 ± 1	49 ± 1	50 ± 1*	48 ± 2†	45 ± 4†
[$HCO3^-$] ($mmol \cdot l^{-1}$)	Control	*a*	23.5 ± 0.7	24.0 ± 0.6	23.1 ± 0.7†	23.0 ± 0.6	22.2 ± 0.6	18.7 ± 0.8
		v	23.6 ± 0.8	23.1 ± 1.1	23.6 ± 0.6	23.6 ± 0.7	23.2 ± 0.8	19.3 ± 0.5
	Dehydration	*a*	23.9 ± 0.7	23.2 ± 1.0	23.0 ± 0.9	23.1 ± 1.3	23.7 ± 1.1	-
		v	23.6 ± 0.8	23.1 ± 1.0	21.6 ± 1.6	23.4 ± 1.6	26.5 ± 0.6	-
	Rehydration	*a*	22.5 ± 0.6	22.4 ± 0.6	22.4 ± 0.6	22.2 ± 0.7	21.6 ± 0.7	19.2 ± 0.9
		v	23.1 ± 0.6	22.7 ± 0.6	22.7 ± 0.7	23.0 ± 0.7	20.5 ± 1.9	21.2 ± 0.4
cBase (ECF) ($mmol \cdot l^{-1}$)	Control	*a*	-1.1 ± 0.9	-0.3 ± 0.7	-1.3 ± 0.8†	-1.5 ± 0.8	-2.7 ± 0.7	-7.4 ± 1.0
		v	0.7 ± 1.0	0.3 ± 1.2	1.0 ± 0.7	1.0 ± 0.9	0.4 ± 1.0	-4.3 ± 0.5
	Dehydration	*a*	-1.7 ± 0.9	-1.7 ± 1.3	-1.9 ± 1.2	-2.0 ± 1.7	-1.4 ± 1.3	-
		v	0.6 ± 0.9	0.0 ± 1.2	-2.0 ± 2.1	-1.1 ± 2.2	2.7 ± 1.4	-
	Rehydration	*a*	-2.4 ± 0.7	-2.3 ± 0.8	-2.4 ± 0.8	-2.8 ± 0.9	-3.7 ± 0.9	-6.9 ± 1.0
		v	0.1 ± 0.8	-0.4 ± 0.8	-0.3 ± 0.8	0.0 ± 0.8	-3.4 ± 2.4	-2.6 ± 0.8
Lactate ($mmol \cdot l^{-1}$)	Control	*a*	0.8 ± 0.1	1.3 ± 0.1*†	1.7 ± 0.1*†	2.8 ± 0.2*†	5.6 ± 0.4*†	11.3 ± 0.7*†
		v	0.9 ± 0.1	1.3 ± 0.1*†	1.6 ± 0.1*†	2.6 ± 0.2*†	5.0 ± 0.4*†	10.1 ± 0.6*†
	Dehydration	*a*	2.1 ± 0.2	1.9 ± 0.2*†	1.6 ± 0.2*†	1.7 ± 0.2*	2.6 ± 0.2*†	-
		v	2.2 ± 0.2	1.9 ± 0.2*†	1.7 ± 0.2*†	1.7 ± 0.2*†	2.4 ± 0.2†	-
	Rehydration	*a*	3.3 ± 0.3	2.9 ± 0.2*†	2.5 ± 0.2*†	2.8 ± 0.2	4.8 ± 0.2*†	8.8 ± 0.3*†
		v	3.3 ± 0.3	2.9 ± 0.2*†	2.5 ± 0.2*†	2.9 ± 0.2†	4.3 ± 0.2*†	8.2 ± 0.3*†
Glucose ($mmol \cdot l^{-1}$)	Control	*a*	6.0 ± 0.2	6.0 ± 0.2	6.0 ± 0.2	5.9 ± 0.2	5.8 ± 0.2†	5.7 ± 0.2
		v	5.4 ± 0.2	5.4 ± 0.2	5.4 ± 0.2	5.3 ± 0.2†	5.2 ± 0.2†	5.0 ± 0.2†
	Dehydration	*a*	6.0 ± 0.2	5.6 ± 0.3*†	5.2 ± 0.3*†	5.0 ± 0.3*†	4.7 ± 0.2*†	-
		v	5.4 ± 0.2	4.9 ± 0.2*†	4.6 ± 0.2*†	4.2 ± 0.3*†	4.0 ± 0.3*†	-
	Rehydration	*a*	12.0 ± 0.7	11.2 ± 0.8*†	10.6 ± 0.8*†	9.7 ± 0.7*†	8.3 ± 0.7*†	6.6 ± 0.9*†
		v	11.0 ± 0.5	10.0 ± 0.5*†	9.4 ± 0.5*†	8.6 ± 0.5*†	7.4 ± 0.5*†	6.2 ± 0.5*†

Values are mean ± SEM for 10 participants. Partial pressure of CO_2 (PCO_2), sodium bicarbonate ([$HCO3^-$]), Acid-base excess (ABE), lactate and glucose for arterial (a) and internal jugular venous (v) blood. Rehydration values at 100% are n=5. * different from rest $P < 0.05$, † different from previous intensity.

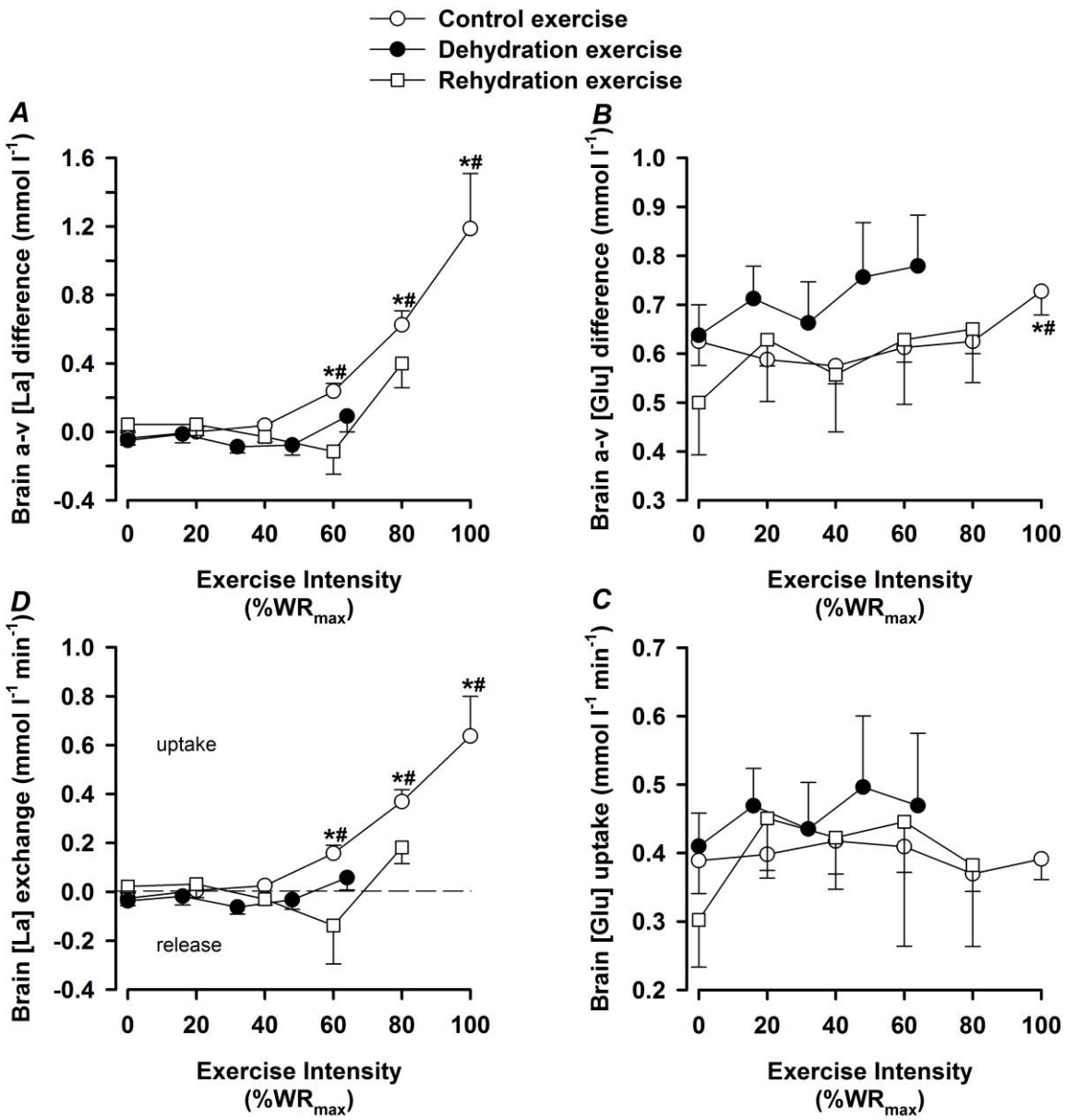


Figure 4-4. Brain a-v difference (A,B) and exchange/uptake (C,D) for lactate and glucose during control, dehydration and rehydration incremental exercise. Both the a-v lactate concentration [La] differences and the lactate exchange across the brain remained stable in the 3 trials up to 80% WRmax, but they increased significantly at maximal exercise in the control and rehydration trials. Similarly, the a-v glucose concentration [Glu] differences and the glucose uptake across the brain remained stable during the 3 incremental tests. Exchange calculated as the product of 2x ICA blood flow and a-v differences. Data are means ± SEM for 7 subjects. * different from rest $P < 0.05$, # $P < 0.05$ *vs.* sub-maximal exercise.

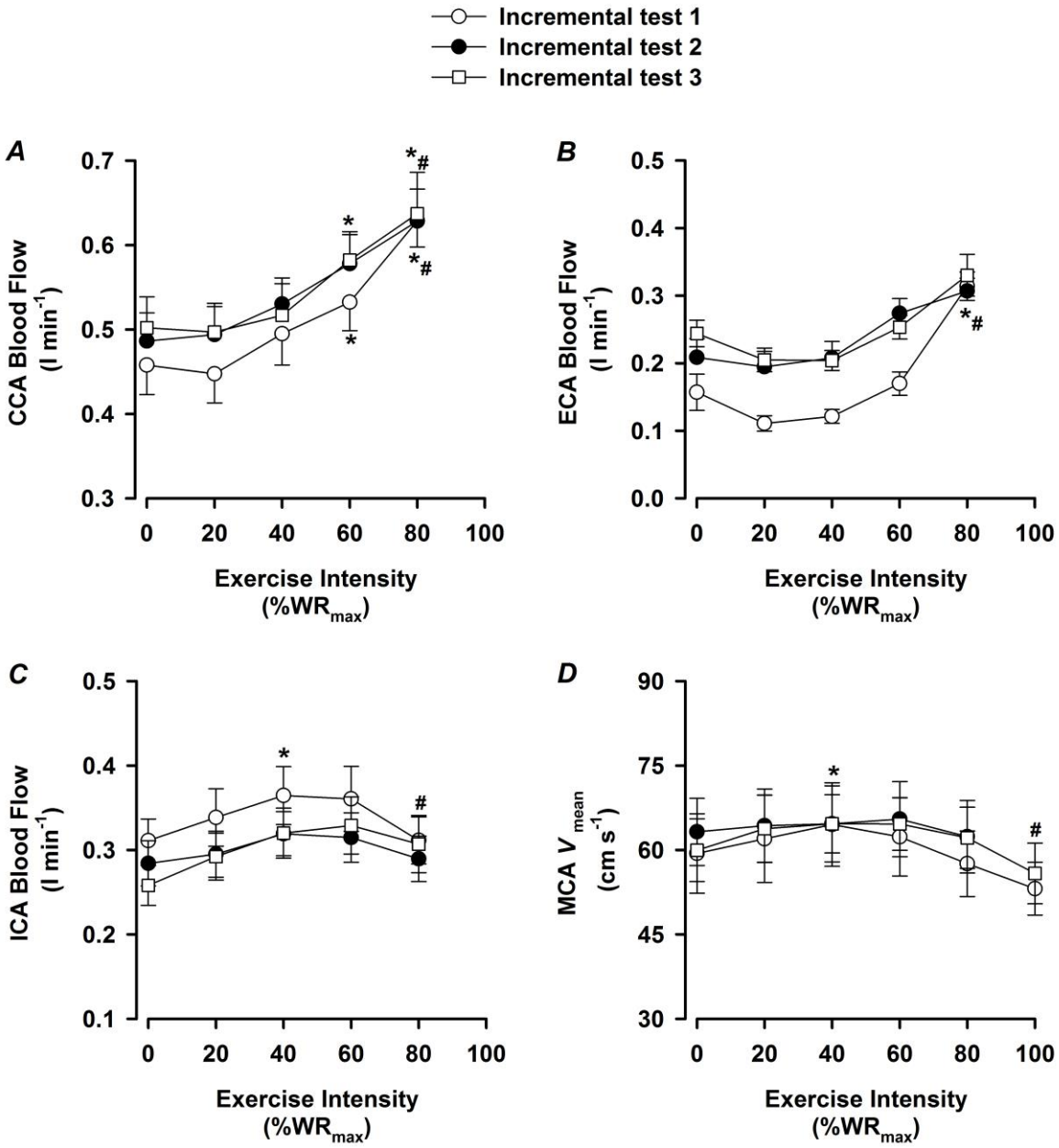


Figure 4-5. Blood flow responses during three incremental exercise tests on the euhydration trial. Common carotid (CCA), calculated external carotid (ECA), internal carotid (ICA) artery blood flow and middle cerebral artery velocity are presented. There were no significant differences for condition (i.e. INC 1, INC 2 and INC 3) for all variables, except calculated ECA blood flow which was significantly higher at rest and sub-maximal, but not at near maximal intensities. Values are mean ± SEM. *P* values represent ANOVA results. * is $P < 0.05$ *vs.* rest, # is $P < 0.05$ *vs.* sub-maximal exercise (i.e. ~40% WR_{max}).

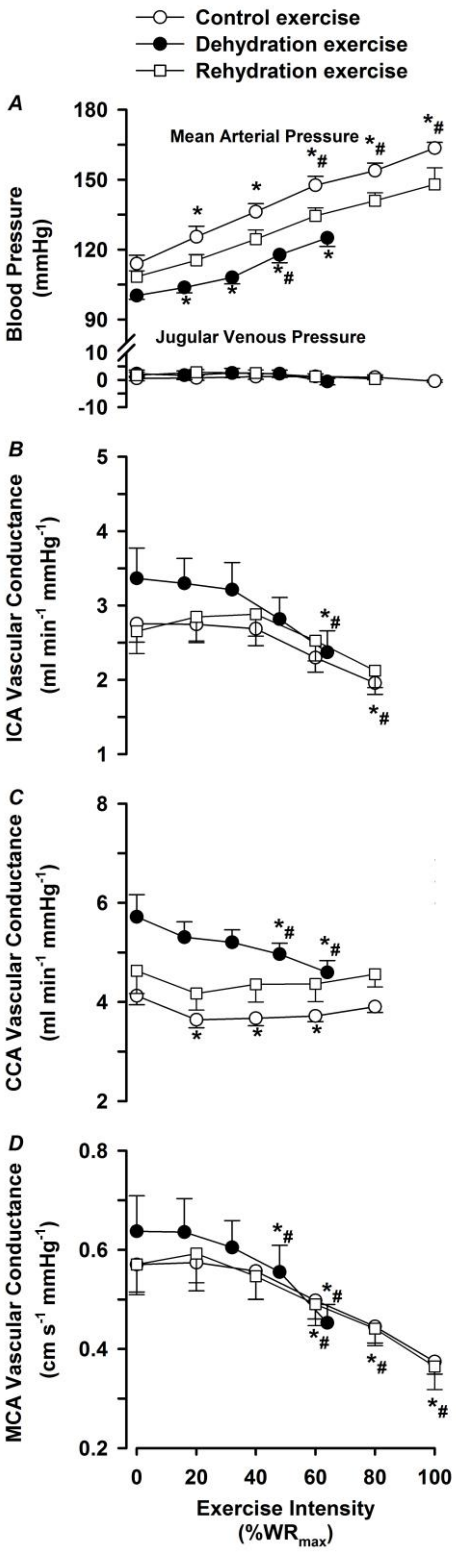


Figure 4-6. Cerebral vascular conductance and perfusion pressure during incremental exercise in different hydration states. Mean arterial and jugular venous pressures (A), internal carotid, common carotid and middle cerebral artery vascular conductance indices (B-D) for control (open circles), dehydration (closed circles) and rehydration (open squares) conditions. Values are mean ± SEM. P values represent ANOVA results. * is $P < 0.05$ *vs.* rest, # is $P < 0.05$ *vs.* sub-maximal exercise (i.e. ~40% WRmax). Significance for control and rehydration were similar in fig A, B and D.

4.4.4 Cerebral blood flow, PCO_2, $ctCO_2$ and temperature

At rest, P_aCO_2 was not different across conditions. The transition from rest to exercise resulted in an increase in P_aCO_2 in all incremental exercise conditions that continued up to 40% WR_{max} in control, whereas in DEH and REH P_aCO_2 was unchanged above 20% WR_{max}. Beyond sub-maximal intensities P_aCO_2 rapidly declined, by 6-7 mmHg, to below resting values in control (and REH), and by 3 mmHg in DEH ($P < 0.05$; Figure 4-7 A). P_vCO_2 increased from rest to 60% WR_{max} in control conditions before declining to baseline values at WR_{max}, whereas in DEH and REH P_vCO_2 was unchanged throughout exercise (Table 4-3). At rest arterial CO_2 content was lower in DEH compared to control and REH (479 ± 22 *vs.* 507 ± 17 and 495 ± 6 $ml \cdot l^{-1}$; Figure 4-7 B). From rest to WR_{max}, arterial CO_2 content declined to below resting values in control (and REH; $P < 0.05$), but a similar decline was not apparent in DEH. Jugular venous CO_2 content declined from rest to WR_{max} (581 ± 15 to 463 ± 11 $ml \cdot l^{-1}$; $P < 0.05$) in control conditions, whereas in DEH and REH CvO_2 content was unchanged throughout exercise (~553 ± 4 $ml \cdot l^{-1}$).

4.4.5 Relationships between cerebral blood flow and PCO_2, $ctCO_2$, pH and temperature

At rest and throughout incremental exercise in all conditions, ICA blood flow (R^2 = 0.41: Figure 4-7 D) and MCA V_{mean} (R^2 = 0.42: Figure 4-7 E) were correlated to changes in P_aCO_2 (both $P < 0.01$). In contrast, only non-significant correlations were observed for C_aCO_2 (R^2 = 0.16), P_vCO_2 (R^2 = 0.15) and $CvCO_2$ (R^2 = 0.19; P = 0.15-0.85). Also, CCA (R^2 = 0.05) and ICA (R^2 = 0.13) blood flow, in all conditions, were not correlated to jugular venous pH (both $P > 0.05$). Lastly, CCA blood flow in control and REH was correlated to changes in jugular venous temperature (R^2 = 0.68; $P < 0.001$: Figure 4-7 F), but not in DEH (R^2 = 0.00; P = 0.74).

4.4.6 Plasma catecholamines and ATP

At rest in DEH, arterial and jugular venous [NA] was higher than control and rehydration (13 ± 4 *vs.* 3 ± 1 and 3 ± 1 $nmol \cdot l^{-1}$ and 12 ± 4 *vs.* 2 ± 0.2 and 6 ± 2 $nmol \cdot l^{-1}$, respectively; $P < 0.05$). From rest to $WR_{max,}$ arterial and jugular venous [NA] increased exponentially in all conditions to a peak of 43 ± 10, 69 ± 19 and 82 ± 21 $nmol \cdot l^{-1}$, and 36 ± 8, 39 ± 10 and 27 ± 5 $nmol \cdot l^{-1}$ in dehydration, control and

rehydration, respectively. The reductions in ICA vascular conductance were correlated to an increased jugular venous [NA] (control R^2 = -0.79, dehydration and rehydration R^2 = -0.66; $P < 0.05$: Figure 4-8 B). On the other hand, arterial and jugular venous [A] was not different among conditions at rest (1.1 ± 0.3 *vs.* 0.8 ± 0.2 and 0.8 ± 0.2 nmol·l^{-1} and 1.0 ± 0.3 *vs.* 0.7 ± 0.1 and 0.6 ± 0.1 nmol·l^{-1}, respectively). Yet, from rest to WR_{max} in dehydration, control and rehydration conditions, [A] increased to a peak of 5.5 ± 1.9, 9.1 ± 2.2 and 7.7 ± 2.8 nmol·l^{-1} in arterial and 6.5 ± 2.4, 8.5 ± 3.6 and 3.3 ± 1.1 nmol·l^{-1} in venous plasma respectively (all $P < 0.05$). Lastly, arterial plasma [ATP] increased in a curvilinear manner from similar values at rest (1058 ± 177 *vs.* 938 ± 128 and 1027 ± 199 nmol·l^{-1}) to WR_{max}, and was higher in dehydration compared to control and rehydration at maximal intensities (1641 ± 189 *vs.* 1403 ± 221 and 1274 ± 188 nmol·l^{-1}; $P < 0.05$).

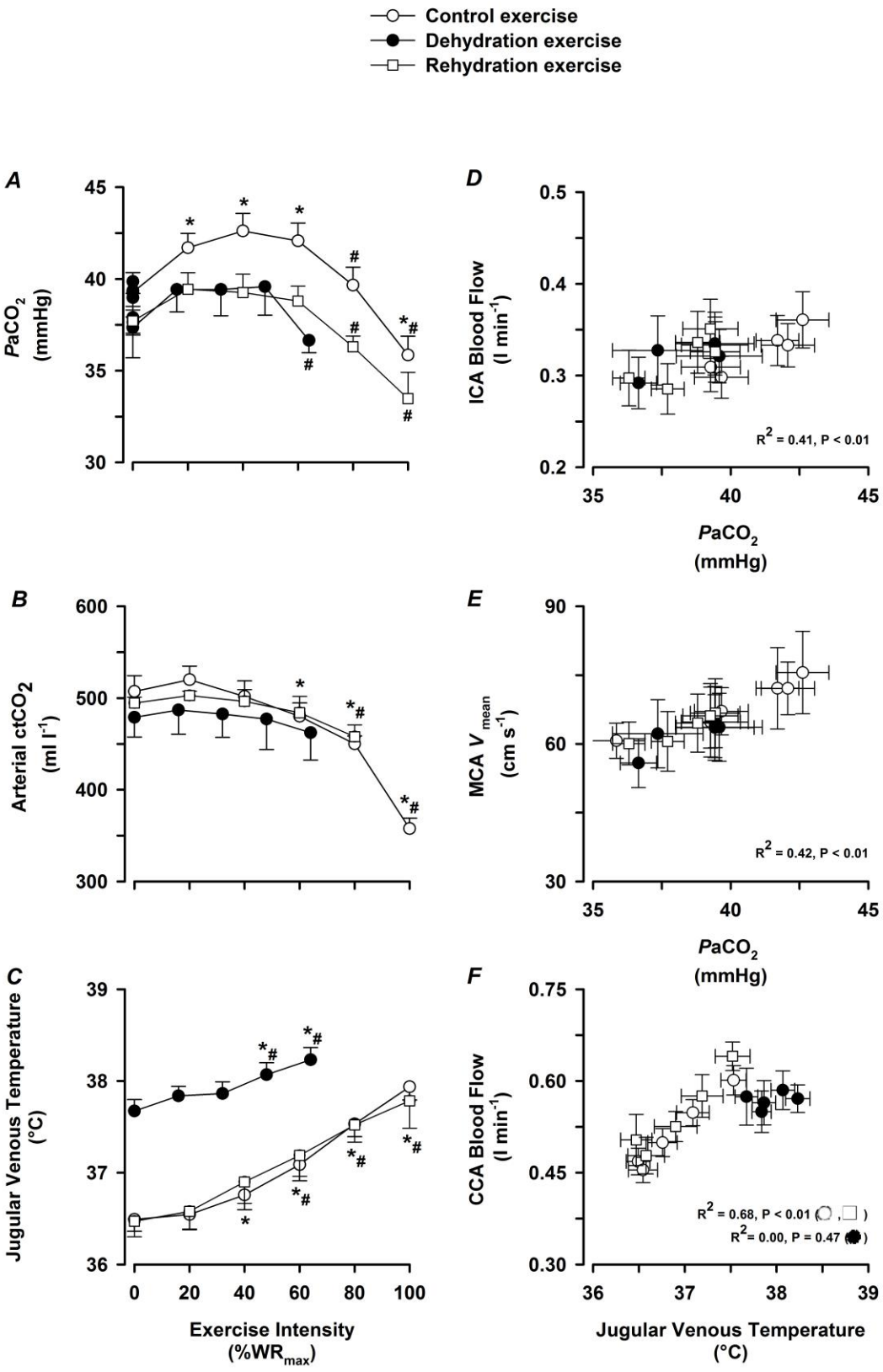


Figure 4-7. Relationships between cerebral perfusion and blood PCO_2 and temperature. Left panel; P_aCO_2 (A), arterial CO_2 content (B), and jugular venous temperature responses to incremental exercise (C). Right panel; ICA blood flow and MCA V_{mean} group mean correlations with P_aCO_2 (D-E), and CCA blood flow group mean correlation to jugular venous temperature (F) in control (open circles), dehydration (closed circles) and rehydration (open squares). * is $P < 0.05$ *vs.* rest, # is $P < 0.05$ *vs.* sub-maximal exercise (i.e. ~40% WRmax). Unless presented, significance for control and rehydration were similar.

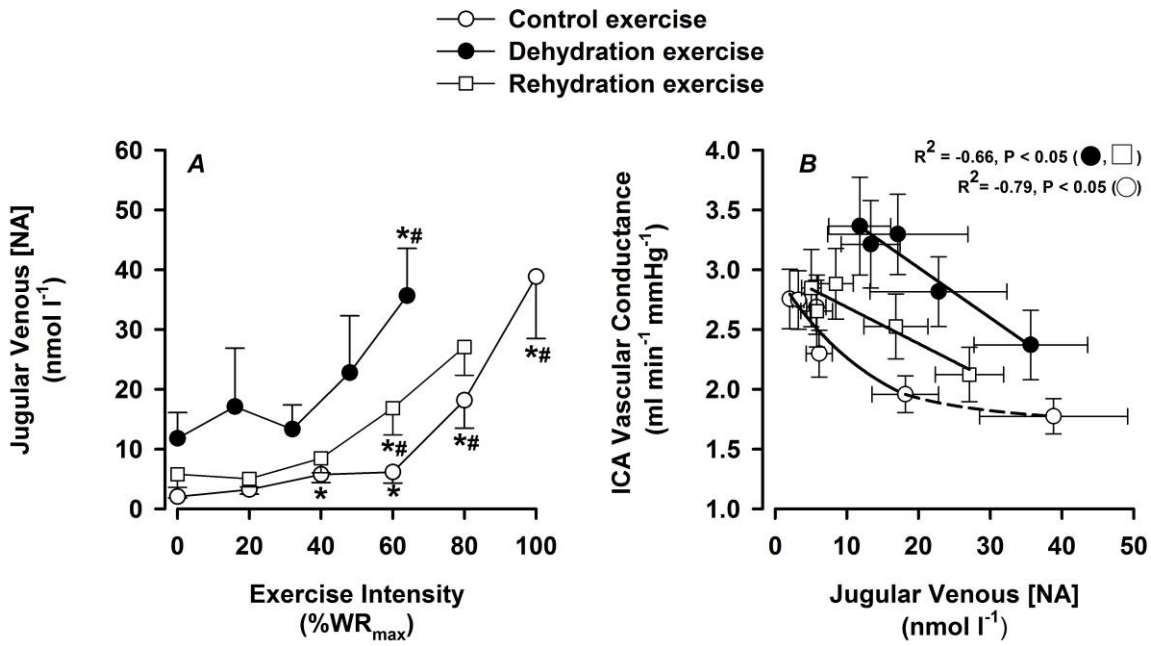


Figure 4-8. Jugular venous [NA] during incremental exercise and relationship of ICA vascular conductance and jugular venous [NA]. Jugular venous [NA] and the relationship between ICA vascular conductance and jugular venous [NA] in control (open circles), dehydration (closed circles) and rehydration (open squares). * is $P < 0.05$ *vs.* rest, # is $P < 0.05$ *vs.* sub-maximal exercise (i.e. ~40% WRmax). Unless presented, significance for control and rehydration were similar.

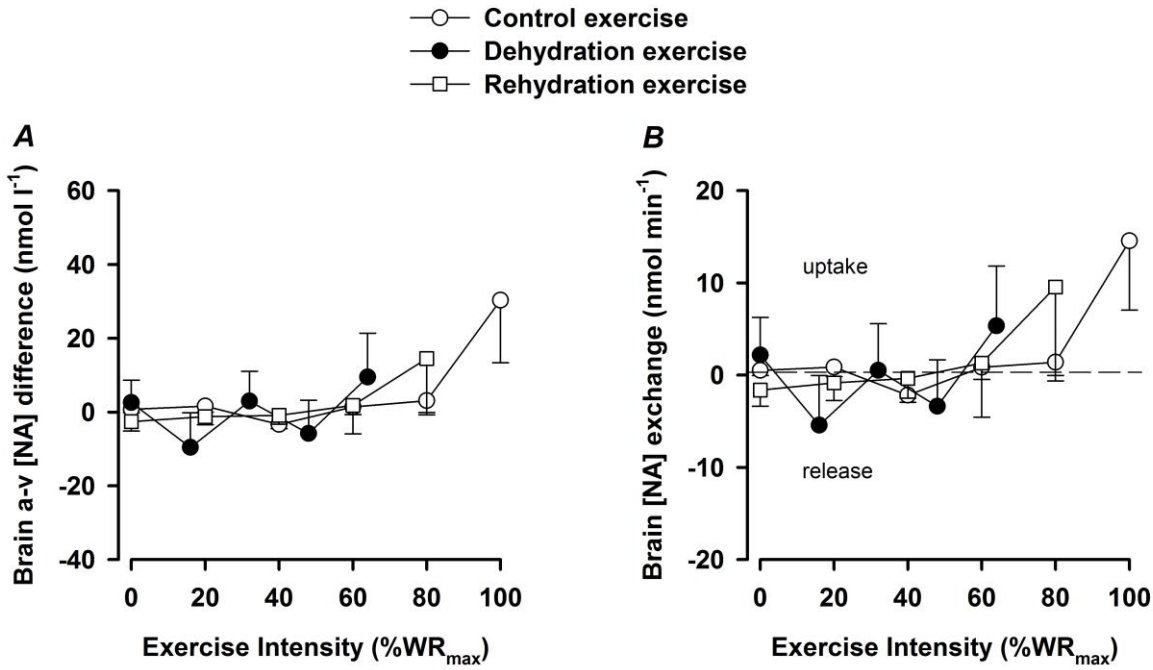


Figure 4-9. Brain [NA] exchange during incremental exercise. The a-v noradrenaline concentration [NA] differences and exchange across the brain remained stable in the 3 trials up to 80% WRmax. Exchange calculated as the product of 2x ICA blood flow and a-v differences. Data are means ± SEM for 7 subjects.

4.5 Discussion

The novel findings of the present study were threefold. Firstly, during exercise in control conditions cerebral perfusion increased from rest to moderate exercise in the heat, before declining to baseline values prior to exhaustion. Secondly, dehydration accelerated the declines in blood flow and O_2 delivery to the brain during incremental cycling exercise to exhaustion in association with a blunted perfusion pressure, reductions in P_aCO_2 and increases in internal jugular venous NA. In contrast to the evident cerebral circulatory strain during the intense exercise stages, common carotid artery blood flow increased from rest to peak exercise in the control and rehydration conditions and remained unchanged with dehydration, indicating that blood flow to extra-cranial tissues increase was related to the increase in temperature (jugular blood). Finally, compensatory increases in brain O_2 extraction maintained $CMRO_2$ throughout exercise in association with a stable or increasing CO_2 production. Collectively these findings suggest that the circulatory strain on the human brain during maximal exercise in the heat, even with dehydration does not compromise $CMRO_2$.

Hydration and perfusion of the head

The current study demonstrates that CBF, blood velocity and O_2 delivery are attenuated prior to the attainment of maximal work rate and that dehydration accelerates this restriction in cerebral perfusion. The decline in cerebral perfusion is in agreement with investigations in humans during graded incremental exercise (Moraine *et al.* 1993; Hellstrom *et al.* 1996; Sato *et al.* 2011) and intense constant load exercise, with and without heat stress (Nybo & Nielsen 2001b; Nybo *et al.* 2002; González-Alonso *et al.* 2004). We have extended these findings by obtaining direct measurements of anterior CBF under conditions that challenge the cardiovascular system to its capacity and examined the functional consequences of a diminished flow on $CMRO_2$ during strenuous exercise.

The common carotid artery forms a major part of the extra-cranial circulation through to the ECA. During all incremental exercise conditions extra-cranial perfusion (CCA and calculated ECA flow; CCA-ICA) increased or was maintained. Strikingly, at rest prior to the dehydration test CCA blood flow was elevated by 25% whereas ICA blood flow was only modestly increased (~6%), indicating a substantially augmented ECA blood flow compared to control when participants'

jugular venous and core temperatures were elevated by 1.2-1.5 °C. Additionally, ECA blood flow increased by ~50% from baseline to 80% WR_{max} (217 ± 30 to 307 ± 22 $ml \cdot min^{-1}$) and achieved a similar peak value across interventions. These findings are consistent with an elevated extra-cranial blood flow with graded exercise in normothermic conditions (Hellstrom *et al.* 1996; Sato *et al.* 2011) and with passive heating at rest (Fan *et al.* 2008; Ogoh *et al.* 2013b). Heat stress, with and without concomitant dehydration, results in a distinct cardiovascular strain (Sawka *et al.* 1979; Montain & Coyle 1992a; 1992b; González-Alonso *et al.* 1997; González-Alonso 1998) and promotes redistribution of blood flow to the skin vascular beds for thermoregulatory purposes (Crandall *et al.* 2008; Crandall & González-Alonso 2010; Johnson & Kellogg 2010). Given that the ECA supplies the majority of the cutaneous circulation of the face and neck, an elevated blood flow to these regions is important for local convective heat exchange. Collectively these findings show contrasting blood flow adjustments across the different vascular beds of the head during strenuous exercise in the heat with both dehydration and euhydration.

Mechanisms of cerebral and extra-cranial blood flow control

In all incremental exercise conditions attenuation in cerebral perfusion was coupled to a decline in cerebral-vascular conductance, indicative of vasoconstriction and thus diminished vessel diameter (Figure 4-6 B, D). Alterations in P_aCO_2 and blood CO_2 content, increased sympathetic nerve activity and concurrent changes in the intra- and extravascular milieu of vasoconstrictor and vasodilator signals may all play a role in restricting CBF (Paulson *et al.* 1990; Ide & Secher 2000; Secher *et al.* 2008; Ogoh & Ainslie 2009b). During strenuous exercise cerebral perfusion was associated with the decrease in P_aCO_2 (Figure 4-7 A, D-E). Given that free CO_2 accounts for only a minor portion of the CO_2 in blood, we reasoned that $ctCO_2$ would indicate whether plasma and/or blood CO_2 is important for the decline in cerebral perfusion. In contrast to the prominent association with P_aCO_2, the correlation with arterial or jugular venous blood $ctCO_2$ was non-significant, indicating that the cerebral circulation is sensitive to changes in free blood PCO_2 rather than to changes in CO_2 bound to haemoglobin or buffered as bicarbonate in the arterial or venous vasculature. There is also controversy in regards to the role of cerebral venous versus arterial PCO_2 on regulation of brain blood flow (Peebles *et al.* 2007). The current study shows that

the relationship between brain flow and P_vCO_2 was not significant because of the maintenance or minimal changes in jugular P_vCO_2. Furthermore, the impact of arterial PO_2 and HbO_2 saturation on CBF is negligible in the present conditions because the changes in these variables during incremental exercise were too small to activate the oxygen sensitive pathways of local CBF control (Willie *et al.* 2012). CO_2 readily crosses the blood brain barrier, altering the extracellular and cerebral spinal fluid (CSF) pH and PCO_2 and there is compelling evidence to suggest that pH has an independent and local effect on cerebral vessel vasoconstriction (with acidosis leading to cerebral vasodilation and alkalosis leading to cerebral vasoconstriction; Kontos *et al*. 1977*a,b*). However, there was no relationship between blood flow to the brain and jugular venous pH. Jugular venous pH may or may not reflect the environment of the extracellular space of the cerebral vasculature and the results suggest that pH is well maintained across the brain. The balance of pH (through the direct effects of CO_2 and the buffering capacity of blood) is therefore important for the CBF response (Willie *et al.* 2014). Together, these findings point to a predominant influence of the arterial over that of the venous and thereby tissue CO_2 in the regulation of CBF.

The present observations are consistent with the concept that the cerebral vasculature is highly sensitive to alterations in P_aCO_2 (Jorgensen *et al.* 1992b; Secher *et al.* 2008), as evidenced by the ~4% change in global and regional CBF per mmHg change in P_aCO_2 (expressed as the “cerebral CO_2 reactivity”) (Madsen *et al.* 1993; Linkis *et al.* 1995; Willie *et al.* 2012), similar to that observed for regional CBF in the present study. The decline in P_aCO_2 beyond moderate exercise intensities occurs in combination with the exponential increase in ventilation, which is accelerated under conditions that induce whole-body hyperthermia (Nybo & Nielsen 2001b; Nybo *et al.* 2002; Wilson *et al.* 2006; Brothers *et al.* 2009b; Brothers *et al.* 2009c; Nelson *et al.* 2011; Ross *et al.* 2012). An important question is whether changing P_aCO_2 levels independently, or in combination with other related vasoconstrictor signals, are restricting CBF during intense exercise. We found that the decline in cerebral vascular conductance was associated with the large increase in jugular venous [NA]. An increase in circulating [NA] may influence cerebrovascular tone (Lee *et al.* 1976; Mitchell *et al.* 2009; Ogoh & Ainslie 2009a; Seifert & Secher 2011) and is associated with enhanced $CMRO_2$ (King *et al.* 1952; Nemoto *et al.* 1996): however, controversy

remains on its role within the cerebral vasculature (Strandgaard & Sigurdsson 2008b; van Lieshout & Secher 2008b). Irrespective of hydration status it appears that increasing jugular venous [NA] during intense exercise reflects increased local sympathetic vasoconstrictor activity and may explain some of the decline in CBF. However, increased circulating [NA] may not directly result in local vasoconstriction and the importance of sympathetic activity above and beyond the role of P_aCO_2 remains unclear.

In contrast to the close coupling between reductions in P_aCO_2 and cerebral perfusion, the relationship does not hold for the extra-cranial circulation (Sato *et al.* 2012; Ogoh *et al.* 2013b), similar to that of peripheral vessels (Ainslie *et al.* 2005; Sato *et al.* 2012). The contrasting responses between the two vascular beds during exercise are interpreted to mean that blood flow is redistributed from the cerebral to the extra-cranial circulation (Sato *et al.* 2011). However, this is an unlikely scenario as preventing the decline in cerebral perfusion during passive hyperthermia through the clamping of end-tidal CO_2 does not alter extra-cranial blood flow (Bain *et al.* 2013). Equally, reducing extra-cranial perfusion, through face cooling, appears to not influence MCA V_{mean} at rest or during light exercise (Miyazawa *et al.* 2012). Whilst P_aCO_2 may not play an important role in the regulation of blood flow to the extra-cranial circulation, mechanisms involving temperature sensitive pathways seem to do so. We observed for the first time a strong correlation between increases in common carotid artery blood flow and internal jugular venous temperature during control and REH incremental exercise (Figure 4-7 F). Additionally, with a rising blood temperature during incremental exercise in all three exercise conditions (up to 1.1 °C), the plasma concentration of the potent intravascular vasodilator ATP increased in arterial blood; a potential mechanism for the temperature-related increase in regional perfusion (Pearson *et al.* 2011; González-Alonso 2012; Kalsi & González-Alonso 2012). Irrespective of the mechanisms, the progressive increase in extra-cranial perfusion may be an important pathway by which heat is locally dissipated to regulate temperature of the tissues within the head (Sato *et al.* 2011). Collectively, these data suggest that cerebral perfusion is restricted with a declining cerebral vascular conductance via a net increase in vasoconstrictor activity. Alterations in P_aCO_2 appear to be the primary mechanism for regulation of cerebrovascular tone, but not extra-cranial vessel conductance.

Is brain oxygen consumption compromised with dehydration during maximal incremental exercise?

An important question is whether central nervous system activity and thus cerebral metabolic demand rise sufficiently during strenuous exercise to increase $CMRO_2$ and whether reductions in flow result in a compromised $CMRO_2$. A major finding of the present study was that $CMRO_2$ was not compromised throughout incremental exercise across exercise conditions in spite of an attenuated perfusion at maximal intensities. This response was met by an increased O_2 extraction during maximal exercise, a response enhanced with dehydration. Our findings of an enhanced O_2 extraction and a maintained $CMRO_2$ are similar to observations during constant load sub-maximal (Ide & Secher 2000; Nybo *et al.* 2002; González-Alonso *et al.* 2004; Secher *et al.* 2008) and maximal exercise (Scheinberg *et al.* 1954; González-Alonso *et al.* 2004). Nevertheless, the possibility exists that $CMRO_2$ is somewhat suppressed during maximal exercise and dehydration due to reduced O_2 supply. In this respect, strenuous exercise with hyperthermia increases $CMRO_2$, a response attributed to the requirement of an increased neuronal activity associated with mental effort and the Arrhenius (Q_{10}) effect of temperature on brain metabolism (Nybo *et al.* 2002). A marked reduction in O_2 supply might lower intracellular PO_2 to the extent that affects metabolic fluxes and challenge cerebral metabolism and motor function (Gjedde *et al.* 2005; Nybo & Rasmussen 2007; Rasmussen *et al.* 2007; Seifert *et al.* 2009; Rasmussen *et al.* 2010). Moreover, in accordance with the Q_{10} effect, a rise in core temperature by 2 °C would be expected to raise the $CMRO_2$ by ~15%; whereas, the $CMRO_2$ is observed to be only one half of the expected (Rasmussen *et al.* 2010). However, in spite of the 20% reductions in perfusion observed across conditions from submaximal to maximal exercise, it is unlikely that the capillary to intracellular PO_2 gradient was reduced to the extent that would compromise $CMRO_2$ given that fractional oxygen extraction increased from 34% at rest to 39% at maximal exercise and was thereby within the range of adequate cerebral tissue oxygenation (Gjedde *et al.* 2005). This notion is consistent with the parallel observations that brain glucose uptake was well-maintained across exercise intensities and hydration conditions and lactate uptake was maintained or elevated (Figure 4-4). Whilst it is difficult to speculate on the alterations within the deep structures of the brain, the current data suggest that brain oxygen consumption is not reduced during intense exercise in the heat, with and without concomitant dehydration.

Methodological considerations

There are several methodological considerations in the present study. Firstly, blood flow measurements were made in the right CCA and ICA, whereas the vessels on the left hand side of the anterior circulation and the vessels of posterior circulation were not measured. In regard to the anterior circulation, side-to-side blood flows at rest and during exercise are similar (Schoning *et al.* 1994; Sato *et al.* 2011; Willie *et al.* 2012). Secondly, blood flow measurements were made by one sonographer. Upon the transition from CCA to ICA ultrasound scans, a temporal lag and minor shift in sample area may occur. Care was taken to ensure a consistent measuring site for each participant and the use of duplex ultrasound allowed the continued monitoring of sample position. Thirdly, in contrast to previous literature observing the right internal jugular vein, we obtained venous blood samples from the left internal jugular vein. Asymmetry may exist in the venous drainage of the brain with the often larger right-internal jugular vein draining the hemispheres and the left-internal jugular vein draining the subcortical areas (Seifert & Secher 2011). However, similar resting values for blood parameters and a-vO_2 difference values are reported in the two jugular veins (Gibbs *et al.* 1942; Munck & Lassen 1957). Moreover, comparable a-vO_2 difference dynamics is observed during incremental exercise based on right jugular vein blood samples (Ide *et al.* 1999b). We therefore assumed equal blood flow and O_2 extraction in the left and right sides of the brain to estimate the $CMRO_2$ index. Thirdly, the $CMRO_2$ index underestimates the global $CMRO_2$ because blood flow through the posterior circulation is not considered. The posterior portion of the brain is supplied by the two vertebral arteries (VA) that anastomose to form the basilar artery before joining the circle of Willis, and their contribution to total brain blood flow is ~20% at rest (Zauner *et al.* 1997). VA flow increases progressively with graded exercise intensities, in contrast to the anterior circulation (ICA) (González-Alonso *et al.* 2004; Sato *et al.* 2011; Sato *et al.* 2012). Thus, if we assume that VA blood flow increases, or follows the same pattern as the ICA, $CMRO_2$ would remain unchanged during exercise in the conditions of the present study. Finally, we were unable to obtain satisfactory ultrasound images during the final stage (100%) in control and rehydration conditions. Blood flow in these stages, used for the calculation of $CMRO_2$, was estimated using the percent decline in MCA V_{mean} from the 80 to 100% work rate. This assumption has been

used to assess changes in flow and $CMRO_2$ during maximal exercise (Fisher *et al.* 2013).

4.6 Conclusion

The present findings demonstrate that dehydration restricts CBF during strenuous exercise. The blunted CBF was associated with a decline in vascular conductance and P_aCO_2 and an in increase in systemic and jugular venous noradrenaline, indications of an enhanced vasoconstrictor activity. Cerebral oxygen extraction was increased during strenuous exercise, more so when perfusion was challenged with dehydration. In contrast, extra-cranial perfusion increased, mirrored by increases in blood temperature. Thus, reductions in cerebral perfusion and cerebral vascular conductance during maximal exercise in different hydration states does not appear to negatively impact $CMRO_2$ because of compensatory increases in cerebral oxygen extraction.

CHAPTER 5 – Dehydration accelerates the reduction in cerebral and extra-cranial blood flow during prolonged exercise in the heat

5.1 Summary

Reductions in oxygen and substrate supply can compromise organ and tissue metabolism during severe stress. Here, we examined whether dehydration during prolonged strenuous exercise in the heat impairs cerebral and extra-cranial blood flow and cerebral metabolism in trained humans. Ten cyclists cycled in a hot environment for ~2 h with and without fluid replacement while measurements of cerebral (internal; ICA) and external (ECA) carotid artery blood flow, arterial and internal jugular venous blood samples and core and blood temperature were obtained. After a rise at the onset of exercise, ICA blood flow declined to baseline values with progressive dehydration ($P < 0.05$). However, cerebral metabolism remained stable through enhanced oxygen extraction and glucose uptake ($P < 0.05$). ECA blood flow increased from rest to 60 min but declined prior to exhaustion. Fluid ingestion sufficient to prevent dehydration maintained cerebral and extra-cranial blood flow throughout non-fatiguing exercise. In conclusion, dehydration during prolonged exercise in the heat induces a circulatory strain on the human brain characterised by a blunted cerebral and extra-cranial blood flow, which is prevented or delayed when hydration is maintained. However, cerebral metabolism is maintained through increases in oxygen and substrate extraction from blood circulating in the brain.

5.2 Introduction

Dehydration and hyperthermia accrued during prolonged exercise in the heat pose a severe challenge to cardiovascular regulation, evidenced by reductions in stroke volume, contracting skeletal muscle perfusion, skin blood flow, cardiac output ($\dot{Q}$) and to a lesser extent mean arterial pressure (Montain & Coyle 1992b; González-Alonso *et al.* 1995; González-Alonso *et al.* 1998). This acute cardiovascular strain might also encompass reductions in cerebral blood flow (CBF) as orthostatic challenges (Ogoh *et al.* 2005b) and pharmacological interventions that depress $\dot{Q}$ (Ide *et al.* 2000), severe passive heat stress (Nelson *et al.* 2011; Bain *et al.* 2013), and combined heat stress with exercise (Nybo & Nielsen 2001b) compromise cerebral perfusion. In the previous chapter (4), it was shown that dehydration led to earlier reductions in CBF during graded exercise to exhaustion, but that compensatory increases in oxygen extraction sufficiently preserved cerebral aerobic metabolism. Thus, during short bouts of high intensity exercise, attenuated cerebral oxygen delivery is unlikely to hinder cerebral function. It is, however, possible that exercise-induced dehydration over a prolonged period may yet impair CBF and metabolism, and might constitute a scenario whereby exercise capacity is reduced by central cerebral mechanisms. However, no study has systematically manipulated hydration status to characterise the effects of prolonged exercise-induced dehydration on the human brain circulation.

Thermoregulatory processes modify the distribution of blood flow, favoring the enhancement of cutaneous perfusion (Crandall *et al.* 2008), to regulate body temperature during exercise, particularly in conditions of heat stress (González-Alonso *et al.* 2008). Exercise (Sato *et al.* 2011) and heat stress (Bain *et al.* 2013; Ogoh *et al.* 2013b) have been shown to independently induce disparate blood flow responses across the head. In light of the possible contrasting distribution of regional blood flow across the head, the mechanisms regulating CBF and extra-cranial blood flow also appear to differ. Altered perfusion pressure, blood gas tensions (particularly the partial pressure of CO_2; P_aCO_2) (Willie *et al.* 2012) and sympathetic activity (Mitchell *et al.* 2009) have been implicated in the control of CBF whereas, body temperature is purported to influence extra-cranial flow (Sato *et al.* 2011; Sato *et al.* 2012; Ogoh *et al.* 2013b).

Reductions in oxygen and substrate supply can compromise organ and tissue metabolism during severe stress (González-Alonso *et al.* 1998) and the circulatory strain induced by dehydration during prolonged exercise in a hot environment may compromise cerebral substrate delivery to the extent that it impairs cerebral metabolism. Alternatively, cerebral metabolism may be maintained through compensatory increases in O_2 extraction (González-Alonso *et al.* 2004) as evidenced by previous reports showing an enhanced (Nybo *et al.* 2002) or stable (Trangmar *et al.* 2014) cerebral metabolic rate for oxygen ($CMRO_2$) during strenuous exercise. Whether a dehydration-induced challenge to cerebral metabolism is an important factor causing early fatigue during prolonged exercise in the heat remains to be determined.

The aim of this study was to assess the effect of dehydration on cerebral and extra-cranial haemodynamics, and cerebral metabolism during prolonged exercise in the heat. A second aim was to gain insight into the mechanisms regulating cerebral and extra-cranial blood flow during prolonged exercise. We hypothesised that dehydration accrued during prolonged exercise in the heat would reduce CBF whereas extra-cerebral blood flow would increase; responses that would be prevented with the maintenance of hydration. Furthermore, we hypothesised that compensatory adjustments in oxygen and substrate extraction from blood would enable the maintenance of the $CMRO_2$ and carbohydrate uptake across the brain.

5.3 Materials and methods

5.3.1 Participants

The participants who took part in the present study were those in the study described in Chapter 4.

5.3.2 Experimental design

The present study formed part of a previous investigation on the effects of dehydration on CBF and metabolism during maximal incremental cycling exercise (Chapter 4; Trangmar *et al.* 2014). Aspects of the experimental design and methodological procedures which are unique to the present chapter are outlined; the reader may refer to Chapter's 3 and 4 for more details where relevant. As

previously outlined, participants visited the laboratory for 3 preliminary sessions followed by 2 experimental sessions, each separated by at least one week.

After the three preliminary (familiarisation) sessions, participants returned to complete two experimental days (visits 4 and 5). On the first experimental trial, participants cycled continuously for two hours and were not permitted to consume fluid whereas, on the second experimental trial (i.e., control trial), participants completed the same exercise protocol, but hydration was maintained through fluid ingestions according to the participant's body mass loss during the previous visit. Fluid was provided in aliquots of ~160 ml every 10 min during exercise. Both experimental trials were performed in the heat (same conditions as in the familiarisation sessions) and pedal cadence was maintained consistently between 70-90 r.p.m.

In the dehydration trial, cerebral haemodynamics and blood samples from the brachial artery and left internal jugular vein were obtained simultaneously at rest and every 30 min during prolonged exercise. Core, skin and jugular venous temperatures and arterial and jugular venous pressures were recorded continuously. The same measures were collected in the control trial, except for the arterial and internal jugular venous blood sampling and intra-arterial/venous blood pressures.

5.3.3 Cerebral haemodynamics

Measurement of vessel blood flow, obtained by a single experienced sonographer, were obtained sequentially at rest and every 30 min from the right internal (ICA), external (ECA) and common carotid arteries (CCA) using an ultrasound system (Vivid 7 Dimension, GE Healthcare, UK) equipped with a 10 MHz linear array transducer. ICA, ECA and CCA measurements were typically taken ~1.0-1.5 cm above and ~1.5 cm below the carotid bifurcation, respectively (Sato *et al.* 2011; Willie *et al.* 2012; Ogoh *et al.* 2013b), and the coefficient of variations for measurements of ICA, ECA and CCA vessel diameter and volume flow at rest (2.8 ± 0.9%, 2.1 ± 1.1% and 4.3 ± 1.0%), and during exercise (5.3 ± 1.6%, 5.1 ± 1.4% and 5.0 ± 1.6%) were considered within acceptable ranges. Calculations of vessel diameter and volume flow were made as previously outlined (see Chapter's 3 and

4). MCA V_{mean} was measured using a 2MHz pulsed trans-cranial Doppler ultrasound system (DWL, Sipplingen, Germany). The right MCA was insonated through the temporal ultrasound window, distal to the MCA-anterior cerebral artery bifurcation, at a depth of 45-60 mm. Signal quality was optimised according to published standards (Aaslid *et al.* 1982).

5.3.4 Catheter placement and blood sampling

Catheters for blood sampling, blood pressure (MAP), internal jugular venous pressure and blood temperature were inserted into the brachial artery of the non-dominant arm and, after local anaesthesia (2% lidocaine), in the left internal jugular vein (Double Lumen Catheter, 16 gauge, 2.3 mm; Multi-Med M2716HE, Edwards Lifesciences, USA) as previously outlined (see Chapter 4).

5.3.5 Blood parameters

Arterial and jugular venous blood gas variables (ABL 800 FLEX, Radiometer, Copenhagen, Denmark) and plasma adrenaline and noradrenaline concentrations were determined as previously outlined (see Chapter 4).

5.3.6 Heart rate, blood pressure and temperatures

Heart rate (Polar Electro, Kempele, Finland), arterial and internal jugular venous pressures (Pressure Monitoring Kit, TruWave, Edwards Lifesciences, Germany), intestinal temperature (HQInc, Palmetto, Florida, USA) and mean skin temperature (TC-2000, Sable Systems, NV, USA) were obtained and analysed as previously outlined (see Chapter 4).

5.3.7 Calculations

Cerebral vascular conductance (CVC) indices, arterial oxygen content, the cerebral metabolic rate for oxygen ($CMRO_2$) and cerebral glucose and lactate uptake were calculated as previously outlined (see Chapter 4). The molar ratio of oxygen to glucose (O_2/glucose index: OGI) and oxygen to carbohydrate (O_2/glucose + ½lactate index: OCI) and whole blood CO_2 content (Douglas *et al.* 1988) were also calculated.

5.3.8 Data analysis

All analyses were made using IBM SPSS Statistics (Version 20, IBM Corporation, Armonk, NY, USA). A one-way repeated-measures ANOVA was used for the assessment of changes over time. Where applicable, measured variables between conditions were analysed using two-way repeated-measures ANOVA in which condition (dehydration and control) and exercise phase (rest, 30, 60, 90 and 120 min) were the main factors. Multiple regressions for within-subject repeated measures were used for the analysis of the relationship between blood flow and blood gas variables and temperatures (Bland & Altman 1995). All data are presented as mean ± SEM and the alpha level for statistical significance was set at $P < 0.05$.

5.4 Results

5.4.1 Temperature response to prolonged exercise

Dehydration exercise resulted in a 3% body mass reduction (78.2 ± 2.7 to 75.8 ± 2.7 kg) and a 10 min reduction in exercise duration (110 ± 8 *vs.* 120 min in control; both $P < 0.001$). Body mass in the control exercise trial was maintained at pre-exercise levels (79 ± 3 kg) through the consumption of fluid at a rate of ~1.2 $l \cdot h^{-1}$. The decline in body mass with dehydration was accompanied by concomitant increases in arterial and venous [Hb] ($P < 0.05$: Table 5-2); indicative of blood volume reductions. Intestinal temperature increased progressively in both trials; but was higher at end exercise in dehydration compared to control (38.6 ± 0.2 *vs.* 38.1 ± 0.1 °C; $P < 0.05$: Table 5-1). Internal jugular venous blood temperature mirrored the rise in intestinal temperature in the dehydration trial (Figure 5-5 B). Mean skin temperature ($\overline{T}_{sk}$) was maintained stable throughout exercise in both the dehydration and control trials (33.0 ± 0.3 and 32.8 ± 0.3 °C: Table 5-1). Heart rate was similar at rest but during exercise was maintained ~12 $beats \cdot min^{-1}$ higher in the dehydration compared to the control trial ($P < 0.05$: Table 5-1).

Table 5-1. Cardiovascular and temperature responses during prolonged exercise.

		Prolonged cycling time (min)				
		Rest	**30**	**60**	**90**	**110/120**
T_I (°C)	Dehydration	37.4 ± 0.1	38.0 ± 0.1*	38.4 ± 0.1*	38.6 ± 0.1*	38.7 ± 0.1*
	Control	37.3 ± 0.1	37.9 ± 0.1*	38.1 ± 0.1*	38.2 ± 0.1*	38.2 ± 0.2*
T_B (°C)	Dehydration	36.9 ± 0.1	37.9 ± 0.1*	38.2 ± 0.1*	38.5 ± 0.1*	38.7 ± 0.1*
$\overline{T}_{sk}$ **(°C)**	Dehydration	34.0 ± 0.3	33.1 ± 0.4	32.7 ± 0.4	32.8 ± 0.4	32.6 ± 0.3
	Control	33.5 ± 0.3	32.6 ± 0.3	32.6 ± 0.3	32.8 ± 0.3	32.3 ± 0.3
HR (beats·min^{-1})	Dehydration	80 ± 3	148 ± 2*	157 ± 2*†	163 ± 2*†	166 ± 3*†
	Control	77 ± 2	142 ± 3*	145 ± 3*	149 ± 3*†	149 ± 3*†

Values are mean ± SEM for 10 subjects. T_I, intestinal temperature; T_B, blood temperature; $\overline{T}_{sk}$, mean skin temperature; HR, heart rate. Data are from the dehydration trial only. * $P < 0.05$ *vs.* rest, † $P < 0.05$ *vs.* 30 min.

Table 5-2. Haematological responses during prolonged exercise.

			Prolonged cycling time (min)				
			Rest	**30**	**60**	**90**	**120**
Hb	Dehydration	a	140 ± 4	149 ± 4*	151 ± 4*	153 ± 4*	152 ± 4*
($g \cdot l^{-1}$)		v	140 ± 4	148 ± 4*	151 ± 4*	153 ± 4*	152 ± 4*
O_2 Sat	Dehydration	a	97.6 ± 0.2	97.3 ± 0.3	97.3 ± 0.3	97.7 ± 0.2	97.7 ± 0.2
(%)		v	58.8 ± 1.0	63.6 ± 1.1*	62.9 ± 2.3	61.7 ± 1.8	61.0 ± 0.7
***P*O_2**	Dehydration	a	96 ± 4	94 ± 3	93 ± 3	97 ± 2	96 ± 2
(mmHg)		v	36 ± 2	40 ± 2*	41 ± 3	40 ± 2	39 ± 2*
ctO_2	Dehydration	a	184 ± 5	196 ± 6*	200 ± 5*†	203 ± 4*†	206 ± 5*†
($ml \cdot l^{-1}$)		v	112 ± 3	127 ± 4*	128 ± 7	127 ± 6	126 ± 4
pH	Dehydration	a	7.39 ± 0.01	7.40 ± 0.01	7.40 ± 0.02	7.44 ± 0.01†	7.45 ± 0.01†
		v	7.34 ± 0.01	7.35 ± 0.01	7.37 ± 0.02	7.38 ± 0.01	7.39 ± 0.01
***P*CO_2**	Dehydration	a	39 ± 1	40 ± 2	39 ± 1	38 ± 1†	37 ± 1†
(mmHg)		v	49 ± 1	50 ± 1	48 ± 2	48 ± 2	49 ± 1
[$HCO3^-$]	Dehydration	a	23.6 ± 0.6	23.7 ± 0.5	23.6 ± 0.9	24.6 ± 0.6*	24.9 ± 0.6*
($mmol \cdot l^{-1}$)		v	23.4 ± 0.6	24.0 ± 0.5	23.9 ± 0.	24.6 ± 0.9	25.0 ± 0.7
cBase(ECF)	Dehydration	a	-1.1 ± 0.7	-1.0 ± 0.7	-1.3 ± 1.1	-0.3 ± 0.8*	0.1 ± 0.7
($mmol \cdot l^{-1}$)		v	0.5 ± 0.6	1.1 ± 0.6	0.8 ± 0.8	1.5 ± 1.2	2.2 ± 0.8

Values are mean ± SEM for 10 participants. Haemoglobin (Hb), oxygen saturation (O_2 sat %), partial pressures of oxygen (PO_2) and oxygen content (ctO_2) for arterial (a) and internal jugular venous (v) blood. * different from rest $P < 0.05$, † different from 30 min value.

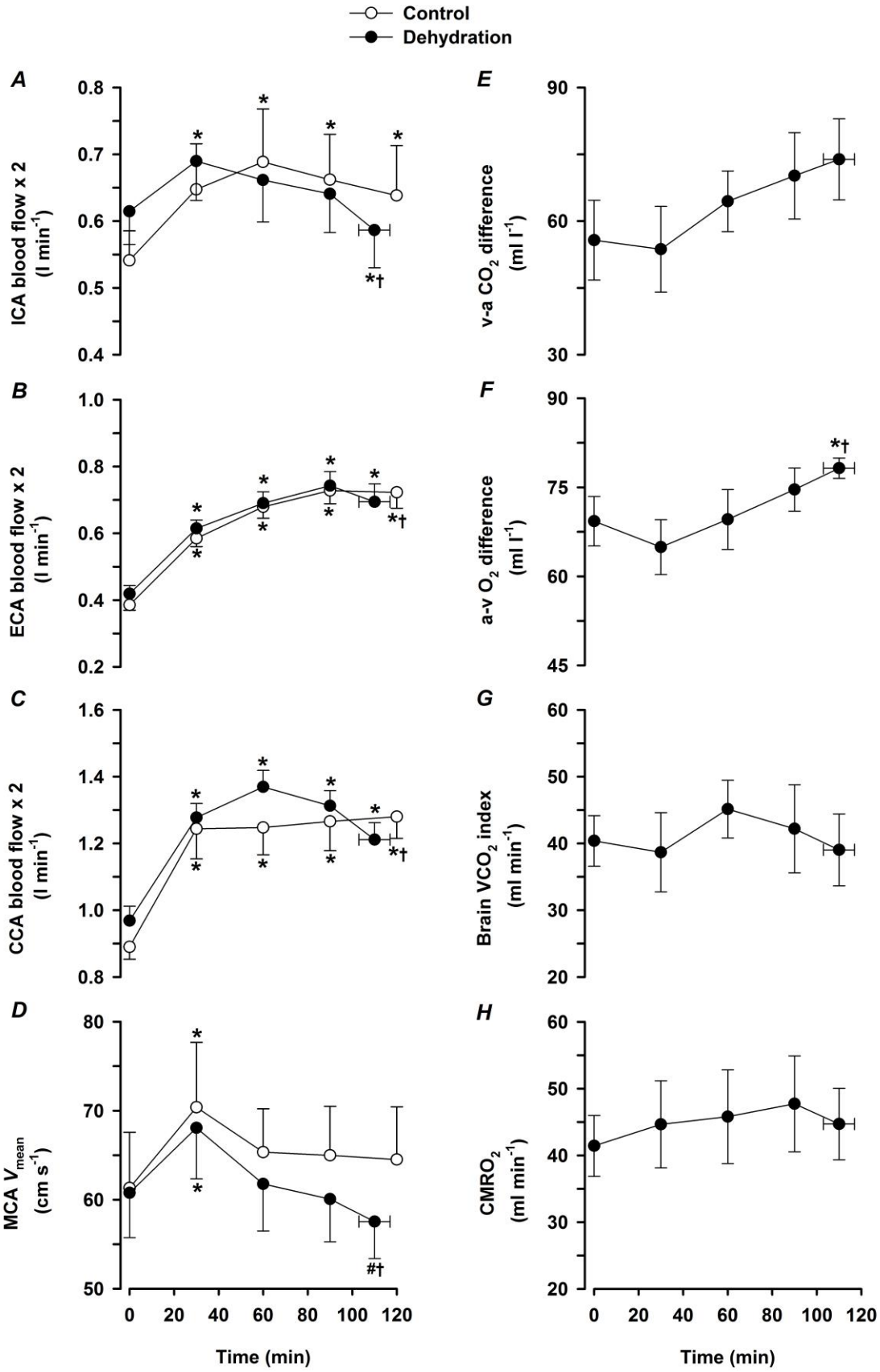


Figure 5-1. Cerebral and extra-cerebral haemodynamics and oxygen parameters during prolonged exercise. Values are means±SEM for 10 subjects. Dehydration and control exercise trials are represented for haemodynamics but not O_2/CO_2 parameters. * different from rest $P < 0.05$, † different from 30 min value.

5.4.2 Brain and extra-cranial haemodynamics and metabolism

In the dehydration trial, ICA blood flow and MCA V_{mean} were increased by ~12% at 30 min ($P < 0.05$), before declining progressively to baseline values at the end of exercise ($P < 0.05$: Figure 5-1 A & D). The decline in ICA blood flow was associated with a marked reduction in blood flow velocity ($P < 0.05$), but not vessel diameter. On the other hand in the control trial, ICA blood flow and MCA V_{mean} increased and remained stable throughout exercise. During the dehydration trial, extra-cranial (CCA and ECA) blood flow increased from rest to 60 min before declining at the end of exercise ($P < 0.05$: Figure 5-1 B & C). In contrast during the control trial, extra-cranial flow increased and was subsequently maintained throughout exercise.

The decline in ICA blood flow at the end of dehydration exercise was accompanied by an increased a-vO_2 difference ($P < 0.05$), but no changes in a-vCO_2 difference or brain $\dot{V}CO_2$ index. Thus, $CMRO_2$ was stable throughout exercise. Both arterial and jugular venous [Glu] gradually declined throughout prolonged exercise (5.4 ± 0.2 to 5.1 ± 0.2 and 5.4 ± 0.2 to 4.4 ± 0.2 mmol·l^{-1}, respectively; $P < 0.05$). Brain a-v [Glu] difference was stable during the early stages of exercise, before increasing prior to the end of exercise (Peak value of 0.7 mmol·l^{-1}; $P < 0.05$: Figure 5-2 A), whilst the brain glucose uptake initially increased (0.33 to 0.43 mmol·min^{-1}; $P < 0.05$: Figure 5-2 B) before remaining stable.

At rest the brain released a small amount of lactate (0.2 ± 0.05 mmol·l^{-1}) and during prolonged exercise with dehydration, arterial and jugular venous [La] gradually declined (3.4 ± to 2.4 ± 0.3 and 3.6 ± 0.5 to 2.4 ± 0.3 mmol·l^{-1}; $P < 0.05$). Brain a-v [La] difference was maintained throughout exercise, as was lactate exchange (Figure 5-2 E). The molar ratio of O_2 to glucose, reflective of the partial and total cerebral carbohydrate metabolism, declined at the onset of exercise (6.1 ± 0.5 *vs.* 4.5 ± 0.3 mmol·l^{-1}; $P < 0.05$: Figure 5-2 C) and thereafter remained stable, with a similar response observed when lactate metabolism was accounted for (Figure 5-2 F).

5.4.3 Brain and extra-cranial vascular conductance

In the dehydration trial, MAP increased ~18% from rest to 30 min, before declining progressively until exercise termination ($P < 0.05$:

Table 5-1). Jugular venous blood pressure was maintained stable throughout dehydration exercise. During prolonged exercise, ICA and MCA V_{mean} vascular conductance were lower in the dehydration compared to the control trial ($P < 0.05$). At the end of exercise in dehydration, both ICA and MCA V_{mean} vascular conductance were reduced ($P < 0.05$: Figure 5-4) whereas both were unchanged throughout control exercise. CCA and ECA vascular conductance were similar between trials during early exercise, but were reduced at the end of exercise in dehydration, compared to control ($P < 0.05$: Figure 5-4).

5.4.4 Blood flow and P_aCO_2, plasma catecholamines and temperature

In the dehydration trial, P_aCO_2 was maintained stable through the early stages of exercise before declining to below baseline values at the end of exercise (~6% reduction from peak value; $P < 0.05$: Figure 5-5) whereas P_vCO_2 remained unchanged throughout exercise. During exercise, the decline in both ICA blood flow ($R^2 = 0.44$; Fig. 5-5 D) and MCA V_{mean} ($R^2 = 0.5$) were correlated to reduced P_aCO_2 (both, $P < 0.01$) and to a weaker extent to arterial [NA] ($R^2 = 0.15$; $P < 0.05$), but not to jugular venous [NA] ($R^2 = 0.02$; $P = 0.58$).

In the dehydration trial, arterial adrenaline concentration ([A]) increased from rest to 30 min (0.7 ± 0.2 to 1.1 ± 0.2 nmol·l^{-1}; $P < 0.05$), before remaining stable throughout exercise (Range 1.1-2.7 nmol·l^{-1}; $P < 0.05$ *vs.* rest) whereas, jugular venous [A] did not increase until after 90 min of exercise (120 min = 2.25 ± 0.74 nmol·l^{-1}; $P < 0.05$ *vs.* rest). Arterial [Na] increased in a curvilinear manner to a peak of 33 ± 8.2 nmol·l^{-1} ($P < 0.05$ *vs.* rest: Figure 5-5 C) whereas, jugular venous [NA], after increasing initially at the onset of exercise (5.6 ± 2.2 to 19.4 ± 4.9 nmol·l^{-1}; $P < 0.01$), was not different from rest at exhaustion. The increases in ECA blood flow and vascular conductance were closely associated with increases in arterial [NA] and internal jugular venous blood temperature ($R^2 = 0.64$; $P < 0.01$: Figure 5-5 B and E and $R^2 = 0.48$; $P < 0.01$: Figure 5-5 F, respectively). However, a consistent relationship was not observed beyond 90 min where the 7% decline in

ECA blood flow (and conductance) was matched with an increased arterial [NA] and blood temperature (Figure 5-5 B & C).

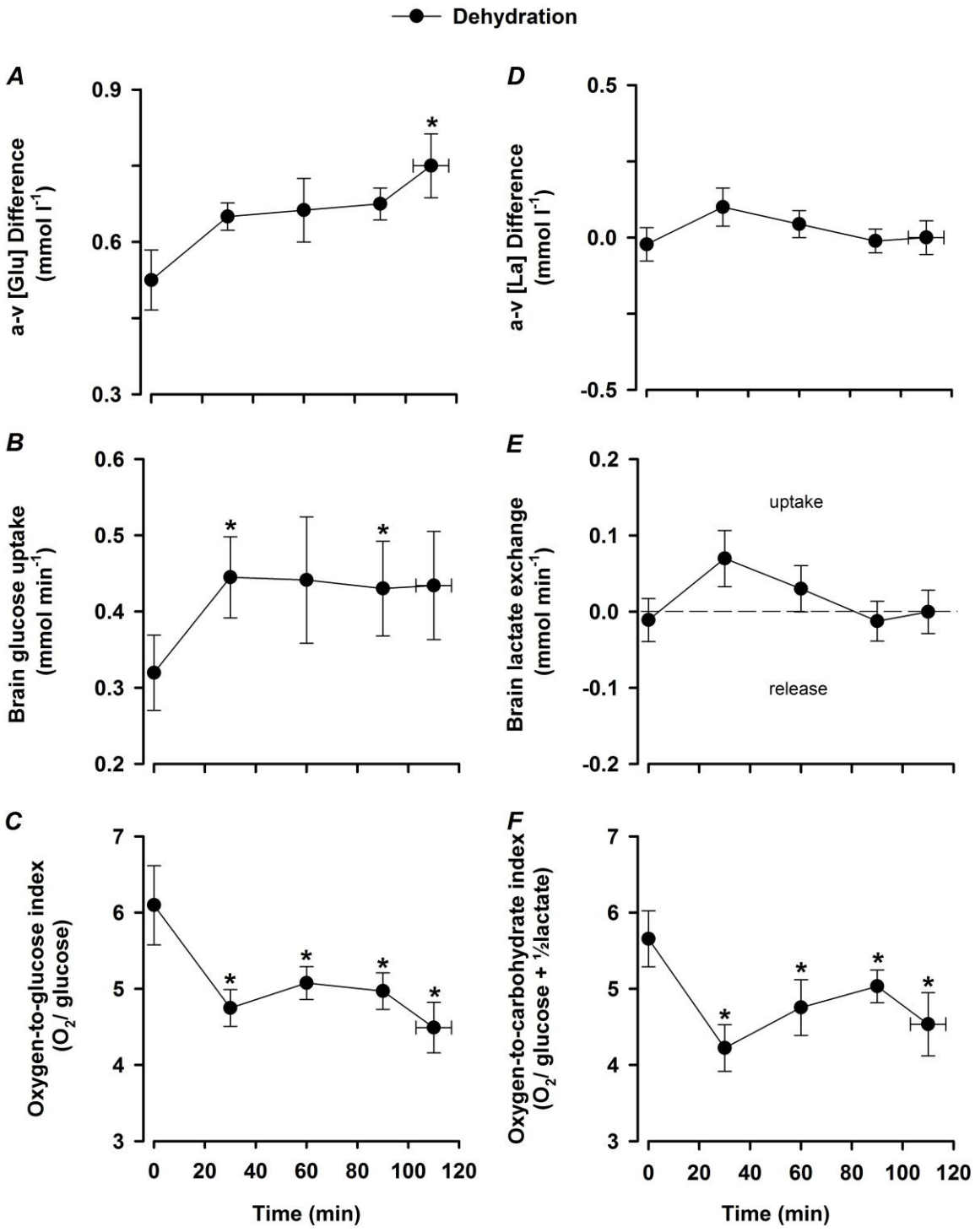


Figure 5-2. Cerebral lactate and glucose variables during prolonged exercise.
Values are means±SEM for 10 subjects. Data presented are from the dehydration trial only. * different from rest $P < 0.05$, † different from 30 min value.

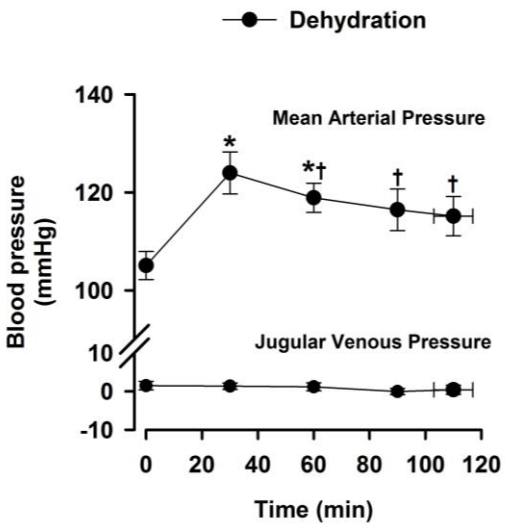


Figure 5-3. Mean arterial and jugular venous pressure during prolonged exercise. Values are means±SEM for 10 subjects. Data presented are from the dehydration trial. * $P < 0.05$ *vs.* rest, † $P < 0.05$ *vs.* 30 min value.

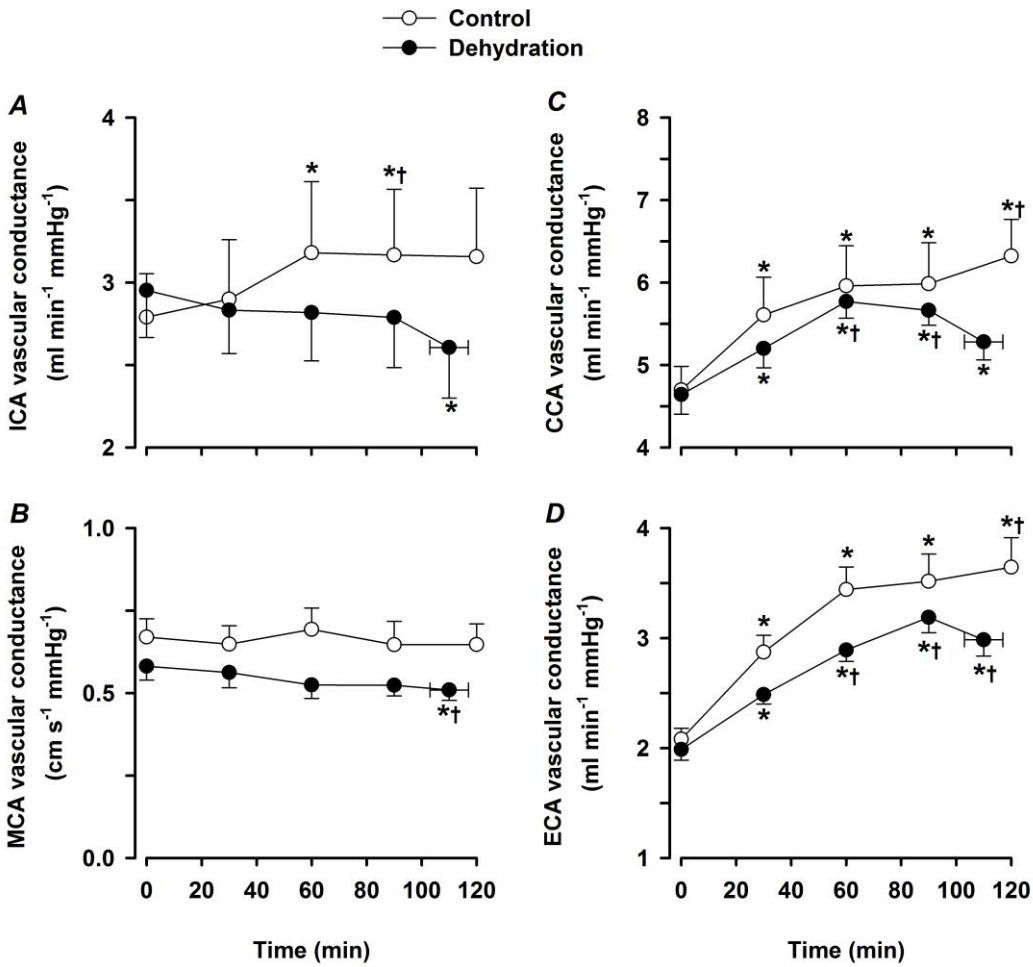


Figure 5-4. Cerebral and extra-cerebral vascular conductance during prolonged exercise. Values are means±SEM for 10 subjects. Dehydration and control exercise trials are represented. VC calculated using MAP/JVP in dehydration and MAP only during control. * different from rest $P < 0.05$, † different from 30 min value.

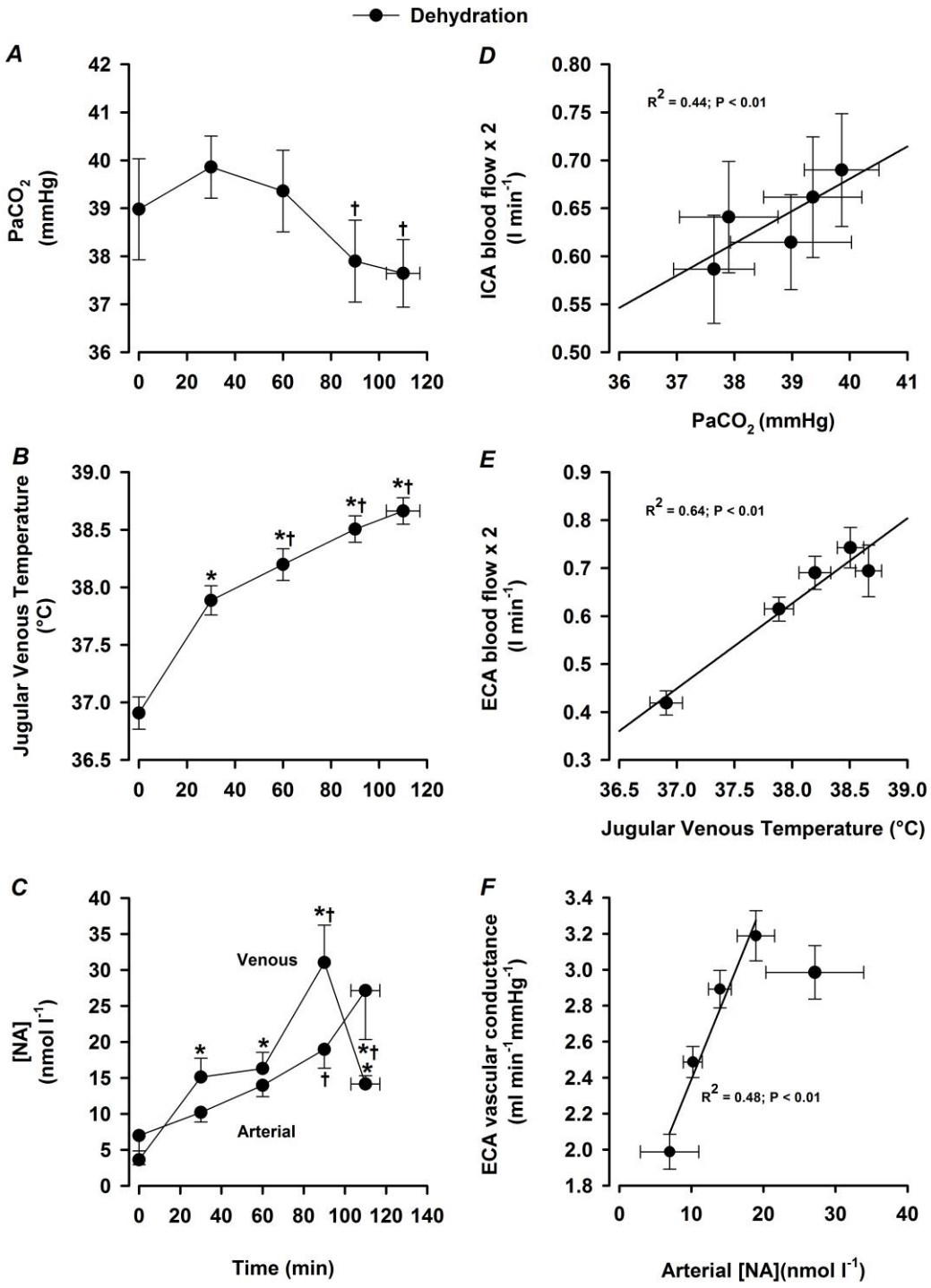


Figure 5-5. Blood temperature, P_aCO_2, systemic noradrenaline concentration and relationships with blood flow during prolonged exercise. Values are means±SEM for 10 subjects. Data presented are from the dehydration trial only. * different from rest $P < 0.05$, † different from 30 min value.

5.5 Discussion

The main finding of the present study is that dehydration impaired prolonged exercise capacity in the heat in association with an accelerated reduction in CBF. However, in spite of the reduced perfusion, cerebral metabolism was preserved as supported by observed elevations in oxygen and glucose extraction from the blood. A second novel finding was that the rise in extra-cranial blood flow in both experimental trials was attenuated prior to volitional exhaustion. Lastly, reductions in CBF were related to a reduced P_aCO_2 and blunted cerebral perfusion pressure, whereas increased extra-cranial conductance was associated with increasing internal temperature and enhanced sympathetic activity as indicated by plasma [NA]. These findings suggest that dehydration, accrued during prolonged strenuous exercise in the heat, affects the regional cranial circulation without impairing cerebral metabolism.

Cerebral and extra-cranial haemodynamics

A first aim of the present study was to characterise the haemodynamic responses of the cerebral and extra-cranial circulations to dehydration and prolonged exercise in the heat. Interestingly, we found that following the well-established increase upon the onset of exercise, CBF and MCA V_{mean} declined gradually to resting values at the point of volitional exhaustion concomitantly with the development of dehydration. Conversely, when hydration was maintained during similar duration exercise, CBF did not decline. These findings with and without dehydration are in agreement with prolonged exercise literature. CBF remains stable during prolonged exercise in thermoneutral environments when the degree of dehydration is negligible (Nybo & Nielsen 2001b; Nybo *et al.* 2002; Nybo *et al.* 2002). However, when exercise in a warm environment causes severe hyperthermia and cardiovascular strain, CBF declines to resting values (Nybo & Nielsen 2001b). These early studies, however, did not establish whether hyperthermia, dehydration, or other factors underpinning volitional exhaustion are responsible for the fall in CBF. Taken together, cerebral perfusion declines with dehydration during strenuous exercise, yet maintenance of euhydration and a stable core temperature slows the rate of CBF decline late in fatiguing exercise.

Another pertinent finding was that blood flow to the extra-cranial tissues displayed a distinct temporal dynamic response to that of the cerebral circulation, but was

also blunted late in exercise in the dehydrated condition. Yet these findings agree with reported 2-3 fold increases in ECA blood flow in response to passive heat stress (Bain *et al.* 2013; Ogoh *et al.* 2013b) and incremental exercise (Sato *et al.* 2011). The ECA primarily supplies blood to the skin circulation of the face and neck. In this light, the enhanced ECA perfusion observed in the present and recent reports may be part of the circulatory adjustments required to meet the thermoregulatory demands for heat transfer to the environment surrounding the head (Ogoh *et al.* 2013b). Collectively, these data suggest dehydration accentuates the rise in internal temperature, reduces extra-cranial blood flow at the point of exhaustion, accelerates the decline in cerebral perfusion, and leads to early exhaustion during prolonged exercise in the heat.

Regional regulation of blood flow

Both local and systemic factors are implicated in regulation of CBF through the modulation of vascular conductance and cerebral perfusion pressure. The decline in perfusion in the dehydration trial was accompanied by a falling cerebrovascular conductance, indicative of augmented net vasoconstriction. Changes in blood gases (Willie *et al.* 2012) and sympathetic nerve activity (Mitchell *et al.* 2009) are thought to play a dominant role in local control of CBF during conditions including exercise (see Chapter 4 for further discussion). In particular, CO_2 is a potent vasoactive substance within the cerebral vasculature with reductions in P_aCO_2 inducing cerebral vasoconstriction and increases leading to vasodilation (Kety & Schmidt 1948a; Willie *et al.* 2012; Bain *et al.* 2013). In support of a role of $PaCO_2$, the decline in vascular conductance with dehydration was associated with reductions in P_aCO_2 (R^2 = 0.44; P < 0.01; Fig. 5-5 D) and cerebral perfusion pressure, but unrelated to the stable arterial and internal jugular venous vascular blood oxygen parameters (Table 5-2). $PaCO_2$, however, accounted for less than one half of the variance in vascular conductance, suggesting that other factors contributed to local vasoconstriction. In this light, we observed a modest relationship between CBF and cerebral perfusion pressure (R^2 = 0.18, P < 0.05). Another potential contributing factor for regulation of CBF is enhanced sympathetic nerve activity. The cerebral vasculature is richly innervated with sympathetic nerves and observations of noradrenaline spillover into the internal jugular venous outflow, as seen here with dehydration, may reflect sympathetic-mediated vasoconstriction of the cerebral vessels (Zhang *et al.* 2002; Mitchell *et al.* 2009).

Despite increases in arterial and jugular venous plasma [NA], the relationships between cerebrovascular tone and plasma NA were weak rendering its role inconclusive (Strandgaard & Sigurdsson 2008a; van Lieshout & Secher 2008a; Willie *et al.* 2014). The current findings suggest that dehydration induced reductions in CBF are dominated by a decline in P_aCO_2 and to a lesser extent cerebral perfusion pressure.

The distinct dynamics of the extra-cranial circulation might involve different regulatory mechanisms. There was a close coupling between the increase in ECA blood flow/conductance and the rise in internal jugular blood temperature (R^2 = 0.64, P < 0.01) and arterial plasma [NA] (R^2 = 0.48, P < 0.01) (Fig. 5-5 E and F). We did not seek to investigate the control of skin blood flow. However, mechanisms associated with increases in local and core temperature are thought to play an important role in cutaneous blood flow regulation. Increases in local tissue temperature elevate skin blood flow, initially through an axon reflex and subsequently via a slower acting NOS mediated pathway (Johnson & Kellogg 2010). NO also enhances α_1-adrenoreceptor sensitivity, promoting involvement of sympathetic mediated vasodilation (Houghton *et al.* 2006; Charkoudian 2010). The role of sympathetic activity in the overall response to local and internal temperature changes is substantiated by a marked elevation in skin and muscle sympathetic nerve activity (Niimi *et al.* 1997; Crandall *et al.* 2008), promoting cutaneous blood distribution and sudomotor function (Kellogg *et al.* 1995; Charkoudian 2010). With the similar ECA profile in both trials, it is more likely that exercise *per se* attenuates cutaneous perfusion as rising internal temperature (above 38 °C) is not matched by further increases in skin perfusion (Brengelmann *et al.* 1977; González-Alonso *et al.* 1999). Dehydration also limits maximal skin perfusion during exercise (Nadel *et al.* 1980) and enhances systemic vascular resistance (González-Alonso *et al.* 1995), leading to attenuated cutaneous blood flow (Kellogg *et al.* 1990). Taken together, elevations in extra-cranial blood flow, in contrast to the CBF response, and the strong correlations observed support that blood flow to these vascular beds is influenced by local and internal temperature and enhanced sympathetic nerve activity. The attenuation in cutaneous blood flow before fatigue is likely due to the development of both dehydration and core hyperthermia.

Impact of dehydration on cerebral metabolism

Reductions in oxygen supply can compromise organ and tissue metabolism, as shown in contracting skeletal muscle with progressive dehydration and hyperthermia during prolonged exercise in the heat (González-Alonso *et al.* 1998). Here, we asked whether the stress of prolonged fatiguing exercise and dehydration is sufficient to compromise cerebral metabolism. Similar to findings during maximal exercise (Trangmar *et al.* 2014), the decline in CBF was met by an equal increase in oxygen extraction such that $CMRO_2$ was maintained during prolonged exercise in the dehydrated state in agreement with other independent metabolic measures obtained in this study. All glucose uptake, cerebral lactate exchange, molar oxygen/glucose ratio (OGI), and the oxygen/carbohydrate index (OCI) remained unchanged, and the cerebral respiratory quotient (~1.03) was stable as previously reported (Dalsgaard *et al.* 2004a). There is contrasting evidence that $CMRO_2$ might be elevated during strenuous exercise and severe hyperthermia compared to control exercise conditions (Nybo *et al.* 2002), thereby arguing that the metabolic demand of the brain increases during strenuous exercise. This conclusion, however, is based on two data points (Nybo *et al.* 2002). To provide a more comprehensive account of the cerebral metabolic responses to exercise and establish whether $CMRO_2$ is altered during fatiguing exercise, we plotted the anterior cerebral blood flow and a-vO_2 difference data from the current prolonged exercise protocol together with the reported baseline and incremental exercise data obtained in the same individuals (Fig. 7-2). This analysis shows that $CMRO_2$ remained stable across a variety of exercise intensities, exercise durations, hydration conditions and rest-to-exercise transitions, as variations in CBF were met by proportional changes in oxygen extraction. Therefore, although regional differences might still exist, the metabolic activity of the brain as a whole does not seem to be either enhanced or compromised during strenuous exercise in healthy trained people.

Methodological considerations

It was not possible to obtain simultaneous blood flow measurements in the CCA, ECA and ICA. Additionally measurements were obtained from the right side of the neck only and an assumption is made that the circulatory adjustments are similar to that of the left side. Venous blood samples were obtained from the left internal

jugular vein and asymmetry in the drainage of the brain may exist between the left and right jugular veins. Finally, blood flow in the posterior circulation (vertebro-basilar system) was not assessed, which may result in underestimation of the calculated cerebral metabolic rate. The posterior circulation contributes a relatively small portion of the total cerebral blood flow (~20%); thus relatively large changes in perfusion (either substantial increase or decreases) would be needed to affect the calculated $CMRO_2$.

5.6 Conclusion

In summary, our findings show that dehydration augments cardiovascular strain and negatively affects cerebral and extra-cranial perfusion during prolonged sub-maximal exercise in the heat. However, despite the circulatory challenge induced by dehydration and the development of hyperthermia, cerebral metabolism is not impaired due to compensatory increases in oxygen and substrate extraction across the cerebral circulation. Thus, reduced cerebral metabolic function is unlikely to explain the reduced exercise capacity with dehydration during prolonged submaximal exercise in the heat.

CHAPTER 6 – Mechanisms restraining exercise capacity in heat stressed humans: contribution of body and skin hyperthermia to brain, limb and systemic circulatory strain

6.1 Summary

Cardiovascular strain and hyperthermia are thought to be important factors limiting exercise capacity in heat-stressed humans, but the contribution of elevations in skin ($\overline{T}_{sk}$) *versus* body temperature remains unknown. Here we assessed cardiovascular capacity and leg, brain and systemic haemodynamic responses to incremental cycling exercise to volitional exhaustion with elevated skin (mild heat-stress; HS_{mild}) and combined core and skin temperatures (moderate heat-stress; HS_{mod}) to ascertain their relationships with the attainment of $\dot{V}O_{2max}$ and the processes of fatigue. $\overline{T}_{sk}$ and blood temperature (T_B), $\dot{V}O_2$ and leg, brain and systemic haemodynamics and haematological parameters were measured. Both heat-stress conditions increased $\overline{T}_{sk}$ *vs.* control (6.2 ± 0.2 °C; $P < 0.001$), however, only HS_{mod} increased resting T_B, blood flow to the legs and $\dot{Q}$ (+0.9 ± 0.1 °C, + 1.1 ±0.1 l·min^{-1} and 4.8 l·min^{-1}; $P < 0.05$). During incremental exercise, $\overline{T}_{sk}$ remained elevated in both HS trials whereas only T_B was greater in H_{Smod}. At exhaustion, exercise capacity and $\dot{V}O_{2max}$ were reduced in HS_{mod} by 13 ± 1% and 6 ± 2% ($P < 0.05$), in association with lower leg blood flow (-11 ± 3%), brain blood velocity (-9 ± 6%), cardiac output (-8 ± 3%) and mean arterial pressure (-14 ± 1%; all $P < 0.05$) but similar maximal heart rate and T_B. These findings demonstrate that whole-body hyperthermia, but not skin hyperthermia alone, compromises exercise capacity in heat-stressed humans through the early attenuation of leg, brain and systemic blood flow. These findings help advance our knowledge and understanding of why exercise performance that involves different times of exposure to hot environments is not universally impaired across all sports and exercise modalities.

6.2 Introduction

It is well documented that aerobic exercise capacity is reduced in hot environments (Rowell *et al.* 1966; Pirnay *et al.* 1970; Rowell 1974; Galloway & Maughan 1997; González-Alonso *et al.* 2008; Sawka *et al.* 2011). The precise mechanisms underpinning the attenuated exercise capacity in the heat remain debated, but may include reduced O_2 delivery compromising muscle and brain metabolism, altered neurotransmitter activity, feedback/reflex mechanisms and attainment of critically high brain, internal and skin temperatures (Nybo & Nielsen 2001a; Nybo *et al.* 2002; Nybo *et al.* 2002; González-Alonso *et al.* 2008; Sawka *et al.* 2012a; Nybo *et al.* 2014; Roelands & Meeusen 2010). It is well established that the contribution of each of the aforementioned factors to early fatigue in the heat is dependent on the task and intensity of the activity (Nybo *et al.* 2014). However, there is a paucity of information on the cardiovascular adjustments to differing extents of heat stress, and thus different levels of skin and body hyperthermia, during maximal incremental exercise to volitional exhaustion. In the first two studies of this thesis, the marked cardiovascular strain invoked by dehydration and a rising internal temperature did not impair cerebral aerobic metabolism. It therefore seems unlikely that metabolic restrictions across the brain contribute to fatigue during maximal incremental exercise (Nybo *et al.* 2014; Trangmar *et al.* 2014).

It is, however, possible that restrictions in active skeletal muscle perfusion might play an important role in the reduced maximal aerobic capacity in heat stress conditions. This is because tight regulation of skeletal muscle O_2 delivery to metabolic demand during sub-maximal exercise (Andersen & Saltin 1985; Delp & Laughlin 1998; Saltin *et al.* 1998; González-Alonso *et al.* 2002; Delp & O'Leary 2004) is lost at high intensities as, prior to volitional exhaustion, systemic and active muscle blood flow are reduced or restricted (González-Alonso & Calbet 2003; Mortensen *et al.* 2005; Mortensen *et al.* 2008). The attenuated leg blood flow per unit of power when approaching maximal exercise intensities occurs concomitantly with enhanced local vasoconstrictor activity and reductions in stroke volume; with changes at the active skeletal muscle mediating these alterations (González-Alonso & Calbet 2003; Calbet *et al.* 2006; Calbet *et al.* 2007; Mortensen *et al.* 2008; Stöhr *et al.* 2011c; Bada *et al.* 2012; Munch *et al.* 2014). Nevertheless, a compromised local aerobic metabolism may ensue as maximal

skeletal muscle O_2 extraction is achieved early in exercise (González-Alonso & Calbet 2003; González-Alonso *et al.* 2004), again in contrast to other regions of the body such the brain (Nybo *et al.* 2002; Trangmar *et al.* 2014; Chapter 4). Yet no study to date has assessed the integrative hemodynamic responses to incremental exercise with manipulations of body temperature designed to tax cardiovascular function and capacity.

An early restriction in regional blood flow may underpin the reduced $\dot{V}O_{2max}$ with heat stress. The magnitude of the decline in $\dot{V}O_{2max}$ is, however, variable and largely dependent on the extent of the heat-stress induced increases in skin and body temperatures (Pirnay *et al.* 1970; Arngrimsson *et al.* 2004; Ely *et al.* 2010; Nybo *et al.* 2014). A critical question is which bodily temperature or combination of temperatures is most closely associated with the attenuation in aerobic capacity in heat stress conditions. On the one hand, brief exposure to heat that does not substantially elevate internal temperature is unlikely to cause a decline in $\dot{V}O_{2max}$ or impair cardiovascular capacity (Arngrimsson *et al.* 2004). In contrast, a reduced aerobic capacity has recently been associated with the attainment of high skin temperatures without significant elevations in internal (core) temperature (Ely *et al.* 2009; Ely *et al.* 2010; Lorenzo *et al.* 2010; Sawka *et al.* 2012a). High skin temperature has therefore been proposed to be a critical factor underpinning reduced aerobic capacity in the heat (Sawka *et al.* 2012a), but this hypothesis has not yet been systematically investigated. Assessment of the haemodynamic and metabolic dynamics during incremental exercise to exhaustion with different degrees of heat stress will elucidate the role of skin *vs.* combined skin and core temperature on exercise capacity and the underpinning cardiovascular, brain and skeletal muscle circulatory and metabolic responses.

The aim of the present study was therefore to investigate the effect of two different grades of heat stress on cardiovascular capacity and brain, leg and systemic blood flow and metabolism during incremental cycling exercise to volitional exhaustion. Brain, leg and systemic haemodynamics and metabolism during incremental exercise were assessed; 1) after a significant heat exposure sufficient to elevate internal and skin temperature, 2) after a brief heat exposure sufficient to elevate skin temperature only and, 3) in control conditions. We hypothesised that

combined core and skin hyperthermia, but not skin hyperthermia alone, would compromise exercise capacity and $\dot{V}O_{2max}$ through attenuated local vascular conductance and early reductions in regional perfusion.

6.3 Methods

6.3.1 Participants

Nine healthy experienced cyclists (mean ± SD; age 26 ± 6 years, stature 181 ± 6 cm, mass 76 ± 9 kg and $\dot{V}O_{2max}$ 60 ± 6 ml·kg^{-1}·min^{-1}) participated in the present study. Participants arrived at the laboratory postprandial with a normal hydration status and were required to abstain from strenuous exercise and alcohol intake for 24 h and caffeine consumption for 12 h.

6.3.2 Experimental design

To accomplish the aim of the study, participants visited the laboratory on 3 occasions, comprising of a preliminary, a main experimental and a control visit. On the first visit (preliminary trial) participants were introduced to the experimental set-up and familiarised with the test methodology. Participants then performed an incremental exercise test on a cycle ergometer (Lode Excalibur, Groningen, Netherlands) to establish $WR_{max,}$ maximal heart rate and $\dot{V}O_{2max}$. The test began at a work rate equivalent to 50% of predicted (see General methods) $\dot{V}O_{2max}$, for 2.5 min, followed by increments of 10% predicted every 2.5 min until the limit of tolerance. Participants were instructed to maintain a cadence between 70-90 r.p.m. and the test was terminated when cycling speed dropped below 60 r.p.m. for more than 3 s, despite strong verbal encouragement to continue. After a 1 h recovery period, participants repeated the incremental test starting with an elevated core temperature, to establish heat stress WR_{max}.

On the second visit (experimental trial), the participants completed three incremental cycling ergometer exercise tests in the upright position; 1) moderate heat stress (with moderate T_c and high $\overline{T}_{sk}$), 2) mild heat stress (with a high $\overline{T}_{sk}$ only) and, 3) control conditions (T_a 18 °C; 36% RH; with fan cooling). In the third and final visit (control trial), the participants completed three incremental cycling

ergometer exercise tests in a thermoneutral environment (20°C; ≤ 50% RH; with fan cooling). Each of the incremental cycling tests consisted of 5 x 2.5 min stages at 20, 40, 60, 80 and 100% WR_{max} but owing to the reduced exercise capacity in the heat stress preliminary test the absolute work rates for moderate heat stress were; 64 ± 2, 128 ± 4, 193 ± 5, 257 ± 7 and 321 ± 9 W and for all other incremental tests were; 74 ± 2, 148 ± 4, 223 ± 7, 297 ± 9 and 371 ± 11 W. On both the experimental and control trials, each incremental test was separated by 1 h of passive recovery, hydration was maintained on each of the visits through the regular consumption of water and cycling pedal cadence during was maintained between 70-90 r.p.m.

To induce the moderate and mild heat stress conditions, core and skin temperatures were elevated, under resting conditions, on the preliminary and experimental visits by the circulation of hot water (50 °C) through a tube lined suit worn by participants. The suit covered the arms, torso and legs at rest whereas, during exercise, only the upper body was heated. Rain trousers were worn to limit heat loss from the legs.

On the experimental trial, brain, leg and systemic haemodynamics and blood samples from the brachial artery and femoral vein were obtained simultaneously at rest and in the final minute of each incremental exercise stage. Skin and femoral venous temperatures and arterial and femoral venous pressures were recorded continuously. The same measures were collected in the control visit, except for the arterio-venous blood sampling, LBF and blood pressure measurements, and with the addition of oesophageal temperature (T_{oes}) (n=5).

6.3.3 Leg, brain and systemic haemodynamics

Leg blood flow during incremental exercise was determined using the constant-infusion thermodilution method (Ganz *et al.* 1964; Andersen & Saltin 1985; González-Alonso *et al.* 1998; González-Alonso *et al.* 2000). Full description of the thermodilution method can be found in the *General Methods* section (3.3.4). At rest, constant-infusion thermodilution may provide inaccurate values. This is because the infusion of ice cold saline may cool the local tissues and not the blood alone (calculation of blood flow using thermodilution requires that temperature

changes are confined to the blood). Resting blood flow data were therefore obtained in 4 participants using duplex Doppler ultrasonography (Vivid 7, Dimension, GE Healthcare, UK). Resting blood flow in the remaining participants was estimated from the directly obtained a-vO_{2diff}, and assuming similar resting leg $\dot{V}O_2$.

MCA V_{mean} was measured using a 2MHz pulsed trans-cranial Doppler ultrasound system (DWL, Sipplingen, Germany). The right MCA was insonated through the temporal ultrasound window, distal to the MCA-anterior cerebral artery bifurcation, at a depth of 45-60 mm. Signal quality was optimised according to Aaslid *et al.* (1982). Cerebral oxygenation was also measured using near-infrared spectroscopy (NIRS) using a commercially available oxygenation monitor (INVOS, Somanetics, Troy, MI, USA).

6.3.4 Catheter placement and blood sampling

Participants rested with a slight head-down tilt whilst catheters for blood sampling, mean arterial pressure (MAP), femoral venous pressure and blood temperature were inserted after local anaesthesia (1% lidocaine) into the brachial artery of the non-dominant arm and anterograde into the right common femoral vein (Logicath Quad lumen, 18 gauge, 2.3 mm; MXA234X16X85, Smiths Medical International LTD), the latter using the Seldinger technique. Catheters were inserted by an experienced clinician under ultrasound guidance and were regularly flushed with normal saline (0.9% NaCl) to maintain patency. The time from catheterisation to the commencement of resting measurements was ~1 h to allow time for the restoration of normal haemodynamics.

6.3.5 Blood variables

Arterial and femoral venous blood samples were drawn into pre-heparinised syringes and analysed immediately for blood gas variables (ABL 800 FLEX, Radiometer, Copenhagen, Denmark) corrected to blood temperature in the femoral vein. The analyser was calibrated (one and two-point) at regular intervals in accordance with manufacturer guidelines.

6.3.6 Heart rate, blood pressure and temperatures

Heart rate was obtained from a chest strap (Polar Electro, Kempele, Finland). Arterial and femoral venous pressure waveforms were recorded using pressure transducers (Pressure Monitoring Kit, TruWave, Edwards Lifesciences, Germany) and were zeroed at the level of the right atrium in the mid-axillary line (arterial) and at the level of the tip of the catheter (femoral venous). Arterial pressure waveforms were amplified (BP amp, ADIstruments) and sampled at 1000 Hz using a data acquisition unit (Powerlab 16/30, ADInstruments, Oxfordshire, UK) for offline analysis. For measurements of femoral venous blood temperature (T_B), a thermistor (T204a, PhysiTemp, Clifton, New Jersey, USA) was inserted through the femoral venous catheter and connected to a thermocouple meter (TC-2000, Sable Systems, NV: USA) and routed through the data acquisition system. In the control trial, oesophageal temperature (T_{oes}) was measured using a thermister (Physitemp, New England, USA), inserted pernasally into the oesophagus at a depth of ¼ standing height. Increases in core temperature during cycling exercise reflect the rise in femoral venous blood temperature, as T_B and T_{oes} have been shown to be within ~0.1 °C (González-Alonso *et al.* 1999). Mean skin temperature ($\overline{T}_{sk}$) from four sites (standard weightings of chest, abdomen, thigh and calf; (Ramanathan 1964) was obtained using a wireless thermocouple system (iButtons®, Maxim Integrated, San José, CA, USA).

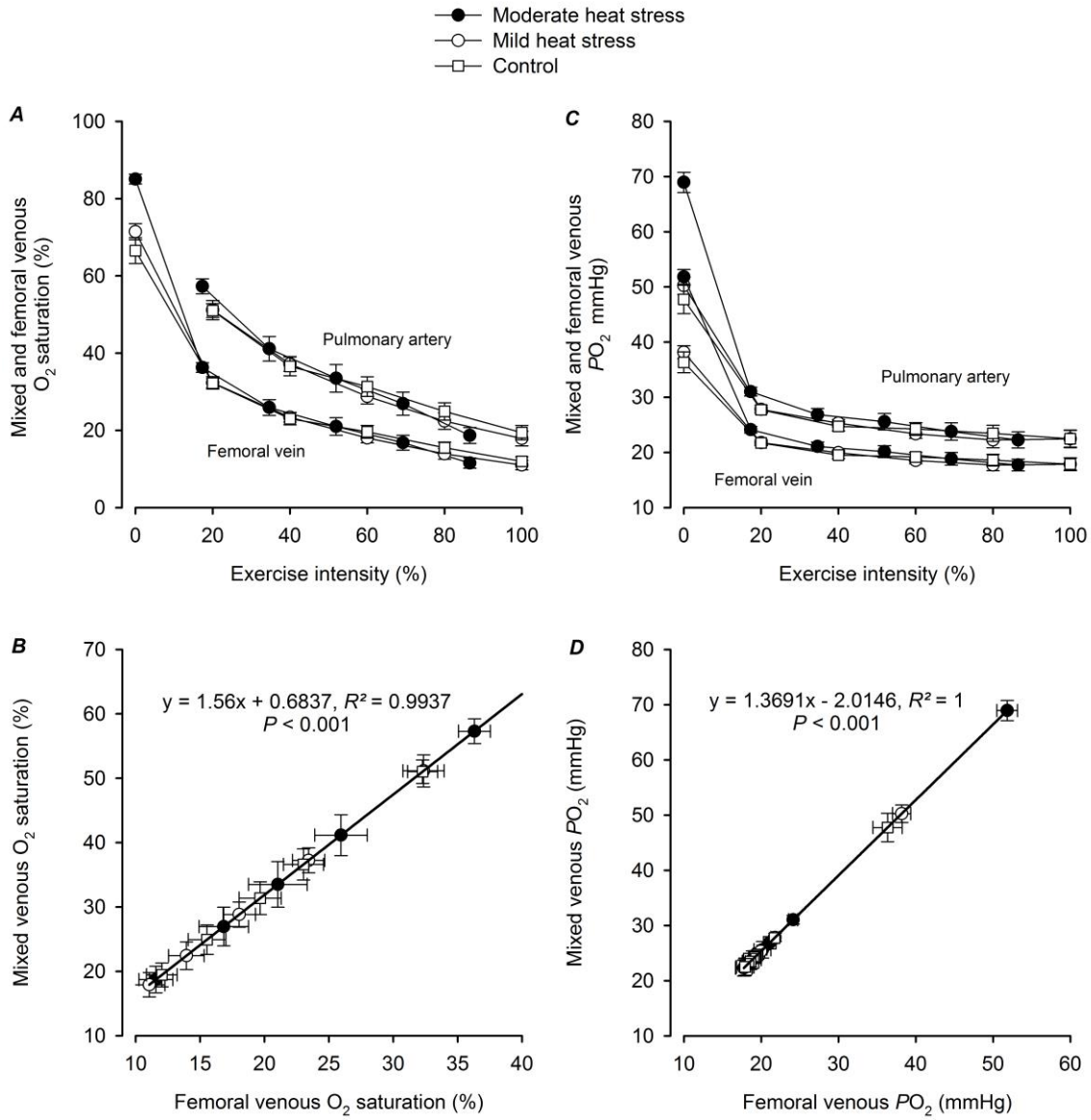


Figure 6-1. Mixed and femoral venous O_2 saturation and PO_2 responses to incremental exercise with different grades of heat stress. Pulmonary artery oxygen saturation (A) and PO_2 (B) were estimated from the femoral venous equivalent, corrected by the slope of the relationship (B and D) between femoral venous and mixed central venous haematological values obtained by Munch *et al.* (Munch *et al.* 2014).

6.3.7 Calculations

In the experimental trials, leg, brain and systemic vascular conductance (VC) indices were calculated by dividing leg blood flow, MCA V_{mean} and leg blood flow by perfusion pressure (MAP-FVP). To calculate cardiac output ($\dot{Q}$) based on the Fick equation, pulmonary artery oxygen saturation and PO_2 (Figure 6-1) were estimated from the femoral venous equivalent, corrected by the slope of the relationship between femoral venous and mixed central venous haematological values obtained by Munch *et al.* (2014). Systemic a-vO_2 difference was then

calculated from the measured arterial oxygen content and the estimated mixed venous O_2 content, using the directly measured haemoglobin concentration in venous blood and the estimated mixed venous oxygen saturations and PO_2. $\dot{Q}$ was subsequently calculated as the ratio of systemic $\dot{V}O_2$ and estimated mixed venous a-vO_2 difference. Resting $\dot{Q}$ was estimated using the Modelflow method (Wessling 1993). When leg blood flow measurements were not possible, LBF was calculated from the estimated Leg $\dot{V}O_2$ (assuming that the increase in pulmonary $\dot{V}O_2$ from baseline reflected only the increase in leg $\dot{V}O_2$) (Mortensen *et al.* 2005; Calbet *et al.* 2007; Mortensen *et al.* 2008; Munch *et al.* 2014) and directly measured leg arterial-to-femoral venous O_2 difference. The agreement between measured LBF and estimated LBF in three participants is presented in Figure 6-2.

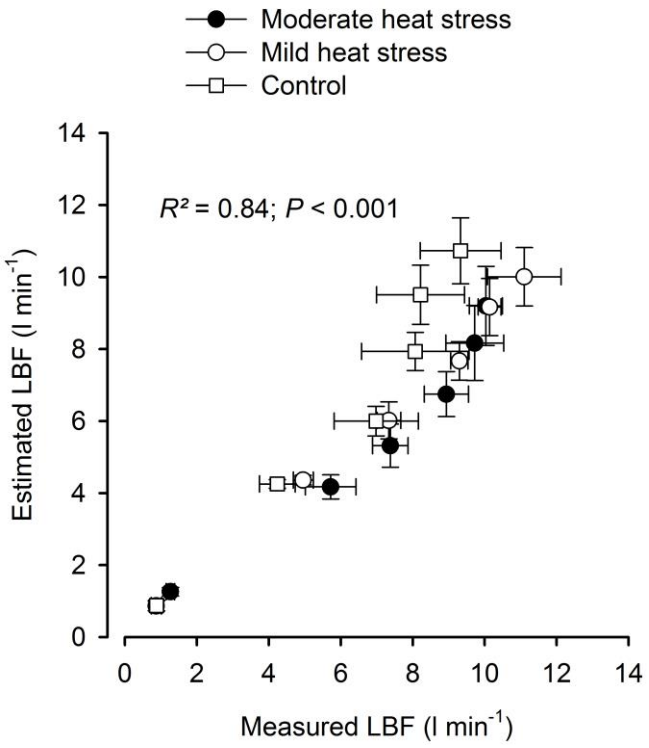


Figure 6-2. Relationship between LBF measured by thermodilution and calculated from the estimated Leg $\dot{V}O_2$ in 3 participants. Estimations of leg $\dot{V}O_2$ were made using the linear relationship between leg and pulmonary $\dot{V}O_2$ from (Mortensen *et al.* 2008). LBF was then calculated as leg $\dot{V}O_2$ over the directly measured arterial-to-femoral venous O_2 difference. The assumption that the increase in pulmonary $\dot{V}O_2$ from baseline reflects only the increase in two-leg $\dot{V}O_2$ was confirmed in the available data from 3 participants (mean difference between systemic and two-leg $\dot{V}O_2$ of ~0.5 l·min^{-1}; P = 0.25).

6.3.8 Statistical analysis

Differences between exercise conditions were assessed using two-way repeated-measures ANOVA in which condition (Moderate heat stress, mild heat stress and control) and exercise phase (rest, 20, 40, 60, 80 and 100%) were the main factors. Where a significant main effect was found, pairwise comparisons were made using the Holm-Bonferroni procedure. Statistical significance was set at $P < 0.05$ and all analyses were made using IBM SPSS Statistics (Version 20, IBM Corporation, Armonk, NY, USA).

6.4 Results

6.4.1 Temperature and cardiorespiratory responses to moderate and mild heat stress

Prior to incremental exercise, T_B was by design elevated in HS_{mod} compared to HS_{mild} and control (37.5 ± 0.1 *vs.* 36.7 ± 0.1 and 37.0 ± 0.1 °C; $P < 0.05$), whereas $\overline{T}_{sk}$ was equally elevated in both heat stress conditions compared to control (38.2 ± 0.3 *vs.* 32.3 ± 0.4 °C; $P < 0.001$: Fig. 6-3 A & B). During incremental exercise in HS_{mod}, T_B was initially unchanged before increasing to a peak of 39.3 ± 0.1 °C ($P < 0.001$ *vs.* rest) whereas, in HS_{mild} and control, T_B increased linearly from rest to WR_{max} (39.1 ± 0.1; $P < 0.001$) and was lower overall compare to HS_{mod}. $\overline{T}_{sk}$ was maintained elevated in both heat stress conditions (36.9 ± 0.4 *vs.* 32.0 ± 0.4 °C; $P < 0.001$) and was subsequently stable throughout exercise. Respiratory variables are presented in Table 6-1. Briefly, respiratory frequency, $\dot{V}CO_2$ and $\dot{V}_E$ increased with exercise intensity and were lower in HS_{mod} compared to HS_{mild} and control (both $P < 0.001$). End-tidal O_2 initially declined before increasing at $WR_{max,}$ with the reverse response observed for CO_2; however, there were no differences between the exercise test conditions ($P = 0.492$). Arterial and venous [Hb] and arterial oxygen content increased with incremental exercise in all conditions, despite a reduction in arterial oxygen saturation (all $P < 0.05$: Table 6-2 & Table 6-3). Arterial oxygen content was elevated by 6% in both heat stress conditions and was higher in HS_{mod} compared to HS_{mild} and control up to 60% WR_{max} ($P < 0.05$: Table 3). During the three incremental tests in the control trial, T_{oes} increase from 36.6 ± 0.1 to 38.3 ± 0.1 °C, but no differences were observed between incremental tests, including cardiorespiratory variables.

Table 6-1. Respiratory responses to incremental exercise with different grades of heat stress.

% WRmax (W)	$\dot{V}E$ ($l \cdot min^{-1}$)	*fr* ($breaths \cdot min^{-1}$)	$PetO_2$ (mmHg)	$PetCO_2$ (mmHg)	$\dot{V}CO_2$ ($l \cdot min^{-1}$)
Moderate heat stress					
0	17 ± 2	18 ± 1	111 ± 3	33 ± 2	0.46 ± 0.05
20 (64)	39 ± 2*	25 ± 2*	103 ± 2*	38 ± 2*	1.35 ± 0.07*
40 (128)	55 ± 2*	29 ± 2*	102 ± 1*	40 ± 1*	1.99 ± 0.08*
60 (193)	77 ± 3*‡	32 ± 2*	106 ± 1	40 ± 1*	2.78 ± 0.07*‡
80 (257)	110 ± 5*‡†	41 ± 2*	111 ± 2	38 ± 1*	3.68 ± 0.11*‡†
100% (321)	148 ± 7*‡†	51 ± 3*	115 ± 1*	34 ± 1	4.42 ± 0.08*‡†
Mild heat stress					
0	14 ± 1	16 ± 2	108 ± 2	34 ± 1	0.39 ± 0.02
20 (74)	39 ± 2*	25 ± 2*	100 ± 2*	39 ± 1*	1.14 ± 0.07*
40 (149)	58 ± 2*	29 ± 2*	102 ± 1*	40 ± 1*	2.12 ± 0.07*
60 (223)	86 ± 3*†	34 ± 2*	106 ± 1	40 ± 1*	3.05 ± 0.09*†
80 (297)	126 ± 4*†	41 ± 2*†	113 ± 1	36 ± 1*	4.10 ± 0.10*†
100% (371)	161 ± 7*	52 ± 3*	116 ± 1*	34 ± 1	4.73 ± 0.16*
Control					
0	14 ± 1	17 ± 2	108 ± 3	33 ± 1	0.40 ± 0.03
20 (74)	39 ± 2*	26 ± 2*	99 ± 1*	38 ± 1*	1.33 ± 0.08*
40 (149)	57 ± 2*	29 ± 2*	102 ± 2*	40 ± 1*	2.06 ± 0.08*
60 (223)	79 ± 3*	32 ± 2*	104 ± 2	40 ± 1*	2.88 ± 0.08*
80 (297)	116 ± 6*	39 ± 2*	110 ± 2	38 ± 1*	3.88 ± 0.11*
100% (371)	165 ± 7*	52 ± 3*	117 ± 1*	33 ± 1	4.73 ± 0.12*

Values are mean ± SEM for 9 participants. Minute ventilation ($\dot{V}_E$), respiratory frequency (*fr*), end-tidal oxygen ($PetO_2$) and carbon dioxide tension ($PetCO_2$) and carbon dioxide production ($\dot{V}CO_2$), * different *vs.* rest $P < 0.05$, ‡ different *vs.* mild heat stress, † different *vs.* control. Presented symbols denote differences between conditions at the same relative percentage of WR_{max}.

6.4.2 Leg, brain and systemic haemodynamics, blood pressure and conductance

At rest, heart rate (88 ± 3 *vs.* ~76 ± 5 beats·min^{-1}), two-legged blood flow (2.1 ± 0.1 *vs.* ~1 ± 0.1 l·min^{-1}) and $\dot{Q}$ (10.4 ± 1.9 *vs.* ~5.1 ± 0.8 l·min^{-1}) were elevated in HS_{mod} compared to HS_{mild} and control, in association with an enhanced leg and systemic vascular conductance (all $P < 0.05$). From rest to sub-maximal exercise, heart rate, $\dot{Q}$ and two-legged blood flow increased linearly with exercise intensity in all conditions ($P < 0.05$ *vs.* rest) and, in HS_{mod} overall, two-legged blood flow and mean arterial pressure were lower ($P < 0.05$) and heart rate and systemic vascular conductance were higher ($P < 0.05$) compared to HS_{mild} and control, whereas there were no differences between groups for $\dot{Q}$. At exhaustion, heart rate increased to similar peak values (189 ± 4, 187 ± 3 and 184 ± 3 beats·min^{-1} in $HS_{mod,}$ HS_{mild} and control, respectively) and, two-legged blood flow (18.5 ± 1.3, 20.3 ± 1.0 and 21.3 ± 1.2 l·min^{-1}), $\dot{Q}$ (21.8 ± 1.4, 23.6 ± 1.1 and 24.7 ± 1.3 l·min^{-1}) and MAP (124 ± 7, 139 ± 7 and 153 ± 7 mmHg) were reduced in HS_{mod} compared to HS_{mild} and control, respectively (all $P < 0.05$), in association with a blunted perfusion pressure. Cerebral perfusion (MCA V_{mean}) declined in all conditions, in association with a decline in cerebrovascular conductance, but was markedly reduced (9%) in HS_{mod} compared to HS_{mild} and control ($P < 0.05$). Furthermore, beyond 60% WR_{max}, mean arterial pressure and $\dot{Q}$ were lower in HS_{mild} compared to control conditions (Figure 6-5), associated with a further elevation in systemic vascular conductance ($P < 0.05$). Femoral venous pressure and leg vascular conductance increased with exercise intensity.

Table 6-2. Blood gases and metabolite responses to incremental exercise with different grades of heat stress.

	pH		Hb ($g \cdot l^{-1}$)		SO_2 (%)		PO_2 (mmHg)		PCO_2 (mmHg)	
% WR_{max}	Arterial	Venous	Arterial	Venous	Arterial	Venous	Arterial	Venous	Arterial	Venous
Moderate heat stress										
Rest	7.46 ± 0.01‡†	7.44 ± 0.01‡†	148 ± 3‡†	151 ± 3‡†	97.5 ± 0.3	85.1 ± 1.3‡†	94.5 ± 2.9	51.8 ± 1.3‡†	38.2 ± 1.3	42.1 ± 1.8
20	7.47 ± 0.01‡†	7.39 ± 0.01*‡†	154 ± 3*‡†	157 ± 3*‡†	98.0 ± 0.2	36.3 ± 1.2*	100.3 ± 2.8	24.1 ± 0.6*‡†	36.2 ± 1.8*	53.7 ± 2.8*
40	7.45 ± 0.01*‡†	7.35 ± 0.01*	154 ± 3*‡†	157 ± 3*‡†	97.8 ± 0.2	25.9 ± 2.0*	99.4 ± 3.1	21.1 ± 0.8*	37.0 ± 1.5‡	60.3 ± 2.7*
60	7.42 ± 0.01*†	7.31 ± 0.01*	155 ± 3*‡†	158 ± 3*‡†	97.5 ± 0.2*	21.0 ± 2.3*	99.3 ± 2.2	20.2 ± 1.1*	38.1 ± 1.2	65.9 ± 2.1*
80	7.40 ± 0.01*‡†	7.26 ± 0.01*‡	156 ± 3*	159 ± 4*‡†	97.2 ± 0.2*	16.8 ± 1.9*‡	98.2 ± 2.9	18.9 ± 1.1*	36.2 ± 0.9	72.1 ± 1.9*
100%	7.36 ± 0.01*‡†	7.19 ± 0.01*‡	157 ± 3*	161 ± 3*‡†	96.7 ± 0.2*	11.6 ± 1.3*	100.3 ± 2.4	17.8 ± 1.1*	33.7 ± 1.0*	78.1 ± 2.1*
Mild heat stress										
Rest	7.44 ± 0.01	7.41 ± 0.01	141 ± 2†	143 ± 3	97.9 ± 0.1	71.5 ± 2.1	95.7 ± 2.2	38.2 ± 1.2	38.2 ± 1.0	44.3 ± 1.2
20	7.44 ± 0.01	7.38 ± 0.01*	147 ± 3*†	149 ± 3*†	97.8 ± 0.1	32.4 ± 1.6*	95.1 ± 2.0	21.8 ± 0.5*	37.7 ± 1.1	52.5 ± 1.6*
40	7.42 ± 0.01*	7.33 ± 0.01*	148 ± 3*†	150 ± 3*†	97.6 ± 0.2	23.4 ± 1.2*	95.9 ± 1.8	20.0 ± 0.5*	39.3 ± 0.9	61.4 ± 1.5*
60	7.41 ± 0.00*	7.29 ± 0.00*	150 ± 3*†	153 ± 3*†	97.3 ± 0.1*	18.0 ± 1.2*	96.0 ± 1.7	18.6 ± 0.6*	38.6 ± 1.0	67.6 ± 1.2*
80	7.38 ± 0.01*	7.23 ± 0.01*†	153 ± 3*	155 ± 3*	97.1 ± 0.2*	13.9 ± 1.4*†	97.3 ± 2.0	17.8 ± 1.0*	36.6 ± 1.2†	74.0 ± 1.5*
100%	7.32 ± 0.01*†	7.15 ± 0.01*†	156 ± 3*†	152 ± 3*†	96.2 ± 0.3*	11.1 ± 1.2*	99.2 ± 2.5	17.9 ± 1.2*	33.3 ± 1.1*	79.0 ± 2.7*
Control										
Rest	7.44 ± 0.01	7.41 ± 0.01	138 ± 3	140 ± 3	97.9 ± 0.1	66.6 ± 3.3	95.8 ± 1.6	36.4 ± 1.9	37.4 ± 1.0	43.9 ± 1.6
20	7.44 ± 0.01	7.39 ± 0.01*	145 ± 3*	146 ± 3*	97.7 ± 0.2	32.3 ± 1.2*	93.5 ± 2.1	21.7 ± 0.3*	37.2 ± 1.0	50.2 ± 1.5*
40	7.42 ± 0.00*	7.34 ± 0.01*	146 ± 3*	147 ± 3*	97.7 ± 0.2	23.0 ± 1.6*	97.5 ± 2.4	19.6 ± 0.6*	38.7 ± 1.0	59.5 ± 1.5*
60	7.40 ± 0.00*	7.29 ± 0.00*	148 ± 3*	151 ± 3*	97.2 ± 0.2*	19.7 ± 1.6*	95.2 ± 1.8	19.2 ± 0.8*	39.4 ± 1.0*	66.0 ± 1.4*
80	7.38 ± 0.01*	7.24 ± 0.01*	150 ± 3*	151 ± 3*	96.8 ± 0.2*	15.5 ± 1.5*	95.6 ± 2.5	18.6 ± 1.1*	38.3 ± 1.4	71.4 ± 1.6*
100%	7.33 ± 0.01*	7.17 ± 0.01*	153 ± 3*	153 ± 3*	96.3 ± 0.3*	12.0 ± 1.2*	98.8 ± 2.6	17.9 ± 1.1*	34.3 ± 1.3*	76.7 ± 2.2*

Values are mean ± SEM for 9 participants. pH, Haemoglobin (Hb), oxygen saturation (SO_2%), partial pressures of oxygen (PO_2) and carbon dioxide (PCO_2) for arterial and femoral venous blood. * different *vs.* rest, ‡ different *vs.* mild heat stress, † different *vs.* control (all $P < 0.05$). Presented symbols denote differences between conditions at the same relative percentage of $WR_{max.}$

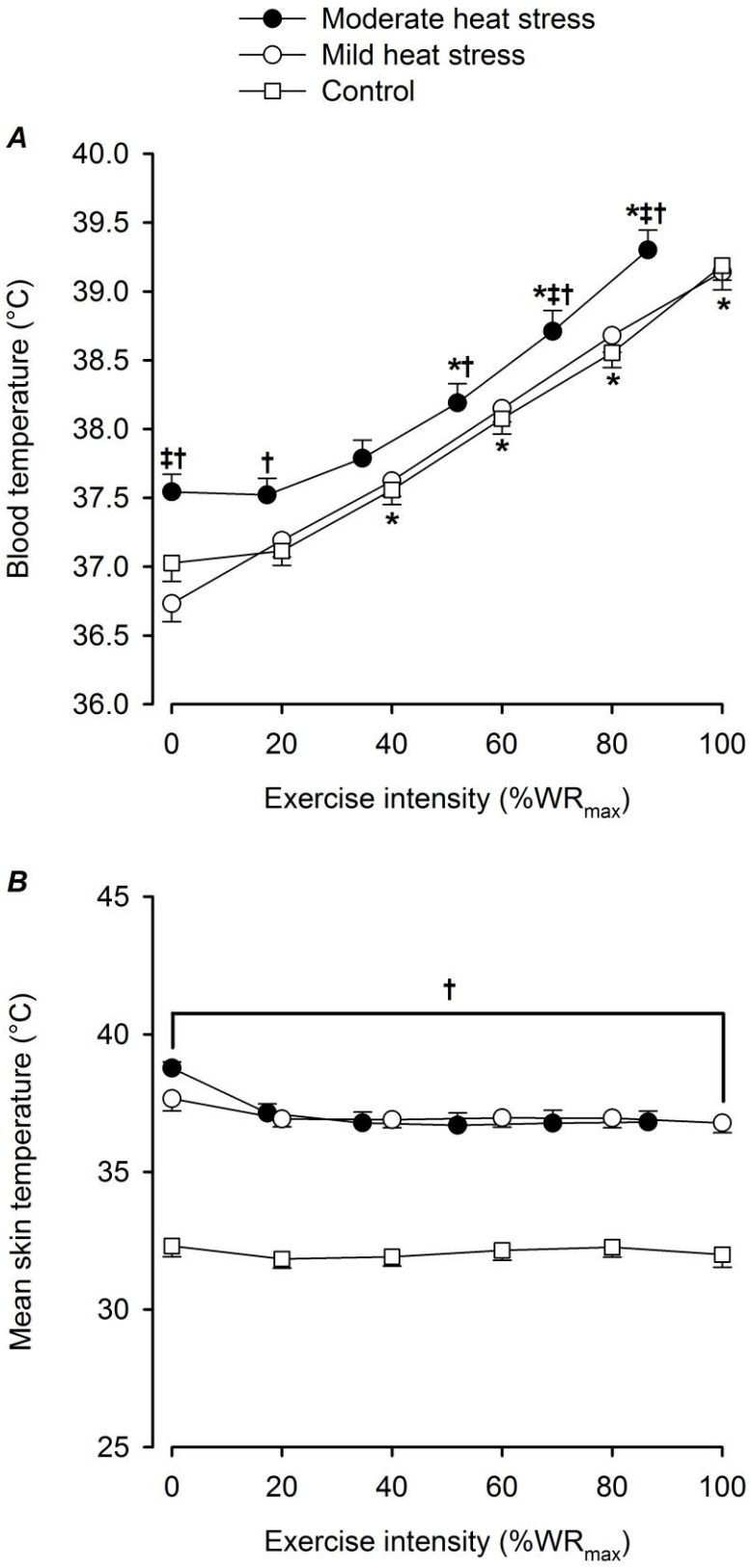


Figure 6-3. Temperature responses to incremental exercise with different grades of heat stress. Values Femoral venous blood (*A*) and mean skin (*B*) temperatures are reported. Values are means ± SEM for 9 subjects. Moderate (internal and skin), mild (skin only) heat stress and control trials are represented. * different *vs.* rest $P < 0.05$, ‡ different *vs.* mild heat stress, † different *vs.* control. Presented symbols denote differences between conditions at the same relative percentage of $WR_{max.}$

Table 6-3. Blood gases and metabolite responses to incremental exercise with different grades of heat stress.

	ctO_2 ($ml \cdot l^{-1}$)		[Lac] ($mmol \cdot l^{-1}$)		[Glu] ($mmol \cdot l^{-1}$)		[HCO_3^-] ($mmol \cdot l$)		**cBase(ECF)**($mmol \cdot l^{-1}$)	
% of WR_{max}	Arterial	Venous	Arterial	Venous	Arterial	Venous	Arterial	Venous	Arterial	Venous
Moderate heat stress										
Rest	199 ± 4‡†	176 ± 3‡†	1.0 ± 0.1	1.1 ± 0.1	5.8 ± 0.1	5.8 ± 0.2	27.3 ± 0.3‡†	27.7 ± 0.3‡†	3.0 ± 0.4‡†	4.1 ± 0.5†
20	209 ± 4*‡†	79 ± 2*‡†	1.7 ± 0.2*	2.1 ± 0.3*	5.9 ± 0.2	5.8 ± 0.3	26.6 ± 0.3*†	27.5 ± 0.4†	2.0 ± 0.5*†	6.5 ± 0.5*†
40	208 ± 4*‡†	56 ± 4*	2.2 ± 0.3*	2.4 ± 0.4*	6.0 ± 0.2	6.0 ± 0.3	26.1 ± 0.5*†	27.0 ± 0.5*	1.5 ± 0.6*	6.8 ± 0.6*†
60	209 ± 4*‡†	46 ± 5*	3.0 ± 0.4*	3.3 ± 0.5*	6.1 ± 0.2‡†	6.0 ± 0.3‡†	25.2 ± 0.5*†	26.0 ± 0.6*†	0.5 ± 0.7*	6.1 ± 0.8*†
80	210 ± 4*	37 ± 4*	4.8 ± 0.5*	5.4 ± 0.5*	6.1 ± 0.3‡†	6.0 ± 0.3‡†	23.3 ± 0.5*‡†	23.8 ± 0.6*‡†	-1.9 ± 0.7*‡	4.2 ± 0.8‡†
100%	210 ± 4*	26 ± 3*	8.6 ± 0.6*	9.7 ± 0.5*	6.3 ± 0.3*‡†	6.3 ± 0.3*‡†	20.1 ± 0.6*‡†	20.3 ± 0.5*‡†	-6.1 ± 0.8*‡†	0.4 ± 0.7*
Mild heat stress										
Rest	191 ± 3†	140 ± 6	1.3 ± 0.2	1.5 ± 0.1	5.9 ± 0.2	5.7 ± 0.3	26.0 ± 0.3†	26.4 ± 0.3	1.7 ± 0.4†	3.4 ± 0.4
20	198 ± 4*†	67 ± 4*	1.5 ± 0.2*	1.6 ± 0.2	5.9 ± 0.3	5.8 ± 0.3	26.0 ± 0.3	26.7 ± 0.3	1.6 ± 0.4	5.6 ± 0.4*
40	199 ± 4*†	49 ± 2*	1.7 ± 0.2*	2.0 ± 0.3*	5.7 ± 0.3*	5.6 ± 0.3	25.7 ± 0.3*	26.3 ± 0.3	1.3 ± 0.4*	6.0 ± 0.5*
60	202 ± 4*†	38 ± 3*	2.8 ± 0.3*	3.3 ± 0.4*	5.4 ± 0.2	5.3 ± 0.2	24.7 ± 0.3*	25.2 ± 0.4*	-0.1 ± 0.5*	5.3 ± 0.5*
80	205 ± 4*	30 ± 3*†	5.7 ± 0.6*	6.3 ± 0.7*	5.3 ± 0.2*	5.2 ± 0.3	22.2 ± 0.5*†	22.5 ± 0.5*	-3.1 ± 0.7*†	2.7 ± 0.7
100%	208 ± 4*	23 ± 2*	10.5 ± 0.8*	11.0 ± 0.8*	5.3 ± 0.3	5.2 ± 0.3	18.3 ± 0.5*†	18.8 ± 0.5*	-8.3 ± 0.7*†	-1.8 ± 0.8*
Control										
Rest	187 ± 4	129 ± 9	1.4 ± 0.2	1.7 ± 0.2	6.1 ± 0.2	5.9 ± 0.1	25.5 ± 0.2	26.0 ± 0.3	1.0 ± 0.3	3.0 ± 0.5
20	195 ± 4*	65 ± 3*	1.6 ± 0.2	1.7 ± 0.2	6.0 ± 0.1*	6.1 ± 0.1	25.5 ± 0.3	26.4 ± 0.3	1.0 ± 0.4	4.9 ± 0.5*
40	197 ± 4*	47 ± 4*	1.7 ± 0.3	2.0 ± 0.3	5.9 ± 0.1	5.9 ± 0.2	25.4 ± 0.3	26.0 ± 0.4	0.9 ± 0.5	5.4 ± 0.6*
60	198 ± 4*	41 ± 4*	2.6 ± 0.3*	3.2 ± 0.4*	5.6 ± 0.2*	5.5 ± 0.2*	24.5 ± 0.4*	24.9 ± 0.5*	-0.1 ± 0.5*	4.8 ± 0.6*
80	201 ± 4*	33 ± 3*	4.8 ± 0.5*	5.5 ± 0.6*	5.3 ± 0.2*	5.2 ± 0.3*	22.7 ± 0.5*	22.8 ± 0.6*	-2.4± 0.8*	2.7 ± 0.8
100%	203 ± 4*	26 ± 3*†	9.3 ± 0.6*	10.3 ± 0.9*	5.2 ± 0.3*	5.1 ± 0.3*	19.1 ± 0.5*	19.4 ± 0.6*	-7.2± 0.8*	-0.8 ± 0.8*

Values are mean ± SEM for 9 participants. Oxygen content (ctO_2), Lactate concentration ([Lac]), Glucose concentration ([Glu]), sodium bicarbonate concentration ([$HCO3^-$])) and acid-base excess (ABE) for arterial and femoral venous blood. * different *vs.* rest $P < 0.05$, ‡ different *vs.* mild heat stress, † different *vs.* control. Presented symbols denote differences between conditions at the same relative percentage of $WR_{max.}$

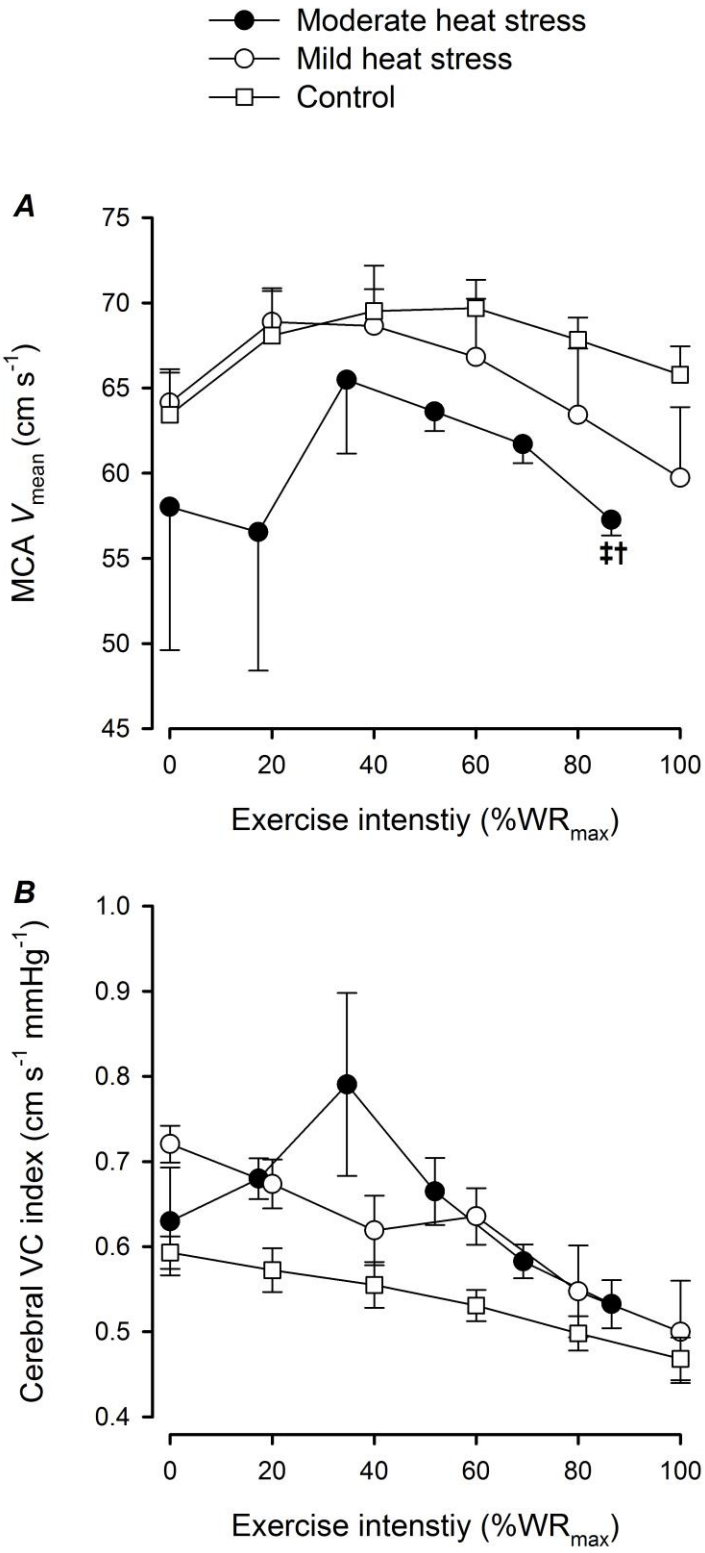


Figure 6-4. Cerebral vascular conductance (VC) responses to incremental exercise with different grades of heat stress. Values are means ± SEM for 9 subjects. Cerebral perfusion (MCA V_{mean}) and cerebral vascular conductance (VC) index are presented. Moderate (internal and skin), mild (skin only) heat stress and control trials are represented. * different *vs.* rest $P < 0.05$, ‡ different *vs.* mild heat stress, † different *vs.* control. Presented symbols denote differences between conditions at the same relative percentage of $WR_{max.}$

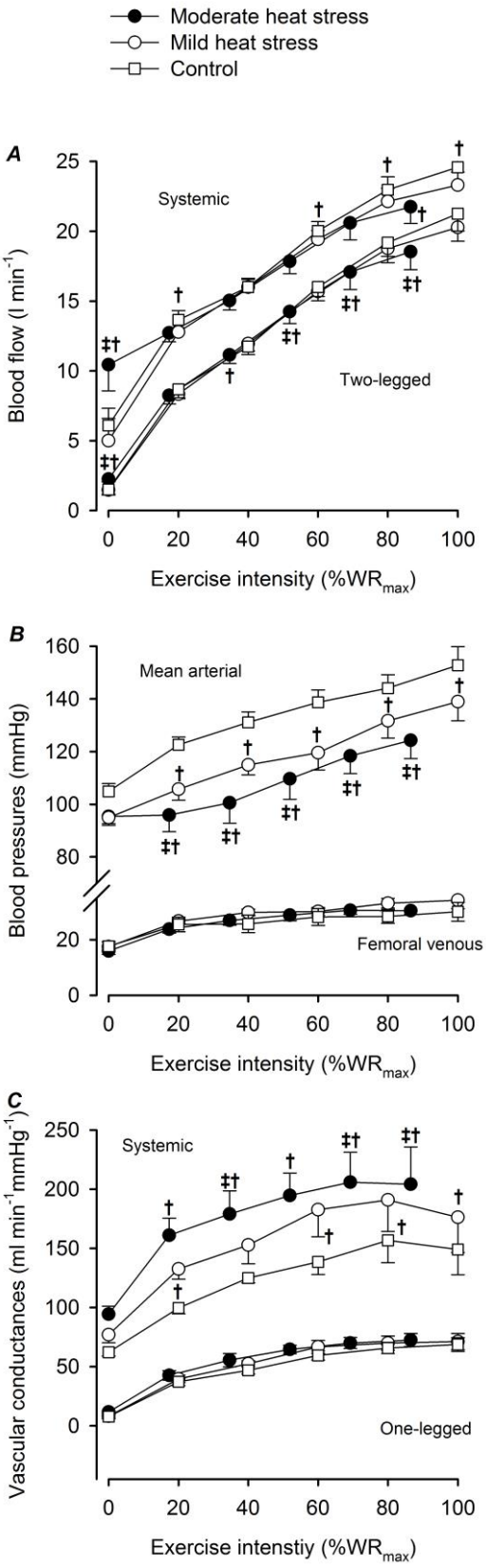


Figure 6-5. Leg and systemic haemodynamic responses to incremental exercise with different grades of heat stress. Blood flow (*A*), systemic and local blood pressure (*B*) and vascular conductance (*C*). Values are means ± SEM for 9 subjects for moderate (internal and skin), mild (skin only) heat stress and control trial. Exercise elevated all variables *vs.* rest (not shown)**,** ‡ different *vs.* mild heat stress, † different *vs.* control (all $P < 0.05$). Presented symbols denote differences between conditions at the same relative percentage of $WR_{max.}$

6.4.3 Leg and systemic oxygen supply and uptake

At rest, leg a-vO_2 difference (24 ± 3 *vs.* ~56 ± 7 ml·l^{-1}) and oxygen extraction (12 ± 1 *vs.* ~32 ± 3%) were lower whereas, leg O_2 delivery (0.42 ± 0.03 *vs.* 0.18 ± 0.02 l·min^{-1}) was elevated in HS_{mod} compared to HS_{mild} and control. However, resting leg $\dot{V}O_2$ (~0.027 ± 0.003 l·min^{-1}) was similar across conditions (P < 0.05; Figure 6-6) as was resting systemic $\dot{V}O_2$ (0.46 ± 0.03 l·min^{-1}) (P = 0.47-0.84). During incremental exercise, leg a-vO_2 difference, leg and systemic oxygen delivery and leg and systemic $\dot{V}O_2$ increased with intensity in all conditions (P < 0.05). At exhaustion, leg a-vO_2 difference and oxygen extraction (87-89%) were not different across conditions, however, leg and systemic O_2 delivery and maximal leg and systemic $\dot{V}O_2$ were reduced in HS_{mod} (P < 0.05) compared to HS_{mild} and control exercise conditions. Lastly, cerebral oxygenation declined by ~13 ± 5Δ% at WR_{max}, but no differences between experimental conditions were observed at exhaustion.

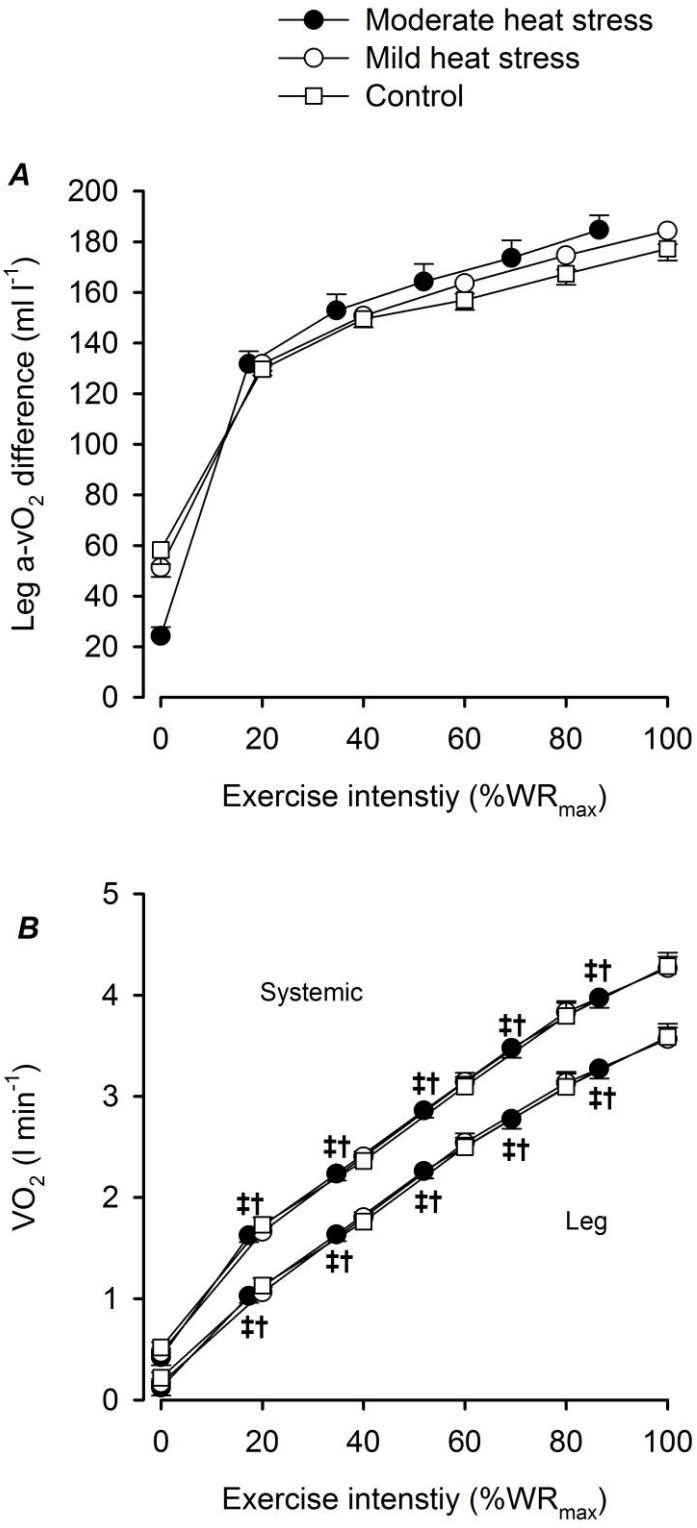


Figure 6-6. Leg and systemic oxygen parameter responses to incremental exercise with two different grades of heat stress. Values are means ± SEM for 9 subjects for moderate (internal and skin), mild (skin only) heat stress and control trial. Exercise elevated all variables *vs.* rest (not shown)**,** ‡ different *vs.* mild heat stress, † different *vs.* control (all $P < 0.05$). Presented symbols denote differences between conditions at the same relative percentage of $WR_{max.}$

6.5 Discussion

This is the first study to systematically alter skin and core temperatures, through different durations of heat stress exposure, to ascertain the role of whole-body *vs.* skin hyperthermia on incremental exercise capacity and gain insight into the potential underlying mechanisms. The novel findings of the present study were threefold. First, a moderate heat stress augmented resting limb blood flow and cardiac output; however, the haemodynamic responses during incremental exercise were similar among the experimental manipulations of skin and core temperatures. Second, only the combination of core and skin hyperthermia compromised maximal aerobic power and exercise capacity. Lastly, the restricted limb and systemic aerobic metabolism during intense exercise with combined core and skin hyperthermia were strongly related to attenuation in the rate of rise in limb perfusion, a plateau in leg and systemic vascular conductance and the attainment of 'maximal' leg O_2 extraction. Taken together, these findings indicate that combined core and skin hyperthermia compromises cardiovascular function and aerobic metabolism during strenuous incremental exercise in trained humans.

Regional and systemic haemodynamics and metabolism at rest and during graded exercise to sub-maximal intensities

A major novel finding of the present study was that, despite the augmented whole-body perfusion under resting core and skin hyperthermia conditions, systemic and limb blood flow increased at a similar rate during incremental exercise, irrespective of the temperature manipulation. Under resting conditions, a moderate heat stress, sufficient to raise core and skin temperature by ~0.8 and 7 °C respectively, resulted in marked elevation in heart rate (+12 beats·min^{-1}), limb blood flow (0.7 l·min^{-1}) and an approximately two-fold increase in cardiac output (from 5 to 10.4 l·min^{-1}), with no elevation in whole-body oxygen uptake. These findings are consistent with the contention that severe passive heat stress invokes a hyperadrenergic state typified by augmented limb, cutaneous and systemic haemodynamics and a substantial thermoregulatory and sympathetic drive (Sawka *et al.* 1985a; Rowell.1993; Niimi *et al.* 1997; Crandall *et al.* 2008; Heinonen *et al.* 2011), and are in general agreement with previous observations of augmented circulatory responses to prolonged passive heat stress (Roddie *et al.* 1956; Rowell *et al.* 1969a; Rowell *et al.* 1969; Rowell 1974; Crandall *et al.* 2008; Pearson *et al.* 2011). In contrast, a brief (mild) heat exposure, with a skin temperature elevation

equivalent to that during moderate heat stress (~37 °C), was an insufficient stimulus to elevate whole-body blood flow compared to control conditions. This was an unexpected observation in the context of Rowell and colleagues' idea that high skin temperature increases the thermoregulatory demand for skin blood flow (Figure 2-7; (Rowell *et al.* 1969a; Rowell 1993). The present data are consistent with the premise that increasing core temperature is the primary stimulus (weighting of 9:1) for the skin hyperaemia (Nadel *et al.* 1971), even though the effector response can be substantially modulated by skin temperature across a wide range of temperature manipulations (Sawka *et al.* 2011).

An important finding of the present study was that during incremental exercise, and in contrast to the observations at rest, limb and systemic blood flow increased at a similar rate up to sub-maximal exercise intensities, irrespective of the temperature manipulation (Figure 6-5). This was the case despite a markedly suppressed mean arterial blood pressure and an elevated systemic vascular conductance. Previous findings on the impact of heat stress on systemic blood flow have been equivocal (Rowell *et al.* 1966; Rowell 1974; González-Alonso & Calbet 2003). On the one hand, it has been shown that stroke volume and cardiac output are substantially reduced at higher intensities during graded exercise (Rowell *et al.* 1966). The implication of this finding was that the competing demand for high skin blood flow compromised systemic (and active muscle) perfusion at high intensities. Contrastingly, during constant-load maximal exercise in the heat, cardiac output is equivalent (if not slightly higher) to normothermic conditions (González-Alonso & Calbet 2003), suggesting that factors other than enhanced skin blood flow *per* se are responsible for early fatigue in the heat. The differences in these findings are probably due to the training status of the participants (untrained *vs.* trained in Rowell *et al.* and González-Alonso *et al.* respectively). Notwithstanding, the current findings do support in part the estimations of Rowell (Figure 2-8) as it was observed that the attainment of maximal HR and a declining SV occurred at a lower absolute oxygen uptake; thus supporting the premise that severe heat stress advances the regulatory limit of the cardiovascular system, concurrently to an early plateau or attenuation in the rate of rise of systemic and limb blood flow prior to volitional exhaustion (Rowell 1974; González-Alonso & Calbet 2003). Moreover, our present findings demonstrate that a high skin temperature (and presumably high blood flow requirements) is unlikely to

compromise active muscle blood flow (Savard *et al.* 1988; Nielsen *et al.* 1990; González-Alonso *et al.* 1999) as cutaneous blood flow plateaus above 37-38 °C (achieved early during graded exercise, and in contrast to the continued elevation in core temperature)(Brengelmann *et al.* 1977) and the circulatory system is able to compensate with sympathetically-mediated redistribution of blood flow from non-active regions (Crandall *et al.* 2008; Kenney *et al.* 2014). Our data demonstrate for the first time that the duration of heat exposure is critical to whether cardiovascular function is impaired during strenuous exercise in the heat stressed human. The regulatory strain only becomes apparent with a combination of skin and core hyperthermia.

Impact of heat stress on maximal aerobic power

To our knowledge this is the first study to simultaneously investigate systemic, brain and active limb perfusion and limb muscle metabolism during incremental exercise in heat stressed individuals. A crucial finding of the present study was that the combination of core and skin hyperthermia prevented the attainment of a comparable $\dot{V}O_{2max}$ to both cool ambient and skin hyperthermia conditions. We observed a reduction in $\dot{V}O_{2max}$ with combined core and skin hyperthermia, but no impact of skin hyperthermia alone. The relative decline in maximal aerobic power (13%) with substantial core and skin hyperthermia is in general agreement with the existing literature (Pirnay *et al.* 1970; Nybo *et al.* 2001; Arngrimsson *et al.* 2004). In contrast, however, others have found a limited impact of heat stress on $\dot{V}O_{2max}$ (decline of ~3-7%) when exercise was performed without a pre-heating protocol (Rowell *et al.* 1965; Rowell *et al.* 1966; Klausen *et al.* 1967; Pirnay *et al.* 1970). These differences are likely due to the different levels of hyperthermia (i.e. skin only *vs.* combined core and skin hyperthermia). Notwithstanding, we observed a similar maximal aerobic power, and systemic and limb haemodynamics and metabolism, with skin hyperthermia alone (Figure 6-5, Figure 6-6).

The purported high skin blood flow requirements with skin heating have been taken to mean that skin temperature alone is an important determinant of aerobic exercise capacity (Ely *et al.* 2009; Ely *et al.* 2010; Lorenzo *et al.* 2010; Sawka *et al.* 2012b). However, it is more likely that a high skin temperature influences aerobic performance through behavioural reflexes associated with thermal comfort, leading to adjustments in power output (and pacing strategies), rather

than a function of circulatory strain *per se* (Schlader *et al.* 2009; Sawka *et al.* 2011; Nybo *et al.* 2014). Our present findings suggest that simply elevating skin temperature does not activate the cascade of events leading to compromised maximal aerobic power. This observation explains why exercise performance in hot environments is not universally impaired across all sports and exercise modalities.

Mechanisms restricting aerobic power with core and skin hyperthermia

In the present study, combined core and skin hyperthermia led to early fatigue during graded exercise in association with an 8% reduction in pulmonary and exercising limbs $\dot{V}O_2$. The lower leg $\dot{V}O_2$ was related to an attenuated limb O_2 delivery (3.9 ± 0.2 *vs.* 4.2 ± 0.2 l·min^{-1}), as 'maximal' leg O_2 extraction (~88%) was similar among the 3 experimental conditions. In contrast, aerobic metabolism was maintained with skin only hyperthermia, with similar haemodynamic alterations compared to control incremental exercise. Whilst this is the first report of different manipulations of temperature on whole-body haemodynamics during incremental exercise, it has previously been shown that restrictions in limb and systemic perfusion, coupled with the simultaneous attainment of a maximal limb O_2 extraction (~90%), of a similar magnitude to that in the present study, markedly reduced leg aerobic metabolism and account for a reduced constant load exercise duration (~5.5 *vs.* 7.6 min) with a significant heat stress (González-Alonso & Calbet 2003; González-Alonso *et al.* 2004). These findings are also in general agreement with observations during normothermic incremental exercise of a marked plateau in limb and systemic vascular conductance and attenuated regional O_2 delivery, eventually leading to a blunted aerobic metabolism and fatigue (Mortensen *et al.* 2005; Calbet *et al.* 2007; Mortensen *et al.* 2008). In the present study, moderate heat stress accentuated the rise in heart rate, to a similar peak value (186 beats·min^{-1}) at reduced absolute work rate. Cardiac tachycardia during incremental exercise, with and without heat stress, can reduce filling time and further accentuates the plateau in stroke volume above 50% $\dot{V}O_{2max}$ (Higginbotham *et al.* 1986; González-Alonso & Calbet 2003; Stöhr *et al.* 2011c). Moreover, it is possible that peripheral mechanisms restricting cardiac filling, rather than maximal heart rate *per se*, are responsible for the attenuated systemic and active muscle blood flow prior to exhaustion during high intensity exercise (Bada *et al.* 2012; Munch *et al.* 2014). Processes at the central nervous system,

particularly in regards to reductions in central drive, are thought to contribute to fatigue processes when heat stressed (Nielsen & Nybo 2003; Nybo & Secher 2004; Todd *et al.* 2005; Nybo 2010; Ross *et al.* 2012; Nybo *et al.* 2014). However, it is unlikely that the moderate reductions in cerebral perfusion, seen here with skin and core hyperthermia and in previous studies (González-Alonso *et al.* 2004; Trangmar *et al.* 2014), are capable of compromising cerebral metabolism to the extent that can explain the reduced aerobic power with substantial heat stressed conditions. Collectively, the present findings are consistent with the idea that a blunted rise in active muscle and systemic blood flow, compromising muscle aerobic metabolism, is a primary contributing factor in the chain of events leading to early fatigue with a significant whole-body hyperthermia.

Methodological considerations

Resting blood flow measurements were made using Doppler ultrasonography, rather than thermodilution. It is known that constant infusion thermodilution technique has limitations when determining LBF in resting conditions since a stable plateau in blood temperature is often not reached. Doppler ultrasound, however, does not have this limitation. Estimated systemic a-vO_2 difference and leg $\dot{V}O_2$ were used to calculate cardiac output (all participants) and LBF (n=5), respectively. It is possible that these estimates may not reflect cardiac output and LBF values at each of the time points. However, a linear relationship is present between systemic and leg a-vO_2 difference (for $\dot{Q}$ estimation), as with systemic and leg $\dot{V}O_2$ (for LBF calculation) during graded exercise (Mortensen *et al.* 2008; Munch *et al.* 2014). We therefore consider our calculations valid under the exercise modality used in the current study.

6.6 Conclusion

In summary, the present findings show that cardiovascular capacity and maximal oxygen uptake are impaired in hot environments which cause both core and skin hyperthermia. The reduced aerobic power was associated with compromised active muscle metabolism due to reduced oxygen delivery. In contrast, skin hyperthermia *alone* does not hinder cardiovascular capacity, maximal oxygen uptake or exercise performance during strenuous whole-body dynamic exercise.

CHAPTER 7 – General discussion

7.1 Introduction

The primary aim of the present thesis was to further understand the circulatory processes contributing to early fatigue in environmentally stressful hot conditions. Specifically, the three studies presented within examined the effects of exercise-induced dehydration and environmental heat stress-mediated skin and body hyperthermia on cerebral, muscle and systemic haemodynamics and metabolism during strenuous exercise. In Chapter 4, cerebral blood flow and metabolism was assessed during maximal incremental exercise in control, dehydrated and rehydrated states. In Chapter 5, blood flow to the cerebral and extra-cranial regions, and cerebral metabolism were investigated during prolonged submaximal exercise with and without progressive dehydration. In Chapter 6, blood flow to the brain, leg and systemic circulations were investigated during maximal incremental cycling exercise to volitional exhaustion, under different grades of heat stress affording the precise manipulation of skin and body temperatures. The following chapter discusses the main findings of each of the three study chapters with integrative discussion of the novel findings in the context of the existing literature. Finally the potential future directions and methodological considerations are discussed.

7.2 Summary of main findings

7.2.1 Impact of dehydration and hyperthermia on CBF and metabolism

The onset of dynamic exercise initiates a chain of events that typically augments regional and global cerebral blood flow; a response that is thought to be required to enhance substrate delivery to the brain and support the elevated neuronal activity (Gjedde *et al.* 2005; Secher *et al.* 2008; Buxton.2009; Rasmussen *et al.* 2010). However, it has been shown that above moderate exercise intensities (Hellstrom *et al.* 1996; Sato *et al.* 2011) and during prolonged strenuous exercise in the heat (Nybo & Nielsen 2001b; Nybo *et al.* 2002), cerebral perfusion can decline towards baseline values. The reductions in O_2 delivery could pose a critical challenge to cerebral metabolism that lead to reductions in central motor output (Nielsen & Nybo 2003; Todd *et al.* 2005; Nybo & Rasmussen 2007), thereby exerting an important role in the chain of events underpinning reduced maximal aerobic and prolonged strenuous exercise capacity with body hyperthermia. In particular, because the loss of body fluids with dehydration during strenuous

exercise in the heat places further strain on circulatory function and the maintenance of systemic and tissue blood flow (Sawka 1992; González-Alonso *et al.* 1995; González-Alonso *et al.* 1997; González-Alonso *et al.* 1998), it was hypothesised that dehydration would substantially attenuate CBF.

The studies contained within this thesis are the first to assess the impact of dehydration on cerebral and extra-cranial blood flow during strenuous exercise in the heat. In Chapter 4, it was observed for the first time that dehydration markedly accelerates the reductions in CBF, above moderate exercise intensities, during graded incremental exercise. The reductions in CBF were accompanied by an increasing cerebral perfusion pressure but falling cerebrovascular conductance, concomitantly with enhanced vasoconstrictor activity, suggesting that local net vasoconstriction underpinned a restricted cerebral blood flow during strenuous exercise. An original finding from Chapter 5 was that dehydration resulted in a progressive decline in CBF. On the other hand, extra-cranial blood flow, which perfuses the skin of the face and neck, increased progressively to 90 min before a plateau occurred. When hydration status was maintained through regular fluid ingestion, CBF was preserved whereas extra-cranial flow responded in a similar fashion. Importantly, the attenuated cerebral perfusion was strongly associated with the decline in P_aCO_2 which is purported to be the primary influencing cerebral vascular tone (Ogoh & Ainslie 2009a; Willie *et al.* 2012). Other factors including sympathetic activity, peripheral reflexes and central haemodynamics (i.e. $\dot{Q}$) may also contribute to cerebrovascular regulation during strenuous exercise in the heat.

No studies to date have explored the impact of dehydration on CBF and metabolism dynamics during strenuous maximal incremental and prolonged sub-maximal exercise in the heat. By using a novel combination of volumetric 'absolute' blood flow, using duplex Doppler ultrasonography, and internal-to-jugular venous blood sampling it was possible to make a reasonable estimate of the absolute $CMRO_2$ across a range of hydration states, exercise intensities and rest-to-exercise transitions. In spite of the circulatory challenge to the human brain during dynamic exercise in the heat, with and without dehydration, compensatory increases in the oxygen extraction fraction afforded a stable $CMRO_2$, suggesting

that impaired aerobic metabolism of the brain as a whole is not a prerequisite for fatigue during intense exercise.

7.2.2 Influence of body temperature on cerebral, systemic and active limb haemodynamics

Uncompensable heat stress has been long known to degrade maximal aerobic power (Rowell 1974; Rowell 1993; González-Alonso & Calbet 2003; Arngrimsson *et al.* 2004). There is, however, discordance in the literature on the impact of high ambient temperatures on maximal exercise capacity; perhaps a consequence of the varied extent of the cardiovascular strain invoked by different manipulations of ambient temperature. It was previously unclear as to whether the reduced exercise capacity is modulated through a regulatory demand for high skin blood flow, due to a substantial elevation in skin temperature alone (Rowell *et al.* 1966; Rowell 1974; Sawka *et al.* 2012a), or whether the concurrent rise in core/internal body temperature is obligatory. Irrespective of heat stress, the attainment of maximal aerobic power during incremental exercise is associated with a marked attenuation in limb and systemic O_2 delivery which compromise active skeletal muscle aerobic metabolism (González-Alonso & Calbet 2003; Mortensen *et al.* 2005; Mortensen *et al.* 2008; Bada *et al.* 2012; Munch *et al.* 2014). No study, however, has systematically assessed the precise regional haemodynamic and metabolic adjustments during maximal incremental exercise in the heat.

In Chapter 6, body temperature was systematically manipulated to provide insight into the circulatory adjustments underpinning incremental exercise capacity under heat stressed conditions. For the first time it was observed that only the combined attainment of a high skin and core hyperthermia was sufficient stimulus to compromise $\dot{V}O_{2max}$. The lower maximal aerobic power was associated with a lower maximal brain, leg and systemic perfusion and a compromised limb aerobic metabolism. These data advance previous findings in normothermic environments (Mortensen *et al.* 2005; Mortensen *et al.* 2008) and during constant load exercise in the heat (González-Alonso & Calbet 2003). Taken together, these studies provide novel information on the circulatory alterations and metabolic consequences which may be important factors limiting strenuous exercise under severe physiological stress. The implications of the present findings are discussed in more detail in the following discussion.

7.2.3 Regional differences in perfusion across the head

For the first time in this thesis, a focal exploration of the regional haemodynamic responses to dehydration across the head was made. In direct contrast to the cerebral circulation, blood flow to the extra-cranial regions was shown to be progressively enhanced with dehydration and hyperthermia during strenuous exercise. Yet, a plateau in ECA blood flow was observed before the end of prolonged exercise in the heat. The discrete extra-cranial blood flow response is mediated by different regulatory mechanisms; however, the physiological consequences for exercise capacity remain unclear.

Extra-cranial blood flow, estimated in Chapter 4 and directly measured in Chapter 5 increased by ~80% over the course of short duration maximal and prolonged sub-maximal exercise in the heat. Similar peak values were obtained irrespective of hydration status. The present findings are in congruence with the limited literature showing a linear increase in ECA flow during graded exercise (Sato *et al.* 2011) and passive elevations in core body temperature (Ogoh *et al.* 2013b). It was suggested that the rise in body temperature associated with heat stress was important for such alterations. We have shown for the first time a strong relationship between the rise in ECA flow and increases in blood temperature and systemic sympathetic activity; hallmarks of the physiological adjustments to exercise hyperthermia. Substantial increases in local and internal body temperature enhance the active vasodilator system and induce a sympathetically-mediated blood flow redistribution (Wallin & Charkoudian 2007; Charkoudian 2010; Johnson *et al.* 2014). This, by extension, could be a mechanism by which the temperature of the tissues of the head is regulated. A further finding in Chapter 5 was that the rise in ECA blood flow and vascular conductance plateaued after ~90 min of exercise (Figure 5-4), concomitant to diminished relationships with the rising temperature and whole-body catecholamines (Figure 5-5). It is possible that the attenuated ECA blood flow, relative to the rising core temperature, is caused by the impact of both exercise and dehydration on cutaneous blood flow. In this light, is has been shown that exercise *per se* restricts skin blood flow above a core temperature of ~38 °C (Brengelmann *et al.* 1977; González-Alonso *et al.* 1999). Dehydration, or more specifically a concomitant hyperosmolality, delays the onset threshold for rising skin blood flow to a higher core temperature (Nadel *et al.* 1980; Shibasaki *et al.* 2009), and results in a lowers SKBF over the course of prolonged

exercise (González-Alonso *et al.* 1995). The precise mechanism by which dehydration restricts ECA blood flow prior to fatigue during prolonged exercise in the heat remains unclear, but could be enhanced vasoconstrictor or diminished active vasodilator activity (Kellogg *et al.* 1998; Charkoudian 2003; Charkoudian 2010; Johnson *et al.* 2014).

A speculative question is whether the increase in extra-cranial perfusion has any bearing on the temperature or haemodynamics of the brain. Face cooling elevates cerebral perfusion at rest and during exercise; however, these findings are confounded by alterations in cardiac output and mean arterial pressure (Miyazawa *et al.* 2012; Miyazawa *et al.* 2013). It is unlikely that 'relieving' the head cutaneous demands for blood flow affect in any way the cerebral circulation as preventing the decline in cerebral perfusion during passive hyperthermia does not lead to further elevations in extra-cranial blood flow (Bain *et al.* 2013). Additionally it remains unlikely that cooling the external surface of the head has an influence on the rise in brain tissue and blood temperature, despite apparent benefits on thermal perception and time to exhaustion (Tyler & Sunderland 2011). Humans do not demonstrate selective brain cooling and brain temperature (indicated by the arterial-to-internal jugular venous temperature difference) is not reduced by face fanning or intranasal cooling in humans (Nybo *et al.* 2002; Nybo 2010; Nybo *et al.* 2014) or rats (Zhu *et al.* 2006; Zhu *et al.* 2009), suggesting that local skin cooling does not determine the absolute cerebral temperature (Nybo *et al.* 2014). Manipulating skin temperature with surface cooling strategies may simply alter the contribution of blood flow to temperature gradients in convective and conductive heat loss from the skin of the head, without affecting deep brain temperature. This phenomenon is well characterised for core temperature in humans exercising in environmental temperature ranging from 5 to 35 °C (Nielsen, 1938). Overall the findings from the present thesis show marked elevations in extra-cranial blood flow with increasing body temperature and systemic sympathetic activity; synonymous with exposure to a marked hyperthermia. The physiological significance of such a response remains to be fully elucidated.

7.3 Mechanistic overview of the present findings

7.3.1 Circulatory limitations to strenuous exercise in the heat

A primary focus of the present thesis was to provide further insight into the circulatory limitations explaining a compromised maximal and sub-maximal exercise capacity. By the use of heat stress and dehydration it was possible to induce a marked strain on cardiovascular function, compromising both maximal incremental and prolonged exercise capacity. On the basis of the findings of the present thesis, and literature pertinent to cerebral and active muscle blood flow during exercise in the heat, a model is presented pertaining to how elevations in core body temperature affect the integrative body systems prior to fatigue during dynamic cycling exercise (Figure 7-1).

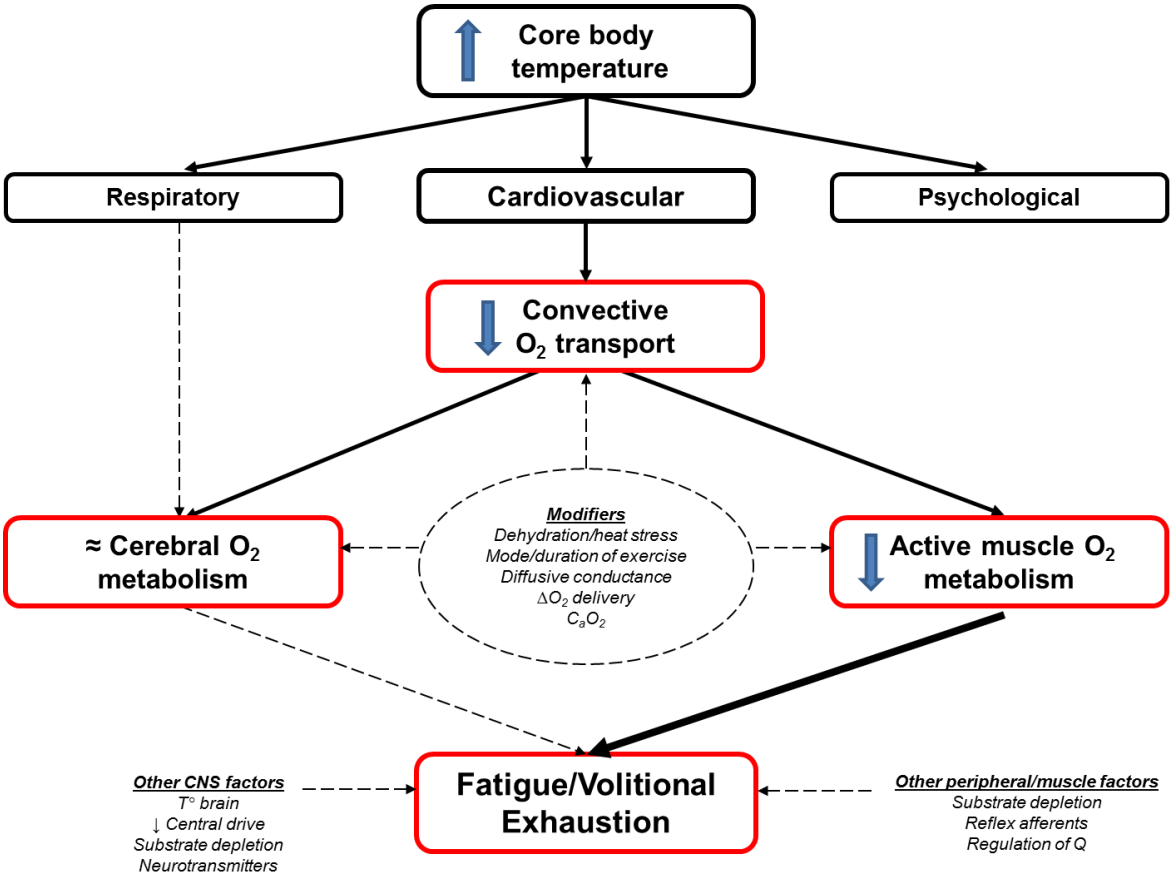


Figure 7-1. Model of the integrative factors contributing to early exhaustion in the heat. Exercise in the heat eliciting high metabolic rates increases both skin and core body temperature and places a significant strain on all physiological systems. The rise in body temperature is dependent on numerous factors including hydration status, exercise intensity and mode and whether the environmental conditions are deemed compensable or uncompensable (Sawka *et al.* 2011; Nybo *et al.* 2014). In the psychological domain, high body temperatures enhance thermal sensation and alter the perception of effort when compared to temperature environments (Gagge *et al.* 1969). Whilst not explored in the present thesis, hyperthermia is associated with a high ventilation rate under resting and exercising conditions (Haldane 1905; White 2006), which influences the perception of effort and alters cerebral O_2 delivery via its effects on P_aCO_2. The findings of the present thesis, in relation to the constituents of the Fick principle, at the level of the brain and limb are highlighted in red and are explored further in the text. Modified from *Nybo et al.* (2014).

In accordance with the Fick principle, the maximal convective O_2 transport and O_2 extraction (a-vO_{2diff}) set the upper limit for local and systemic aerobic metabolism ($\dot{V}O_2$). In light of the findings of chapters four through six, the impact of the present manipulations of body temperature and hydration on systemic and local perfusion is explored. Second, the present observations of different O_2 extraction capacities and the impact on local aerobic metabolism are discussed. Lastly, an appreciation is made for the other integrative processes contributing to early fatigue in the heat.

Convective O_2 transport

A primary finding of the present thesis was that dehydration accelerated the decline in CBF and O_2 delivery during maximal incremental and prolonged strenuous exercise in a hot environment (Chapters 4 & 5). Moreover, exercise in a high ambient temperature invoking combined skin and core hyperthermia substantially reduced maximal aerobic power, in association with and early attenuation in both systemic and active limb blood flow (Chapter 6).

Reductions in systemic and regional blood flow can independently impair local aerobic metabolism through a decline in convective O_2 transport. In the present thesis, both dehydration and hyperthermia invoked an early reduction (cerebral) or attenuation (limb) in convective oxygen transport which could impair local aerobic metabolism when the compensatory capacity to further extract oxygen from the arterial blood is exhausted. The present findings are in general agreement with previous observations that the rate of rise in active muscle blood flow is attenuated prior to exhaustion, concomitant to a substantial increase in sympathetic activity, blunted systemic and limb vascular conductance and an attenuation in central cardiovascular haemodynamics (Rosenmeier *et al.* 2004; Mortensen *et al.* 2005; Calbet *et al.* 2007; Mortensen *et al.* 2008; Stöhr *et al.* 2011c; Trinity *et al.* 2012). It is also clear that the cerebral circulation is similarly affected by a general reduction in systemic perfusion that manifests during exhaustive exercise in the heat (Nybo *et al.* 2001; Nybo & Nielsen 2001b; González-Alonso *et al.* 2004), or when dehydration is developed during prolonged sub-maximal exercise (González-Alonso *et al.* 1995; González-Alonso *et al.* 1997; González-Alonso *et al.* 1998). The present thesis extends these findings by showing that 1) the decline in CBF at high intensities is markedly accelerated by the superimposition of dehydration and 2) that core and skin hyperthermia accelerates the attainment of maximal circulatory adjustments which in part contributes to the reduced $\dot{V}O_{2max}$ in hot ambient conditions.

An interesting finding in Chapter 6 was that brief exposure to exogenous heat stress, without increasing core temperature, did not negatively impact on systemic and active limb blood flow or maximal exercise capacity during incremental exercise. In general, prolonged sub-maximal exercise invoking the attainment of a high (‘critical’) core temperature does not reduce systemic and active limb blood

flow compared to normothermic conditions (Savard *et al.* 1988; Nielsen *et al.* 1990; Smolander & Louhevaara 1992; Nielsen *et al.* 1993; González-Alonso *et al.* 1998). This suggests that, at least at sub-maximal exercise intensities, the additional circulatory demands requiring enhanced cutaneous blood flow are supported without compromising blood pressure (and flow) regulation (Rowell *et al.* 1966; Kenney *et al.* 2014). However, in similar exercise and environmental conditions, the progressive loss of body fluids with dehydration augments core temperature and reduces active muscle perfusion (González-Alonso *et al.* 1998). As shown for the first time in Chapter 5, progressive dehydration similarly compromises CBF during prolonged exercise in the heat whereas, when euhydrated, cerebral perfusion remains stable until such time that a substantial hyperthermia-induced hypocapnia develops (Nybo & Nielsen 2001b). At higher exercise intensities, approaching $\dot{V}O_{2max}$, $\dot{Q}$ was shown to decline in untrained men during graded exercise in the heat (Rowell *et al.* 1966), leading to the hypothesis that high skin blood flow requirements compromise systemic (and therefore active muscle) O_2 delivery (Rowell 1974). However, more recently, systemic perfusion is shown to be equivalent in both high and normothermic ambient conditions in trained athletes (González-Alonso & Calbet 2003). Nevertheless, when core and skin temperature rise considerably during exercise eliciting maximal aerobic power (as in Chapter 6), the regulatory limit of the cardiovascular system (i.e. maximal HR, $\dot{Q}$ and O_2 extraction) is attained more quickly and, in the novel findings of the present thesis, at a lower percentage of normothermic $\dot{V}O_{2max}$.

Whilst subject to much debate, the findings of the present thesis are in general agreement with the view that the attainment of $\dot{V}O_{2max}$ during dynamic exercise, with or without heat stress, involving a large muscle mass is preceded by an attenuation in systemic, brain and active muscle blood flow (González-Alonso & Calbet 2003; González-Alonso *et al.* 2004; Mortensen *et al.* 2005; González-Alonso 2006; Saltin & Calbet 2006; Mortensen *et al.* 2008). It has long been shown that the maximal vasodilation of the skeletal muscle would 'outstrip' the pumping capacity of the heart, as maximal recruitment of more than half of the skeletal musculature is unattainable during a prolonged period in normally active individuals (Secher *et al.* 1977; Andersen & Saltin 1985; Rowell.1993; Mortensen *et al.* 2005; Calbet *et al.* 2007; González-Alonso *et al.* 2008; Mortensen *et al.*

2008). This premise is further evidenced by the observation that inspiratory loading and assisted ventilation reduce and increase respectively, blood flow to the active limbs (Harms *et al.* 1997; Harms *et al.* 1998); however, it is unlikely that the respiratory musculature is spared from the overall reduction in systemic perfusion during maximal whole-body exercise (Secher & Richardson 2009; Vogiatzis *et al.* 2009). In contrast to dynamic, whole-body exercise, when exercise is isolated to a small fraction of muscle systemic and active muscle blood flow has been shown to increase linearly with exercise intensity (Andersen & Saltin 1985; Mortensen *et al.* 2005; Mortensen *et al.* 2008), indicating that the volume of muscle mass recruited has a significant bearing on whether exercise capacity is reduced by a perfusion limitation.

The mechanisms attributed to the decline in cerebral perfusion during maximal and prolong sub-maximal exercise were explored in detail in Chapters 4 and 5. The precise mechanisms regulating systemic and limb perfusion at high intensities remain to be fully elucidated in the context of the present thesis. Maximal heart rate appears not to place a limiting influence on O_2 delivery (Bada *et al.* 2012; Munch *et al.* 2014); however, because peripheral blood flow appears to be primarily controlled at the local level through the balance of vasodilator and vasoconstrictor activity (Mortensen *et al.* 2007; González-Alonso *et al.* 2008; Mortensen *et al.* 2009; Mortensen *et al.* 2009; González-Alonso 2012; Hellsten *et al.* 2012), it is possible that overriding vasoconstrictor activity at the active muscle vasculature is responsible for the compromised systemic perfusion (Bada *et al.* 2012). Furthermore, both heat stress and maximal exercise independently augment systemic sympathetic activity concomitant to a reduced limb and systemic vascular conductance, even when only a small muscle mass exercise is employed (Mortensen *et al.* 2008). It is therefore possible that the early attenuation of systemic and active muscle perfusion with high core and skin temperatures in Chapter 6 is related to enhanced vasoconstrictor tone.

In addition to the decline in $\dot{Q}$, any reduction in arterial oxygen content will narrow the arterio-venous gradient and limit $\dot{V}O_{2max}$ (Rowell.1993). Elevating C_aO_2 acutely via manipulation of red blood cell mass enhances maximal aerobic power and $\dot{Q}$ (Ekblom *et al.* 1972; Ekblom *et al.* 1975; Ekblom *et al.* 1976; Gledhill *et al.* 1999), whereas reductions attenuate aerobic power (Calbet *et al.* 2009). Reductions in

arterial oxygen saturation can occur in maximally exercising elite athletes by alveolar-to-arterial diffusion limitations, intra-pulmonary shunt and ventilation perfusion mismatch (Wagner 1996; Dempsey *et al.* 2008a; Dempsey *et al.* 2008b; Wagner 2011); however, this is not a universal finding in normally active individuals exercising maximally with a normal inspired O_2 fraction (Rowell.1993). As typically observed, dehydration and hyperthermia (Chapters 4 and 5), and exercise heat stress (Chapter 6), resulted in an arterial haemoconcentration, and with no clear arterial desaturation prior to volitional exhaustion it is unlikely that O_2 delivery in the heat was restricted through this mechanism.

Oxygen extraction and local aerobic metabolism

Because the active musculature (inclusive of respiratory, cardiac and skeletal muscle) accounts for ~90% (leg $\dot{V}O_2$ = ~84%) of the increase in systemic oxygen uptake during incremental exercise (Rowell.1993; Calbet *et al.* 2007), the attenuated and early decline in systemic, cerebral and active limb O_2 delivery can compromise local aerobic metabolism.

The observations in the present thesis argue against impairment of cerebral aerobic metabolism prior to volitional exhaustion, during maximal incremental and prolonged strenuous exercise in the heat, irrespective of hydration status. More specifically Chapters 4 and 5 have shown that fractional oxygen extraction increased to compensate for the decline in O_2 delivery, regardless of hydration status. Moreover, on further analysis it is apparent that these acute alterations are proportional to the reduction in blood flow, such that the $CMRO_2$ was not compromised before fatigue (Figure 7-2).

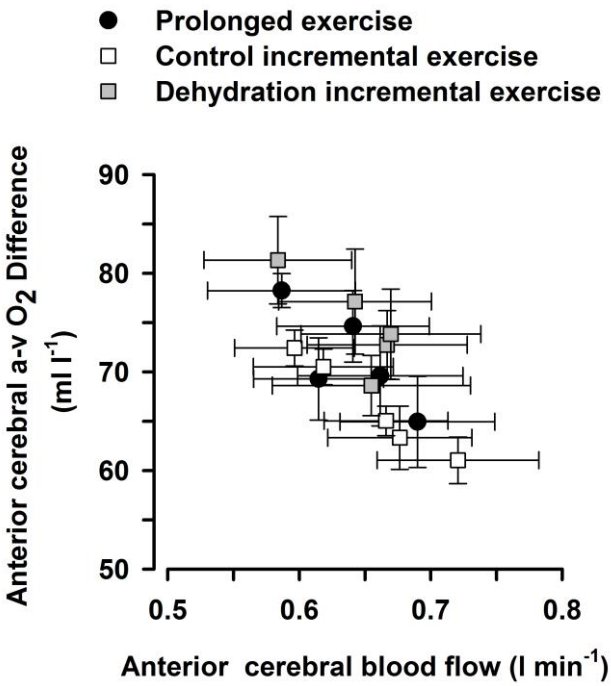


Figure 7-2. Relationship between cerebral perfusion and a-vO_2 difference. Note that regardless of the exercise condition, the decline in anterior cerebral blood flow was equally compensated for by adjustments in oxygen extraction, such that CMRO2 was maintained at ~45 ml·min^{-1}. Data from dehydration during prolonged exercise, and in control and dehydration conditions during maximal incremental exercise.

The capacity to enhance oxygen extraction in the face of a compromised O_2 delivery could be considered an important functional adaptation to ensure the appropriate and stable oxygenation of the brain. However, a contrasting theory has evolved suggesting that reductions in O_2 delivery with hyperthermia can challenge the maintenance of cerebral oxygenation (Rasmussen *et al.* 2010). In the study by Rasmussen and colleagues it was observed that hyperthermia during prolonged exercise, 1) increased the $CMRO_2$ by ~10% in association with the Q_{10} effect, 2) concurrently, CBF declined by ~15% and, 3) oxygen extraction increased by ~8%. It was suggested that these events led to a reduction in mitochondrial oxygen tension ($P_{mito}O_2$) which could impair cerebral oxygen availability; implying a diffusional limitation to O_2 transport to the cerebral tissue (Gjedde *et al.* 1999; Gjedde *et al.* 2005). Whilst this is an attractive hypothesis, it is unlikely to explain the reduced exercise capacities in the present thesis. Despite the marked reductions in CBF observed in Chapters 4 and 5, a rise in $CMRO_2$ was not evident, and the increase in O_2 extraction (< 40%) was far below the 'theoretically maximal' levels at volitional exhaustion (McHenry *et al.* 1961; Bain & Ainslie 2014; Bain *et*

al. 2014; Lewis *et al.* 2014a). A reduction in CBF on the order of 50-60% is required to lower the $P_{mito}O_2$ to below ~5 mmHg; a threshold that is considered not to be compensated for by further increases in O_2 extraction (Gjedde *et al.* 2005; Nybo & Rasmussen 2007; Secher *et al.* 2008; Bain *et al.* 2014). Lastly, whilst cerebral oxygenation (indexed in the frontal cortex) is reduced with hypoxemia, restoration of cerebral O_2 delivery with hyperoxia and CO_2 clamping does not increase maximal incremental power (Subudhi *et al.* 2009; Subudhi *et al.* 2009; Olin *et al.* 2010; Olin *et al.* 2011; Subudhi *et al.* 2011; Subudhi *et al.* 2011). Taken together these findings support the premise that reductions in cerebral oxygenation are not a major factor explaining the reduction in maximal aerobic power with hyperthermia.

In contrast, the findings in Chapter 6 provide evidence that attenuated oxygen delivery to the working muscles, which is advanced with high skin and core temperatures, can suppress active muscle $\dot{V}O_2$. Specifically we observed a marked reduction in maximal aerobic power, with combined skin and core hyperthermia, concomitant to a higher heart rate and core temperature (Figure 6-3), and lower systemic and active limb perfusion (Figure 6-5). The early attenuation in the rate of O_2 delivery was temporally matched by a similar peak $a\text{-}vO_{2difference}$ and oxygen extraction (~88%); that is, there was no increase in O_2 extraction above values observed under normothermic control conditions. The present findings are in congruence with previous observations during constant power maximal exercise in the heat where it was shown that a reduced exercise capacity occurred in the presence of a maximal oxygen extraction of ~90%; which again was similar to control conditions (González-Alonso & Calbet 2003). Unlike the brain, therefore, active skeletal muscle aerobic metabolism is restricted prior to exhaustion by the transient attenuation in O_2 supply (Figure 7-3). The present novel findings extend these observations by showing for the first time that whole-body heat stress leads to the early attainment of the circulatory regulatory limit during maximal incremental exercise. Restricted active muscle aerobic metabolism, but not $CMRO_2$, is an important factor contributing to the cascade of events leading to early fatigue during maximal aerobic exercise under heat stressed conditions.

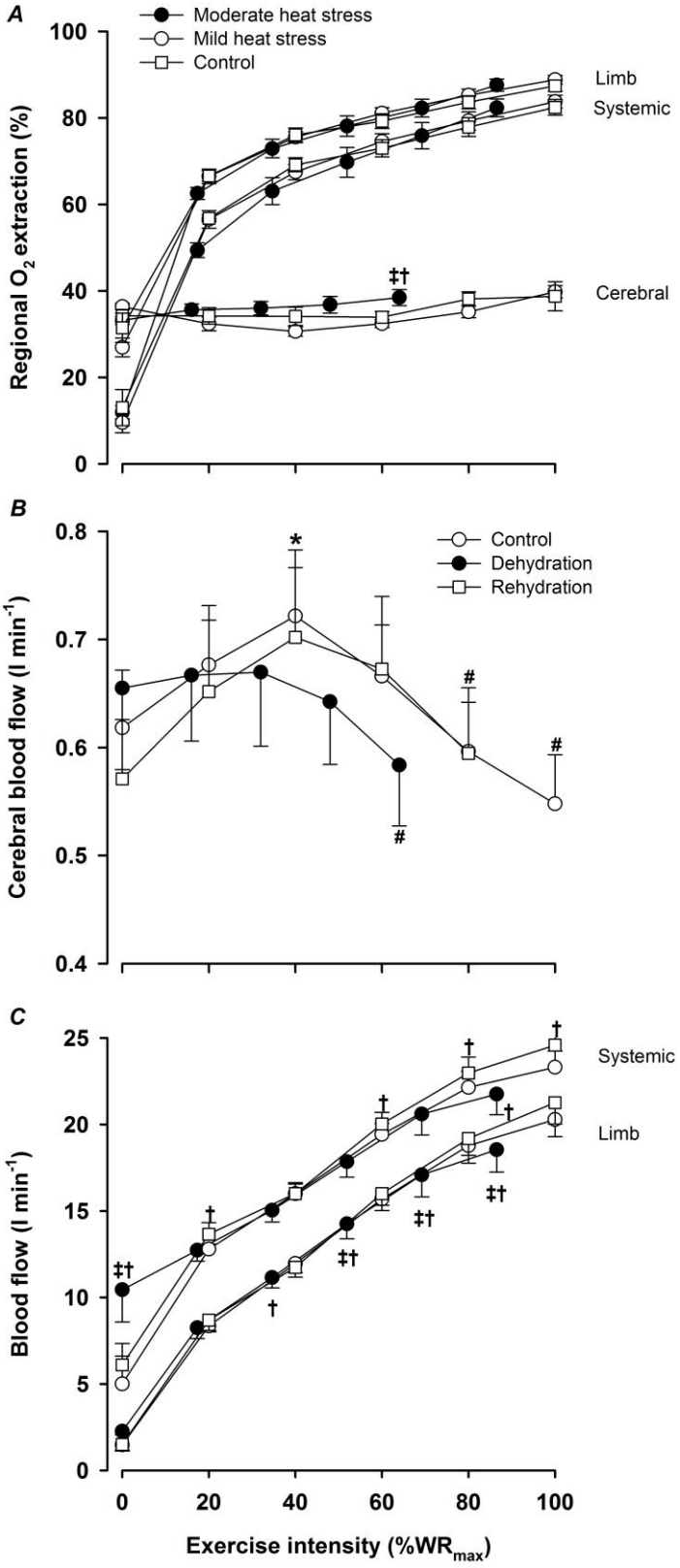


Figure 7-3. Integrative systemic and regional blood flow and metabolism in studies 1-3. Note that maximal oxygen extraction across the limb and systemic circulations is achieved earlier in moderate heat stress compared to mild and control conditions (A), in association with an accelerated attenuation in limb and systemic blood flow per unit of power (C). Across the brain, peak oxygen extraction was ~40% irrespective of dehydration (A), and accompanied a similar fall in cerebral blood flow (B).

Other CNS factors

In respect to the model presented in Figure 7-1, it remains possible that other processes are impaired by dehydration and hyperthermia and explain the early fatigue in hot environments. At the level of the central nervous system, elevations in core body temperature during prolonged exercise in the heat increase the rate of cerebral and body heat storage (Nybo *et al.* 2002). Cerebral tissue temperature during strenuous exercise is primarily influenced by the increasing arterial blood temperature and the reductions in cerebral blood flow (reducing convective heat transfer via the circulation), as heat loss through the cranium is minimal (Nybo *et al.* 2002; Zhu *et al.* 2006; Zhu *et al.* 2009). Although direct cerebral tissue temperature measurements are unsuitable in healthy, maximally exercising humans, it is possible that attainment of a high brain or hypothalamic temperature might be a mechanism by which central drive to the locomotor muscles is impaired with body hyperthermia; although the current associations are not causal (Nybo & Nielsen 2001c; Nielsen & Nybo 2003; Cheung & Sleivert 2004; Ross *et al.* 2012). This is, however, an unlikely scenario in the present thesis as the extent of body hyperthermia was lower, in comparison to other reports (Nybo & Nielsen 2001b; Nybo *et al.* 2002; Nybo *et al.* 2002).

Reductions in brain glycogen stores might constitute a limiting factor for impaired metabolism during intense neuronal activation (Dalsgaard *et al.* 2004a; Dalsgaard & Secher 2007). During maximal exercise, glucose is taken up by the brain in surplus to the oxidative rate; it is considered that this occurs to replenish glycogen stores which be initially depleted during cerebral activation (Dalsgaard *et al.* 2002). There remains limited understanding of the contribution of these alterations to fatigue during exercise; however, limited data in rats suggests that the brain 'super-compensates' glycogen stores after exercise in certain brain regions (e.g. hippocampus, cortex), and may constitute a metabolic adaptation to training (Matsui *et al.* 2012). A further possibility is that disturbances in brain neurotransmitters (e.g. dopamine and serotonin), that are associated with feelings of lethargy under heat stressed conditions, precipitate the development of 'central fatigue' and advance the early curtailment of exercise (Nielsen & Nybo 2003; Meeusen *et al.* 2006). Identification of the precise cerebral alterations that may be of importance for the development of fatigue during maximal or prolonged strenuous exercise remains challenging.

7.4 Methodological considerations

Unilateral blood flow measurements were made in all studies. An assumption was made that extra-cranial volume flow measured on one side is similar to that of the contralateral side (Schoning *et al.* 1994). A limitation to CBF measurements in Chapters 4 & 5 was that it was only possible to assess anterior cerebral blood flow (i.e. the internal carotid artery). It is estimated that the two ICAs perfuse ~70-80% of the brain, with the remaining supplied by two vertebral arteries within the posterior circulation of the head. It is acknowledged that the VAs may have a different CO_2 reactivity and respond in a different manner during graded exercise by increasing linearly up to 80% WR_{max} (Sato *et al.* 2011). This is possibly due to the enhanced metabolic demand of the regions perfused by the posterior circulation (for example the cardiorespiratory centre) which may become increasingly more active during high intensity exercise (Delp *et al.* 2001). It remains to be established whether this relationship holds with dehydration and during strenuous exercise in the heat.

Changes in CBF were underestimated through the omission of measurements of VA blood flow (purported to contribute ~25% of total CBF), which in turn led to an underestimation of $CMRO_2$. However, assuming a similar relative increase observed by Sato and colleagues, the relatively small changes in VA flow do not seem to influence the $CMRO_2$ to an extent that would undermine the findings of the present thesis. It is important to point out that the present measures of CBF and the independently assessed a-vO_2 differences were inversely related across a variety of conditions, providing assurance of the sensitivity of these measures. Notwithstanding this, it is acknowledged that there is evidence, although limited, that global $CMRO_2$ may be augmented during strenuous exercise (Nybo *et al.* 2002). Future studies should therefore aim to also measure VA flow as well as posterior venous blood oxygenation to conclusively establish whether $CMRO_2$ indeed remains stable or increases during exhaustive exercise.

A final consideration from studies 1 and 2 was that jugular venous blood samples were obtained unilaterally from the left internal jugular vein. Despite an asymmetry existing in the venous drainage of the brain, comparable blood haematological and gas values have been reported in both the left and right internal jugular veins. Moreover, the jugular vein might be partially collapsed, in favour of the spinal

venous plexus (Gisolf *et al.* 2004; Olesen *et al.* 2014), and it therefore is possible that this influence the metabolic processes (Valdueza *et al.* 2000). It is, however, unlikely that the calculated $CMRO_2$ would be altered substantially by sampling from the alternate vein (Gibbs *et al.* 1942; Munck & Lassen 1957). Moreover, comparable a-vO_2 difference dynamics is observed during incremental exercise based on right jugular vein blood samples (Ide *et al.* 1999b). Placement of the catheter in the retrograde position to the jugular bulb is designed to preclude any extra-cerebral contamination, although this cannot be completely excluded.

Lastly, estimations of mixed venous (pulmonary artery) PO_2 and O_2 saturation were made to calculate cardiac output in study 3. It is acknowledged that these estimations may not reflect the blood gas variables that would have been obtained in the present thesis. PO_2 only contributes a negligible part of the calculated oxygen content whereas discrepancies in SO_2 would significantly influence the estimation. There is a strong linear relationship (R^2 = 0.99; P = 0.00) between femoral-venous and central mixed venous blood gases during incremental exercise (Munch *et al.* 2014), which make our current estimations reasonable in the circumstances of the present thesis.

7.5 Significance of findings and future directions

For the first time this thesis has characterised the haemodynamic and metabolic responses to exercise-induced dehydration and heat stress-mediated skin and core hyperthermia and provides novel insight into the impact of reductions in convective O_2 delivery on brain and active limb metabolism during both maximal and prolonged exercise in trained humans. These data further support the literature on the detrimental effects of dehydration and hyperthermia on physiological function and exercise capacity (González-Alonso *et al.* 1995; González-Alonso *et al.* 1997; González-Alonso *et al.* 1998; González-Alonso & Calbet 2003; González-Alonso *et al.* 2008; Cheuvront & Kenefick 2014; Nybo *et al.* 2014). Understanding the physiological processes at the level of the brain and active limb are of upmost importance to understanding the functional limitations of the human body during severe stress. The ability to 'cope' with the circulatory strain imposed by such conditions appears to region specific, with the brain having a greater functional oxygen extraction reserve than the active skeletal muscles to offset dehydration and hyperthermia induced reductions in convective O_2 delivery. Moreover, appreciation of the impact of dehydration and varying extents of heat stress are of great importance to athletes and coaches who are often exposed to similar environmental challenges during performance.

Studies 1 and 2 have shown that the brain is able to use its 'oxygen reserve' to compensate for transient cerebral hypoperfusion. This provides further support for the fact that, at least in the exercise paradigms explored in the present thesis, the $CMRO_2$ is largely unaltered. Others have suggested that reductions in CBF are capable of reducing the diffusive capacity for oxygen into the neuronal mitochondria and that cerebral oxygenation can become compromised (Gjedde *et al.* 1999; Rasmussen *et al.* 2007; Rasmussen *et al.* 2010). The latter mechanism, coupled with a reduction in the cerebral metabolic ratio (Dalsgaard & Secher 2007) could yet be a contributing factor to the development of central fatigue observed prior to exhaustion during dynamic whole-body exercise, particularly with additional challenges to convective O_2 transport (e.g. hypoxia) (Nybo & Rasmussen 2007; Goodall *et al.* 2012). Further studies should try to delineate these distinct pathways and use novel techniques (e.g. arterial spin labelling with MRI, PET) to further explore the cerebral changes to dynamic exercise in stressful

conditions. Specifically, studies invoking significant challenges to CBF (for example severe hyperthermia, haemorrhage and orthostatic challenge) coupled with the advanced measurement techniques will further our understanding of the cerebral adjustments during physiological stress.

Dehydration invokes whole-body fluid displacement and volume changes, which have also been shown to affect the brain (Kempton *et al.* 2009; Kempton *et al.* 2011). To this end, dehydration invoked volume changes may influence neuronal function during cognitive tasks (Kempton *et al.* 2011). If it is considered that exercise, particularly with dehydration and concomitant hyperthermia, invokes changes in cerebral activation, it is possible that direct changes in cerebral morphology might negatively influence cerebral processes and contribute to fatigue. Combination magnetic resonance imaging (MRI) and arterial spin labelling (ASL), ultrasound and haematological measurements under such conditions could be an avenue to assess the deep structural changes at the level of the brain.

Modest elevations in core temperature, invoking reductions in P_aCO_2 and CBF to levels less than those presented in the current thesis, have been shown to reduce voluntary activation during sustained MVC (Nybo & Nielsen 2001a) and during cortical voluntary activation assessed with trans-cranial magnetic stimulation (Todd *et al.* 2005; Ross *et al.* 2012). As highlighted in Chapter 4, reduced convective O_2 delivery does not negatively impact on the cerebral metabolic rate whereas, a high CNS temperature *per se* might influence central nervous drive to the muscles and could explain the 'central fatigue' associated with exercise hyperthermia. Future studies should combine exploration of central fatigue with hyperthermia, concomitantly with the assessment of cerebral haemodynamics and metabolism.

There are still many unresolved questions on the regulation of cerebral blood flow during exercise. In particular, the functional role of sympathetic and cholinergic innervation within the cerebral vessels remains contested. Due to the clearly important role of P_aCO_2 on cerebral vasoconstriction, sympathetic activity may be a redundant mechanism. In Chapter 4 it was observed that cerebral catecholamine uptake was enhanced whereas, recent evidence of NA spillover from the brain has been shown (Mitchell *et al.* 2009; Seifert & Secher 2011). Designing a paradigm

whereby a critical role for sympathetic activity can be identified is challenging because of the influence of such manipulations on other factors that contribute to CBF (e.g. MAP and $\dot{Q}$). Future studies, perhaps utilising direct/global α_2-adrenergic blockade (supressing sympathetic outflow) and cerebral NA spillover, combined with ultrasound derived measurements of vessel diameter, are required to further our knowledge of the role of sympathetic activity on the regulation of CBF.

It has been considered that the increase in extra-cranial blood flow with heat stress, and during exercise, is related to thermoregulatory mechanisms (Sato *et al.* 2011). This assumption is supported by the present and direct association with blood temperature during prolonged exercise; supported by indirect evidence of important elevations in ECA flow for local cutaneous perfusion (Miyazawa *et al.* 2012; Ogoh *et al.* 2013b). Whilst we now highlight a clear relationship with blood temperature, the regulatory mechanisms underpinning the elevated extra-cranial flow remain unclear. Local and systemic increases in temperature initiate enhanced skin perfusion through neural (axon-reflex) and metabolic (i.e. NO) pathways (Kellogg *et al.* 1998; Charkoudian 2010; Johnson & Kellogg 2010; Johnson *et al.* 2014). Furthermore, a rising temperature *per se* stimulates the release of ATP from red blood cells (Kalsi & González-Alonso 2012), which has been shown to be a potent vasodilator at the active skeletal muscle *in vivo* (González-Alonso *et al.* 2008; Mortensen *et al.* 2009; González-Alonso 2012). It can be speculated that the head cutaneous vasculature is controlled by similar mechanisms. Whether elevations in ATP are important for the increase in extra-cranial flow, or for alterations in CBF remain unknown. Further research to tease out the mechanisms underpinning regional head blood flow redistribution is warranted.

7.6 Hypotheses

Chapter 4 - Dehydration will accelerate the attenuation in cerebral blood flow during graded incremental exercise to exhaustion, but not impair the cerebral metabolic rate for oxygen ***(Accepted)***

The cerebral metabolism would, however, be maintained through compensatory increases in substrate metabolism ***(Accepted)***

Chapter 5 - Dehydration would accentuate the increase in internal temperature and lead to early exhaustion with concomitant reductions in cerebral and extra-cranial blood flow. ***(Accepted)***

Maintaining hydration status would prevent the rise in internal temperature, prolong submaximal exercise capacity and prevent the decline in regional blood flow and the potential for impaired metabolism. ***(Accepted)***

Chapter 6 - Combined elevations in internal and skin temperature would reduce maximal aerobic capacity with an associated attenuation of brain, muscle and systemic blood flow. ***(Accepted)***

Elevations in skin temperature alone would not be sufficient to reduce maximal aerobic capacity and constrain regional haemodynamics. ***(Accepted)***

7.7 Summary

The present thesis provides new information on the impact of dehydration and hyperthermia on cardiovascular function during strenuous exercise in trained humans. In Chapter 4 it is shown that dehydration markedly accelerates the reduction in CBF, above sub-maximal exercise intensities. However, compensatory increases in O_2 extraction preserved the $CMRO_2$. A similar finding was observed in Chapter 5 when dehydration was progressively developed during prolonged sub-maximal exercise in the heat. Interestingly, the distribution of blood flow is different during prolonged exercise and seems to be governed by different regulatory mechanisms. In Chapter 6 it is shown that exogenous heat stress, sufficient to elevate core and skin temperature, reduces maximal aerobic power concomitant to reductions in systemic, brain and limb blood flow, leading to a blunting of exercising limb and systemic oxygen metabolism.

Future studies should continue to explore the mechanisms regulating cerebral and active muscle blood flow to further understand the regulatory limits to human cardiovascular function and capacity. Understanding these mechanisms may help to develop strategies to prevent or delay the circulatory impairment in athletic performance, ageing and disease.

7.8 Conclusion

The findings of the present study show that dehydration and hyperthermia induce a marked cardiovascular strain, characterised by reductions in blood flow to the brain and active skeletal muscle during strenuous exercise. In contrast to the exercising musculature, the brain displays a greater functional oxygen extraction reserve capacity to cope with reduced perfusion and therefore cerebral haemodynamic and metabolic disturbances do not appear to play a role in limiting aerobic exercise performance. However, a reduction in convective O_2 transport to the exercising limb with whole-body hyperthermia is a crucial factor in the cascade of events underpinning reduced aerobic power in the heat. This is because the skeletal muscle oxygen extraction reserve is quickly exhausted in these settings.

REFERENCES

Association for the Advancement of Medical Instrumentation (1993). American national standard. Electronic or automated sphygmomanometers. ANSI/AAMI SP 10-1992. AAMI: VA, USA.

Aaslid R, Lindegaard KF, Sorteberg W & Nornes H (1989). Cerebral autoregulation dynamics in humans. *Stroke* **20**, 45-52.

Aaslid R, Markwalder TM & Nornes H (1982). Noninvasive transcranial Doppler ultrasound recording of flow velocity in basal cerebral arteries. *J Neurosurg* **57**, 769-774.

Adolph EF & Dill DB (1938). Observations on water metabolism in the desert. *American Journal of Physiology* **123**, 369-378.

Ainslie PN,(2009). Have a safe night: intimate protection against cerebral hyperperfusion during REM sleep. *J Appl Physiol* **106**, 1031-1033.

Ainslie PN, Ashmead JC, Ide K, Morgan BJ & Poulin MJ (2005). Differential responses to CO2 and sympathetic stimulation in the cerebral and femoral circulations in humans. *J Physiol* **566**, 613-624.

Ainslie PN & Ogoh S (2010). Regulation of cerebral blood flow in mammals during chronic hypoxia: a matter of balance. *Exp Physiol* **95**, 251-262.

Amann M & Calbet JA (2008). Convective oxygen transport and fatigue. *J Appl Physiol* **104**, 861-870.

American College of Sports Medicine, Sawka MN, Burke LM, Eichner ER, Maughan RJ, Montain SJ & Stachenfeld NS (2007). American College of Sports Medicine position stand. Exercise and fluid replacement. *Med Sci Sports Exerc* **39**, 377-390.

Andersen P & Saltin B (1985). Maximal perfusion of skeletal muscle in man. *J Physiol* **366**, 233-249.

Arngrimsson SA, Petitt DS, Borrani F, Skinner KA & Cureton KJ (2004). Hyperthermia and maximal oxygen uptake in men and women. *Eur J Appl Physiol* **92**, 524-532.

Astrand PO, Cuddy TE, Saltin B & Stenberg J (1964). Cardiac Output during Submaximal and Maximal Work. *J Appl Physiol* **19**, 268-274.

Bada AA, Svendsen JH, Secher NH, Saltin B & Mortensen SP (2012). Peripheral vasodilatation determines cardiac output in exercising humans: insight from atrial pacing. *J Physiol* **590**, 2051-2060.

Bain AR & Ainslie PN (2014). On the limits of cerebral oxygen extraction. *J Physiol* **592**, 2917-2918.

Bain AR, Morrison SA & Ainslie PN (2014). Cerebral oxygenation and hyperthermia. *Front Physiol* **5**, 92.

Bain AR, Smith KJ, Lewis NC, Foster GE, Wildfong KW, Willie CK, Hartley GL, Cheung SS & Ainslie PN (2013). Regional changes in brain blood flow during severe passive hyperthermia: effects of PaCO2 and extracranial blood flow. *J Appl Physiol* **115**, 653-659.

Bassett DR,Jr & Howley ET (2000). Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Med Sci Sports Exerc* **32**, 70-84.

Below PR, Mora-Rodriguez R, González-Alonso J & Coyle EF (1995). Fluid and carbohydrate ingestion independently improve performance during 1 h of intense exercise. *Med Sci Sports Exerc* **27**, 200-210.

Bishop CC, Powell S, Rutt D & Browse NL (1986). Transcranial Doppler measurement of middle cerebral artery blood flow velocity: a validation study. *Stroke* **17**, 913-915.

Bland JM & Altman DG (1995). Calculating correlation coefficients with repeated observations: Part 1--Correlation within subjects. *BMJ* **310**, 446.

Boushel R,(2010). Muscle metaboreflex control of the circulation during exercise. *Acta Physiol* **199**, 367-383.

Boushel R, Gnaiger E, Calbet JA, González-Alonso J, Wright-Paradis C, Sondergaard H, Ara I, Helge JW & Saltin B (2011). Muscle mitochondrial capacity exceeds maximal oxygen delivery in humans. *Mitochondrion* **11**, 303-307.

Bouzier-Sore AK, Voisin P, Canioni P, Magistretti PJ & Pellerin L (2003). Lactate is a preferential oxidative energy substrate over glucose for neurons in culture. *J Cereb Blood Flow Metab* **23**, 1298-1306.

Brengelmann GL, Johnson JM, Hermansen L & Rowell LB (1977). Altered control of skin blood flow during exercise at high internal temperatures. *J Appl Physiol* **43**, 790-794.

Brothers RM, Wingo JE, Hubing KA & Crandall CG (2009a). The effects of reduced end-tidal carbon dioxide tension on cerebral blood flow during heat stress. *J Physiol* **587**, 3921-3927.

Brothers RM, Zhang R, Wingo JE, Hubing KA & Crandall CG (2009b). Effects of heat stress on dynamic cerebral autoregulation during large fluctuations in arterial blood pressure. *J Appl Physiol* **107**, 1722-1729.

Brothers RM, Wingo JE, Hubing KA & Crandall CG (2009c). The effects of reduced end-tidal carbon dioxide tension on cerebral blood flow during heat stress. *J Physiol* **587**, 3921-3927.

Buskirk ER, Iampietro PF & Bass DE (1958). Work performance after dehydration: effects of physical conditioning and heat acclimatization. *Wilderness Environ Med* **12**, 189-194.

Buxton RB & Frank LR (1997). A model for the coupling between cerebral blood flow and oxygen metabolism during neural stimulation. *J Cereb Blood Flow Metab* **17**, 64-72.

Buxton, RB (2009). *An introduction to functional magnetic resonance imaging : principles and techniques*. Cambridge University Press, Cambridge.

Byrne C & Lim CL (2007). The ingestible telemetric body core temperature sensor: a review of validity and exercise applications. *Br J Sports Med* **41**, 126-133.

Calbet JA, González-Alonso J, Helge JW, Sondergaard H, Munch-Andersen T, Boushel R & Saltin B (2007). Cardiac output and leg and arm blood flow during incremental exercise to exhaustion on the cycle ergometer. *J Appl Physiol* **103**, 969-978.

Calbet JA, Jensen-Urstad M, van Hall G, Holmberg HC, Rosdahl H & Saltin B (2004). Maximal muscular vascular conductances during whole body upright exercise in humans. *J Physiol* **558**, 319-331.

Calbet JA, Lundby C, Sander M, Robach P, Saltin B & Boushel R (2006). Effects of ATP-induced leg vasodilation on VO2 peak and leg O2 extraction during maximal exercise in humans. *Am J Physiol Regul Integr Comp Physiol* **291**, R447-53.

Calbet JA, Rådegran G, Boushel R & Saltin B (2009). On the mechanisms that limit oxygen uptake during exercise in acute and chronic hypoxia: role of muscle mass. *J Physiol* **587**, 477-490.

Carter R,3rd, Cheuvront SN, Vernieuw CR & Sawka MN (2006). Hypohydration and prior heat stress exacerbates decreases in cerebral blood flow velocity during standing. *J Appl Physiol* **101**, 1744-1750.

Casaburi R, Barstow TJ, Robinson T & Wasserman K (1989). Influence of work rate on ventilatory and gas exchange kinetics. *J Appl Physiol* **67**, 547-555.

Charkoudian N,(2010). Mechanisms and modifiers of reflex induced cutaneous vasodilation and vasoconstriction in humans. *J Appl Physiol* **109**, 1221-1228.

Charkoudian N,(2003). Skin blood flow in adult human thermoregulation: how it works, when it does not, and why. *Mayo Clin Proc* **78**, 603-612.

Cheung SS & Sleivert GG (2004). Multiple triggers for hyperthermic fatigue and exhaustion. *Exerc Sport Sci Rev* **32**, 100-106.

Cheuvront SN & Kenefick RW (2014). Dehydration: physiology, assessment, and performance effects. *Compr Physiol* **4**, 257-285.

Cheuvront SN, Kenefick RW, Montain SJ & Sawka MN (2010). Mechanisms of aerobic performance impairment with heat stress and dehydration. *J Appl Physiol* **109**, 1989-1995.

Coyle EF, Coggan AR, Hemmert MK & Ivy JL (1986). Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate. *J Appl Physiol* **61**, 165-172.

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

Crandall CG & González-Alonso J (2010). Cardiovascular function in the heat-stressed human. *Acta Physiologica* **199**, 407-423.

Crandall CG, Wilson TE, Marving J, Vogelsang TW, Kjaer A, Hesse B & Secher NH (2008). Effects of passive heating on central blood volume and ventricular dimensions in humans. *J Physiol* **586**, 293-301.

Dalsgaard MK,(2006). Fuelling cerebral activity in exercising man. *J Cereb Blood Flow Metab* **26**, 731-750.

Dalsgaard MK, Ide K, Cai Y, Quistorff B & Secher NH (2002). The intent to exercise influences the cerebral O(2)/carbohydrate uptake ratio in humans. *J Physiol* **540**, 681-689.

Dalsgaard MK, Ogoh S, Dawson EA, Yoshiga CC, Quistorff B & Secher NH (2004a). Cerebral carbohydrate cost of physical exertion in humans. *Am J Physiol Regul Integr Comp Physiol* **287**, R534-40.

Dalsgaard MK, Quistorff B, Danielsen ER, Selmer C, Vogelsang T & Secher NH (2004b). A reduced cerebral metabolic ratio in exercise reflects metabolism and not accumulation of lactate within the human brain. *J Physiol* **554**, 571-578.

Dalsgaard MK & Secher NH (2007). The brain at work: a cerebral metabolic manifestation of central fatigue?. *J Neurosci Res* **85**, 3334-3339.

Dalsgaard MK, Volianitis S, Yoshiga CC, Dawson EA & Secher NH (2004). Cerebral metabolism during upper and lower body exercise. *J Appl Physiol* **97**, 1733-1739.

Delp MD, Armstrong RB, Godfrey DA, Laughlin MH, Ross CD & Wilkerson MK (2001). Exercise increases blood flow to locomotor, vestibular, cardiorespiratory and visual regions of the brain in miniature swine. *J Physiol* **533**, 849-859.

Delp MD & Laughlin MH (1998). Regulation of skeletal muscle perfusion during exercise. *Acta Physiol Scand* **162**, 411-419.

Delp MD & O'Leary DS (2004). Integrative control of the skeletal muscle microcirculation in the maintenance of arterial pressure during exercise. *J Appl Physiol* **97**, 1112-1118.

Dempsey JA,(1986). J.B. Wolffe memorial lecture. Is the lung built for exercise?. *Med Sci Sports Exerc* **18**, 143-155.

Dempsey JA, McKenzie DC, Haverkamp HC & Eldridge MW (2008a). Update in the understanding of respiratory limitations to exercise performance in fit, active adults. *Chest* **134**, 613-622.

Dempsey JA, Miller JD, Romer L, Amann M & Smith CA (2008b). Exercise-induced respiratory muscle work: effects on blood flow, fatigue and performance. *Adv Exp Med Biol* **605**, 209-212.

Dempsey JA & Wagner PD (1999). Exercise-induced arterial hypoxemia. *J Appl Physiol* **87**, 1997-2006.

Detry JM, Brengelmann GL, Rowell LB & Wyss C (1972). Skin and muscle components of forearm blood flow in directly heated resting man. *J Appl Physiol* **32**, 506-511.

Douglas AR, Jones NL & Reed JW (1988). Calculation of whole blood CO2 content. *J Appl Physiol* **65**, 473-477.

Eisenhofer G, Goldstein DS, Ropchak TG, Nguyen HQ, Keiser HR & Kopin IJ (1988). Source and physiological significance of plasma 3,4-dihydroxyphenylglycol and 3-methoxy-4-hydroxyphenylglycol. *J Auton Nerv Syst* **24**, 1-14.

Ekblom B, Goldbarg AN & Gullbring B (1972). Response to exercise after blood loss and reinfusion. *J Appl Physiol* **33**, 175-180.

Ekblom B, Huot R, Stein EM & Thorstensson AT (1975). Effect of changes in arterial oxygen content on circulation and physical performance. *J Appl Physiol* **39**, 71-75.

Ekblom B, Wilson G & Astrand PO (1976). Central circulation during exercise after venesection and reinfusion of red blood cells. *J Appl Physiol* **40**, 379-383.

Ely BR, Cheuvront SN, Kenefick RW & Sawka MN (2010). Aerobic performance is degraded, despite modest hyperthermia, in hot environments. *Med Sci Sports Exerc* **42**, 135-141.

Ely BR, Ely MR, Cheuvront SN, Kenefick RW, Degroot DW & Montain SJ (2009). Evidence against a 40 degrees C core temperature threshold for fatigue in humans. *J Appl Physiol* **107**, 1519-1525.

Esler M, Jennings G, Korner P, Blombery P, Sacharias N & Leonard P (1984). Measurement of total and organ-specific norepinephrine kinetics in humans. *Am J Physiol* **247**, E21-8.

Esler M, Jennings G, Korner P, Willett I, Dudley F, Hasking G, Anderson W & Lambert G (1988). Assessment of human sympathetic nervous system activity from measurements of norepinephrine turnover. *Hypertension* **11**, 3-20.

Esler M, Jennings G, Lambert G, Meredith I, Horne M & Eisenhofer G (1990). Overflow of catecholamine neurotransmitters to the circulation: source, fate, and functions. *Physiol Rev* **70**, 963-985.

Esler MD, Jennings GL & Lambert GW (1988). Release of noradrenaline into the cerebrovascular circulation in patients with primary hypertension. *J Hypertens Suppl* **6**, S494-6.

Fadel PJ,(2013). Neural control of the circulation during exercise in health and disease. *Front Physiol* **4**, 224.

Fan JL, Cotter JD, Lucas RA, Thomas K, Wilson L & Ainslie PN (2008). Human cardiorespiratory and cerebrovascular function during severe passive hyperthermia: effects of mild hypohydration. *J Appl Physiol* **105**, 433-445.

Faraci FM & Heistad DD (1998). Regulation of the cerebral circulation: role of endothelium and potassium channels. *Physiol Rev* **78**, 53-97.

Faraci FM, Heistad DD & Mayhan WG (1987). Role of large arteries in regulation of blood flow to brain stem in cats. *J Physiol* **387**, 115-123.

Febbraio MA, Murton P, Selig SE, Clark SA, Lambert DL, Angus DJ & Carey MF (1996). Effect of CHO ingestion on exercise metabolism and performance in different ambient temperatures. *Med Sci Sports Exerc* **28**, 1380-1387.

Ferrier C, Jennings GL, Eisenhofer G, Lambert G, Cox HS, Kalff V, Kelly M & Esler MD (1993). Evidence for increased noradrenaline release from subcortical brain regions in essential hypertension. *J Hypertens* **11**, 1217-1227.

Fisher JP, Hartwich D, Seifert T, Olesen ND, McNulty CL, Nielsen HB, van Lieshout JJ & Secher NH (2013). Cerebral perfusion, oxygenation and metabolism during exercise in young and elderly individuals. *J Physiol* **591**, 1859-1870.

Fog M,(1938). The Relationship between the Blood Pressure and the Tonic Regulation of the Pial Arteries. *J Neurol Psychiatry* **1**, 187-197.

Forster HV, Haouzi P & Dempsey JA (2012). Control of breathing during exercise. *Compr Physiol* **2**, 743-777.

Fortney SM, Nadel ER, Wenger CB & Bove JR (1981). Effect of acute alterations of blood volume on circulatory performance in humans. *J Appl Physiol Respir Environ Exerc Physiol* **50**, 292-298.

Fortney SM, Vroman NB, Beckett WS, Permutt S & LaFrance ND (1988). Effect of exercise hemoconcentration and hyperosmolality on exercise responses. *J Appl Physiol* **65**, 519-524.

Fortney SM, Wenger CB, Bove JR & Nadel ER (1984). Effect of hyperosmolality on control of blood flow and sweating. *J Appl Physiol Respir Environ Exerc Physiol* **57**, 1688-1695.

Fox PT & Raichle ME (1986). Focal physiological uncoupling of cerebral blood flow and oxidative metabolism during somatosensory stimulation in human subjects. *Proc Natl Acad Sci U S A* **83**, 1140-1144.

Fritzsche RG, Switzer TW, Hodgkinson BJ & Coyle EF (1999). Stroke volume decline during prolonged exercise is influenced by the increase in heart rate. *J Appl Physiol* **86**, 799-805.

Gagge AP, Stolwijk JA & Saltin B (1969). Comfort and thermal sensations and associated physiological responses during exercise at various ambient temperatures. *Environ Res* **2**, 209-229.

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

Ganio MS, Wingo JE, Carrolll CE, Thomas MK & Cureton KJ (2006). Fluid ingestion attenuates the decline in VO2peak associated with cardiovascular drift. *Med Sci Sports Exerc* **38**, 901-909.

Ganz V, Hlavova A, Fronek A, Linhart J & Prerovsky I (1964). Measurement of Blood Flow in the Femoral Artery in Man at Rest and during Exercise by Local Thermodilution. *Circulation* **30**, 86-89.

Gibbs EL, Lennox WG, Nims LF & Gibbs FA (1942). Arterial and cerebral venous blood: arterial-venous difference in man. *The Journal of biological chemistry* **144**, 325-332.

Gisolf J, van Lieshout JJ, van Heusden K, Pott F, Stok WJ & Karemaker JM (2004). Human cerebral venous outflow pathway depends on posture and central venous pressure. *J Physiol* **560**, 317-327.

Gjedde A, Johannsen P, Cold GE & Ostergaard L (2005). Cerebral metabolic response to low blood flow: possible role of cytochrome oxidase inhibition. *J Cereb Blood Flow Metab* **25**, 1183-1196.

Gjedde A, Poulsen PH & Ostergaard L (1999). On the oxygenation of hemoglobin in the human brain. *Adv Exp Med Biol* **471**, 67-81.

Gledhill N, Warburton D & Jamnik V (1999). Haemoglobin, blood volume, cardiac function, and aerobic power. *Can J Appl Physiol* **24**, 54-65.

González-Alonso J,(2006). Comment on point: counterpoint "in health and in a normoxic environment, VO2 max is/is not limited primarily by cardiac output and locomotor muscle blood flow". Vol 100: 744-8, 2006. Debate does not answer the key question of whether the circulatory or the muscle diffusive O2 transport and oxidative system reaches its capacity in any human being performing maximal exercise. *J Appl Physiol* **100**, 1088-1089.

González-Alonso J,(1998). Separate and combined influences of dehydration and hyperthermia on cardiovascular responses to exercise. *Int J Sports Med* **19 Suppl 2**, S111-4.

González-Alonso J & Calbet JA (2003). Reductions in systemic and skeletal muscle blood flow and oxygen delivery limit maximal aerobic capacity in humans. *Circulation* **107**, 824-830.

González-Alonso J, Calbet JA & Nielsen B (1999). Metabolic and thermodynamic responses to dehydration-induced reductions in muscle blood flow in exercising humans. *J Physiol* **520 Pt 2**, 577-589.

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

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

González-Alonso J, Dalsgaard MK, Osada T, Volianitis S, Dawson EA, Yoshiga CC & Secher NH (2004). Brain and central haemodynamics and oxygenation during maximal exercise in humans. *J Physiol* **557**, 331-342.

González-Alonso J, Mora-Rodriguez R, Below PR & Coyle EF (1997). Dehydration markedly impairs cardiovascular function in hyperthermic endurance athletes during exercise. *J Appl Physiol* **82**, 1229-1236.

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

González-Alonso J, Mora-Rodriguez R & Coyle EF (2000). Stroke volume during exercise: interaction of environment and hydration. *Am J Physiol Heart Circ Physiol* **278**, H321-30.

González-Alonso J, Mortensen SP, Jeppesen TD, Ali L, Barker H, Damsgaard R, Secher NH, Dawson EA & Dufour SP (2008). Haemodynamic responses to exercise, ATP infusion and thigh compression in humans: insight into the role of muscle mechanisms on cardiovascular function. *J Physiol* **586**, 2405-2417.

González-Alonso J, Olsen DB & Saltin B (2002). Erythrocyte and the regulation of human skeletal muscle blood flow and oxygen delivery: role of circulating ATP. *Circ Res* **91**, 1046-1055.

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

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

González-Alonso J,(2012). ATP as a mediator of erythrocyte-dependent regulation of skeletal muscle blood flow and oxygen delivery in humans. *J Physiol* **590**, 5001-5013.

Goodall S, Gonzalez-Alonso J, Ali L, Ross EZ & Romer LM (2012). Supraspinal fatigue after normoxic and hypoxic exercise in humans. *J Physiol* **590**, 2767-2782.

Goodman DA, Kenefick RW, Cadarette BS & Cheuvront SN (2009). Influence of sensor ingestion timing on consistency of temperature measures. *Med Sci Sports Exerc* **41**, 597-602.

Gorman MW, Marble DR, Ogimoto K & Feigl EO (2003). Measurement of adenine nucleotides in plasma. *Luminescence* **18**, 173-181.

Grant EG, Benson CB, Moneta GL, Alexandrov AV, Baker JD, Bluth EI, Carroll BA, Eliasziw M, Gocke J, Hertzberg BS, Katanick S, Needleman L, Pellerito J, Polak JF, Rholl KS, Wooster DL & Zierler RE (2003). Carotid artery stenosis: gray-scale and Doppler US diagnosis--Society of Radiologists in Ultrasound Consensus Conference. *Radiology* **229**, 340-346.

Haldane JS,(1905). The Influence of High Air Temperatures: No. 1. *J Hyg* **5**, 494-513.

Hardebo JE & Owman C (1980). Characterization of the in vitro uptake of monoamines into brain microvessels. *Acta Physiol Scand* **108**, 223-229.

Harms CA, Babcock MA, McClaran SR, Pegelow DF, Nickele GA, Nelson WB & Dempsey JA (1997). Respiratory muscle work compromises leg blood flow during maximal exercise. *J Appl Physiol* **82**, 1573-1583.

Harms CA, Wetter TJ, McClaran SR, Pegelow DF, Nickele GA, Nelson WB, Hanson P & Dempsey JA (1998). Effects of respiratory muscle work on cardiac output and its distribution during maximal exercise. *J Appl Physiol* **85**, 609-618.

Hedlund S, Nylin G & Regnstrom O (1962). The behaviour of the cerebral circulation during muscular exercise. *Acta Physiol Scand* **54**, 316-324.

Heinonen I, Brothers RM, Kemppainen J, Knuuti J, Kalliokoski KK & Crandall CG (2011). Local heating, but not indirect whole body heating, increases human skeletal muscle blood flow. *J Appl Physiol* **111**, 818-824.

Hellsten Y, Nyberg M, Jensen LG & Mortensen SP (2012). Vasodilator interactions in skeletal muscle blood flow regulation. *J Physiol* **590**, 6297-6305.

Hellstrom G, Fischer-Colbrie W, Wahlgren NG & Jogestrand T (1996). Carotid artery blood flow and middle cerebral artery blood flow velocity during physical exercise. *J Appl Physiol* **81**, 413-418.

Herholz K, Buskies W, Rist M, Pawlik G, Hollmann W & Heiss WD (1987). Regional cerebral blood flow in man at rest and during exercise. *J Neurol* **234**, 9-13.

Hermansen L, Hultman E & Saltin B (1967). Muscle glycogen during prolonged severe exercise. *Acta Physiol Scand* **71**, 129-139.

Higginbotham MB, Morris KG, Williams RS, McHale PA, Coleman RE & Cobb FR (1986). Regulation of stroke volume during submaximal and maximal upright exercise in normal man. *Circ Res* **58**, 281-291.

Hill A & Lupton H (1923). Muscular exercise, lactic acid, and the supply and utilization of oxygen. *Quarterly Journal of Sports Medicine* **16**, 135-171.

Houghton BL, Meendering JR, Wong BJ & Minson CT (2006). Nitric oxide and noradrenaline contribute to the temperature threshold of the axon reflex response to gradual local heating in human skin. *J Physiol* **572**, 811-820.

Hughson RL & Tschakovsky ME (1999). Cardiovascular dynamics at the onset of exercise. *Med Sci Sports Exerc* **31**, 1005-1010.

Hunt AP & Stewart IB (2008). Calibration of an ingestible temperature sensor. *Physiol Meas* **29**, N71-8.

Ide K, Boushel R, Sorensen HM, Fernandes A, Cai Y, Pott F & Secher NH (2000). Middle cerebral artery blood velocity during exercise with beta-1 adrenergic and unilateral stellate ganglion blockade in humans. *Acta Physiol Scand* **170**, 33-38.

Ide K, Gullov AL, Pott F, Van Lieshout JJ, Koefoed BG, Petersen P & Secher NH (1999a). Middle cerebral artery blood velocity during exercise in patients with atrial fibrillation. *Clin Physiol* **19**, 284-289.

Ide K, Horn A & Secher NH (1999b). Cerebral metabolic response to submaximal exercise. *J Appl Physiol* **87**, 1604-1608.

Ide K, Pott F, Van Lieshout JJ & Secher NH (1998). Middle cerebral artery blood velocity depends on cardiac output during exercise with a large muscle mass. *Acta Physiol Scand* **162**, 13-20.

Ide K & Secher NH (2000). Cerebral blood flow and metabolism during exercise. *Prog Neurobiol* **61**, 397-414.

Jakobsen M & Enevoldsen E (1989). Retrograde catheterization of the right internal jugular vein for serial measurements of cerebral venous oxygen content. *J Cereb Blood Flow Metab* **9**, 717-720.

Johnson JM & Kellogg DL,Jr (2010). Local thermal control of the human cutaneous circulation. *J Appl Physiol* **109**, 1229-1238.

Johnson JM, Minson CT & Kellogg DL,Jr (2014). Cutaneous vasodilator and vasoconstrictor mechanisms in temperature regulation. *Compr Physiol* **4**, 33-89.

Jorgensen LG,(1995). Transcranial Doppler ultrasound for cerebral perfusion. *Acta Physiol Scand Suppl* **625**, 1-44.

Jorgensen LG, Perko G & Secher NH (1992a). Regional cerebral artery mean flow velocity and blood flow during dynamic exercise in humans. *J Appl Physiol* **73**, 1825-1830.

Jorgensen LG, Perko M, Hanel B, Schroeder TV & Secher NH (1992b). Middle cerebral artery flow velocity and blood flow during exercise and muscle ischemia in humans. *J Appl Physiol* **72**, 1123-1132.

Jorgensen LG, Perko M, Hanel B, Schroeder TV & Secher NH (1992c). Middle cerebral artery flow velocity and blood flow during exercise and muscle ischemia in humans. *J Appl Physiol* **72**, 1123-1132.

Kalsi KK & González-Alonso J (2012). Temperature-dependent release of ATP from human erythrocytes: mechanism for the control of local tissue perfusion. *Exp Physiol* **97**, 419-432.

Kellogg DL,Jr., Crandall CG, Liu Y, Charkoudian N & Johnson JM (1998). Nitric oxide and cutaneous active vasodilation during heat stress in humans. *J Appl Physiol* **85**, 824-829.

Kellogg DL,Jr., Pergola PE, Piest KL, Kosiba WA, Crandall CG, Grossmann M & Johnson JM (1995). Cutaneous active vasodilation in humans is mediated by cholinergic nerve cotransmission. *Circ Res* **77**, 1222-1228.

Kellogg DL,Jr, Johnson JM & Kosiba WA (1990). Baroreflex control of the cutaneous active vasodilator system in humans. *Circ Res* **66**, 1420-1426.

Kempton MJ, Ettinger U, Foster R, Williams SC, Calvert GA, Hampshire A, Zelaya FO, O'Gorman RL, McMorris T, Owen AM & Smith MS (2011). Dehydration affects brain structure and function in healthy adolescents. *Hum Brain Mapp* **32**, 71-79.

Kempton MJ, Ettinger U, Schmechtig A, Winter EM, Smith L, McMorris T, Wilkinson ID, Williams SC & Smith MS (2009). Effects of acute dehydration on brain morphology in healthy humans. *Hum Brain Mapp* **30**, 291-298.

Kenefick RW, Cheuvront SN, Palombo LJ, Ely BR & Sawka MN (2010a). Skin temperature modifies the impact of hypohydration on aerobic performance. *J Appl Physiol* **109**, 79-86.

Kenefick RW, Cheuvront SN, Palombo LJ, Ely BR & Sawka MN (2010b). Skin temperature modifies the impact of hypohydration on aerobic performance. *J Appl Physiol* **109**, 79-86.

Kenefick RW, Mahood NV, Hazzard MP, Quinn TJ & Castellani JW (2004). Hypohydration effects on thermoregulation during moderate exercise in the cold. *Eur J Appl Physiol* **92**, 565-570.

Kenney WL, Stanhewicz AE, Bruning RS & Alexander LM (2014). Blood pressure regulation III: what happens when one system must serve two masters: temperature and pressure regulation?. *Eur J Appl Physiol* **114**, 467-479.

Kety SS & Schmidt CF (1948a). The Effects of Altered Arterial Tensions of Carbon Dioxide and Oxygen on Cerebral Blood Flow and Cerebral Oxygen Consumption of Normal Young Men. *J Clin Invest* **27**, 484-492.

Kety SS & Schmidt CF (1948b). The Nitrous Oxide Method for the Quantitative Determination of Cerebral Blood Flow in Man: Theory, Procedure and Normal Values. *J Clin Invest* **27**, 476-483.

Kety SS & Schmidt CF (1946). Measurement of cerebral blood flow and cerebral oxygen consumption in man. *Fed Proc* **5**, 264.

King BD, Sokoloff L & Wechsler RL (1952). The effects of l-epinephrine and l-norepinephrine upon cerebral circulation and metabolism in man. *J Clin Invest* **31**, 273-279.

Klausen K, Dill DB, Phillips EE,Jr & McGregor D (1967). Metabolic reactions to work in the desert. *J Appl Physiol* **22**, 292-296.

Kontos HA, Wei EP, Navari RM, Levasseur JE, Rosenblum WI & Patterson JL,Jr (1978). Responses of cerebral arteries and arterioles to acute hypotension and hypertension. *Am J Physiol* **234**, H371-83.

Krogh A & Lindhard J (1913). The regulation of respiration and circulation during the initial stages of muscular work. *J Physiol* **47**, 112-136.

Lafrenz AJ, Wingo JE, Ganio MS & Cureton KJ (2008). Effect of ambient temperature on cardiovascular drift and maximal oxygen uptake. *Med Sci Sports Exerc* **40**, 1065-1071.

Lassen NA,(1974). Control of cerebral circulation in health and disease. *Circ Res* **34**, 749-760.

Lassen NA,(1959). Cerebral blood flow and oxygen consumption in man. *Physiol Rev* **39**, 183-238.

Lee TJ, Su C & Bevan JA (1976). Neurogenic sympathetic vasoconstriction of the rabbit basilar artery. *Circ Res* **39**, 120-126.

Lewis NC, Bain AR, MacLeod DB, Wildfong KW, Smith KJ, Willie CK, Sanders ML, Numan T, Morrison SA, Foster GE, Stewart JM & Ainslie PN (2014a). Impact of hypocapnia and cerebral perfusion on orthostatic tolerance. *J Physiol*.

Lewis NC, Messinger L, Monteleone B & Ainslie PN (2014b). Effect of acute hypoxia on regional cerebral blood flow: effect of sympathetic nerve activity. *J Appl Physiol* **116**, 1189-1196.

Linkis P, Jorgensen LG, Olesen HL, Madsen PL, Lassen NA & Secher NH (1995). Dynamic exercise enhances regional cerebral artery mean flow velocity. *J Appl Physiol* **78**, 12-16.

Lorenzo S, Halliwill JR, Sawka MN & Minson CT (2010). Heat acclimation improves exercise performance. *J Appl Physiol* **109**, 1140-1147.

Lucas RA, Pearson J, Schlader ZJ & Crandall CG (2013). Hypercapnia-induced increases in cerebral blood flow do not improve lower body negative pressure tolerance during hyperthermia. *Am J Physiol Regul Integr Comp Physiol* **305**, R604-9.

Lucas SJ, Tzeng YC, Galvin SD, Thomas KN, Ogoh S & Ainslie PN (2010). Influence of changes in blood pressure on cerebral perfusion and oxygenation. *Hypertension* **55**, 698-705.

Lundby C, Boushel R, Robach P, Moller K, Saltin B & Calbet JA (2008). During hypoxic exercise some vasoconstriction is needed to match O2 delivery with O2 demand at the microcirculatory level. *J Physiol* **586**, 123-130.

Madsen PL & Secher NH (1999). Near-infrared oximetry of the brain. *Prog Neurobiol* **58**, 541-560.

Madsen PL, Sperling BK, Warming T, Schmidt JF, Secher NH, Wildschiodtz G, Holm S & Lassen NA (1993). Middle cerebral artery blood velocity and cerebral blood flow and O2 uptake during dynamic exercise. *J Appl Physiol* **74**, 245-250.

Matsui T, Ishikawa T, Ito H, Okamoto M, Inoue K, Lee MC, Fujikawa T, Ichitani Y, Kawanaka K & Soya H (2012). Brain glycogen supercompensation following exhaustive exercise. *J Physiol* **590**, 607-616.

Maughan R & Shirreffs S (2004). Exercise in the heat: challenges and opportunities. *J Sports Sci* **22**, 917-927.

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

McHenry LC,Jr, Fazekas JF & Sullivan JF (1961). Cerebral hemodynamics of syncope. *Am J Med Sci* **241**, 173-178.

Meeusen R, Watson P, Hasegawa H, Roelands B & Piacentini MF (2006). Central fatigue: the serotonin hypothesis and beyond. *Sports Med* **36**, 881-909.

Minson CT, Wladkowski SL, Cardell AF, Pawelczyk JA & Kenney WL (1998). Age alters the cardiovascular response to direct passive heating. *J Appl Physiol* **84**, 1323-1332.

Mitchell D & Wyndham CH (1969). Comparison of weighting formulas for calculating mean skin temperature. *J Appl Physiol* **26**, 616-622.

Mitchell DA, Lambert G, Secher NH, Raven PB, van Lieshout J & Esler MD (2009). Jugular venous overflow of noradrenaline from the brain: a neurochemical indicator of cerebrovascular sympathetic nerve activity in humans. *J Physiol* **587**, 2589-2597.

Miyazawa T, Horiuchi M, Ichikawa D, Subudhi AW, Sugawara J & Ogoh S (2012). Face cooling with mist water increases cerebral blood flow during exercise: effect of changes in facial skin blood flow. *Front Physiol* **3**, 308.

Miyazawa T, Horiuchi M, Komine H, Sugawara J, Fadel PJ & Ogoh S (2013). Skin blood flow influences cerebral oxygenation measured by near-infrared spectroscopy during dynamic exercise. *Eur J Appl Physiol* **113**, 2841-2848.

Montain SJ & Coyle EF (1992a). Fluid ingestion during exercise increases skin blood flow independent of increases in blood volume. *J Appl Physiol* **73**, 903-910.

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

Montain SJ, Sawka MN, Latzka WA & Valeri CR (1998a). Thermal and cardiovascular strain from hypohydration: influence of exercise intensity. *Int J Sports Med* **19**, 87-91.

Montain SJ, Smith SA, Mattot RP, Zientara GP, Jolesz FA & Sawka MN (1998b). Hypohydration effects on skeletal muscle performance and metabolism: a 31P-MRS study. *J Appl Physiol* **84**, 1889-1894.

Moraine JJ, Lamotte M, Berre J, Niset G, Leduc A & Naeije R (1993). Relationship of middle cerebral artery blood flow velocity to intensity during dynamic exercise in normal subjects. *Eur J Appl Physiol Occup Physiol* **67**, 35-38.

Moralez G, Romero SA, Rickards CA, Ryan KL, Convertino VA & Cooke WH (2012). Effects of dehydration on cerebrovascular control during standing after heavy resistance exercise. *J Appl Physiol* **112**, 1875-1883.

Mortensen SP, Damsgaard R, Dawson EA, Secher NH & 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**, 2621-2635.

Mortensen SP, Dawson EA, Yoshiga CC, Dalsgaard MK, Damsgaard R, Secher NH & González-Alonso J (2005). Limitations to systemic and locomotor limb muscle oxygen delivery and uptake during maximal exercise in humans. *J Physiol* **566**, 273-285.

Mortensen SP, González-Alonso J, Bune LT, Saltin B, Pilegaard H & Hellsten Y (2009). ATP-induced vasodilation and purinergic receptors in the human leg: roles of nitric oxide, prostaglandins, and adenosine. *Am J Physiol Regul Integr Comp Physiol* **296**, R1140-8.

Mortensen SP, González-Alonso J, Damsgaard R, Saltin B & Hellsten Y (2007). Inhibition of nitric oxide and prostaglandins, but not endothelial-derived hyperpolarizing factors, reduces blood flow and aerobic energy turnover in the exercising human leg. *J Physiol* **581**, 853-861.

Mortensen SP, Nyberg M, Thaning P, Saltin B & Hellsten Y (2009). Adenosine contributes to blood flow regulation in the exercising human leg by increasing prostaglandin and nitric oxide formation. *Hypertension* **53**, 993-999.

Munch GD, Svendsen JH, Damsgaard R, Secher NH, González-Alonso J & Mortensen SP (2014). Maximal heart rate does not limit cardiovascular capacity in healthy humans: insight from right atrial pacing during maximal exercise. *J Physiol* **592**, 377-390.

Munck O & Lassen NA (1957). Bilaterial cerebral blood flow and oxygen comsumption in man by use of krypton 85. *Circ Res* **5**, 163-168.

Nadel ER,(1980). Circulatory and thermal regulations during exercise. *Fed Proc* **39**, 1491-1497.

Nadel ER, Bullard RW & Stolwijk JA (1971). Importance of skin temperature in the regulation of sweating. *J Appl Physiol* **31**, 80-87.

Nadel ER, Fortney SM & Wenger CB (1980). Effect of hydration state of circulatory and thermal regulations. *J Appl Physiol Respir Environ Exerc Physiol* **49**, 715-721.

National Institute for Clinical Excellence (2002). Guidance on the Use of Ultrasound Locating Devices for Placing Central Venous Catheters **49**.

Nelson MD, Haykowsky MJ, Stickland MK, Altamirano-Diaz LA, Willie CK, Smith KJ, Petersen SR & Ainslie PN (2011). Reductions in cerebral blood flow during passive heat stress in humans: partitioning the mechanisms. *J Physiol* **589**, 4053-4064.

Nemoto EM, Klementavicius R, Melick JA & Yonas H (1996). Norepinephrine activation of basal cerebral metabolic rate for oxygen (CMRO2) during hypothermia in rats. *Anesth Analg* **83**, 1262-1267.

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

Nielsen B, Hyldig T, Bidstrup F, González-Alonso J & Christoffersen GR (2001). Brain activity and fatigue during prolonged exercise in the heat. *Pflugers Arch* **442**, 41-48.

Nielsen B & Nybo L (2003). Cerebral changes during exercise in the heat. *Sports Med* **33**, 1-11.

Nielsen B, Rowell LB & Bonde-Petersen F (1984). Cardiovascular responses to heat stress and blood volume displacements during exercise in man. *Eur J Appl Physiol Occup Physiol* **52**, 370-374.

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

Nielsen HB, Bredmose PP, Stromstad M, Volianitis S, Quistorff B & Secher NH (2002). Bicarbonate attenuates arterial desaturation during maximal exercise in humans. *J Appl Physiol* **93**, 724-731.

Niimi Y, Matsukawa T, Sugiyama Y, Shamsuzzaman AS, Ito H, Sobue G & Mano T (1997). Effect of heat stress on muscle sympathetic nerve activity in humans. *J Auton Nerv Syst* **63**, 61-67.

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

Nybo L,(2003). CNS fatigue and prolonged exercise: effect of glucose supplementation. *Med Sci Sports Exerc* **35**, 589-594.

Nybo L, Jensen T, Nielsen B & González-Alonso J (2001). Effects of marked hyperthermia with and without dehydration on VO(2) kinetics during intense exercise. *J Appl Physiol* **90**, 1057-1064.

Nybo L, Moller K, Volianitis S, Nielsen B & Secher NH (2002). Effects of hyperthermia on cerebral blood flow and metabolism during prolonged exercise in humans. *J Appl Physiol* **93**, 58-64.

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

Nybo L & Nielsen B (2001b). Middle cerebral artery blood velocity is reduced with hyperthermia during prolonged exercise in humans. *J Physiol (Lond)* **534**, 279-286.

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

Nybo L & Rasmussen P (2007). Inadequate cerebral oxygen delivery and central fatigue during strenuous exercise. *Exerc Sport Sci Rev* **35**, 110-118.

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

Nybo L & Secher NH (2004). Cerebral perturbations provoked by prolonged exercise. *Prog Neurobiol* **72**, 223-261.

Nybo L, Secher NH & Nielsen B (2002). Inadequate heat release from the human brain during prolonged exercise with hyperthermia. *J Physiol (Lond)* **545**, 697-704.

Nybo L, Wanscher M & Secher NH (2014). Influence of intranasal and carotid cooling on cerebral temperature balance and oxygenation. *Front Physiol* **5**, 79.

Ogoh S, Brothers RM, Barnes Q, Eubank WL, Hawkins MN, Purkayastha S, O-Yurvati A & Raven PB (2005a). The effect of changes in cardiac output on middle cerebral artery mean blood velocity at rest and during exercise. *J Physiol* **569**, 697-704.

Ogoh S, Brothers RM, Barnes Q, Eubank WL, Hawkins MN, Purkayastha S, O-Yurvati A & Raven PB (2005b). The effect of changes in cardiac output on middle cerebral artery mean blood velocity at rest and during exercise. *J Physiol* **569**, 697-704.

Ogoh S, Brothers RM, Eubank WL & Raven PB (2008). Autonomic neural control of the cerebral vasculature: acute hypotension. *Stroke* **39**, 1979-1987.

Ogoh S, Sato K, Nakahara H, Okazaki K, Subudhi AW & Miyamoto T (2013a). Effect of acute hypoxia on blood flow in vertebral and internal carotid arteries. *Exp Physiol* **98**, 692-698.

Ogoh S, Sato K, Okazaki K, Miyamoto T, Hirasawa A, Morimoto K & Shibasaki M (2013b). Blood flow distribution during heat stress: cerebral and systemic blood flow. *J Cereb Blood Flow Metab* **33**, 1915-1920.

Ogoh S & Ainslie PN (2009a). Cerebral blood flow during exercise: mechanisms of regulation. *J Appl Physiol* **107**, 1370-1380.

Ogoh S & Ainslie PN (2009b). Regulatory Mechanisms of Cerebral Blood Flow During Exercise: New Concepts. *Exerc Sport Sci Rev* **37**, 123-129.

Olesen J,(1971). Contralateral focal increase of cerebral blood flow in man during arm work. *Brain* **94**, 635-646.

Olesen J, Paulson OB & Lassen NA (1971). Regional cerebral blood flow in man determined by the initial slope of the clearance of intra-arterially injected 133Xe. *Stroke* **2**, 519-540.

Olesen ND, van Lieshout JJ, Fisher JP, Seifert T, Nielsen HB & Secher NH (2014). Case report: (Pre)syncopal symptoms associated with a negative internal jugular venous pressure. *Front Physiol* **5**, 317.

Olin JT, Dimmen AC, Subudhi AW & Roach RC (2011). Cerebral blood flow and oxygenation at maximal exercise: The effect of clamping carbon dioxide. *Respir Physiol Neuro* **175**, 176-180.

Olin JT, Dimmen AC, Subudhi AW & Roach RC (2010). Cerebral Oxygenation and Blood Flow Velocity at Maximal Exercise: The Effect of Clamping Carbon Dioxide. *Med Sci Sports Exerc* **42**, 508-508.

Paulson OB, Strandgaard S & Edvinsson L (1990). Cerebral autoregulation. *Cerebrovasc Brain Metab Rev* **2**, 161-192.

Pearson J, Kalsi KK, Stohr EJ, Low DA, Barker H, Ali L & Gonzalez-Alonso J (2013). Haemodynamic responses to dehydration in the resting and exercising human leg. *Eur J Appl Physiol* **113**, 1499-1509.

Pearson J, Low DA, Stöhr E, Kalsi 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**, R663-73.

Peebles K, Celi L, McGrattan K, Murrell C, Thomas K & Ainslie PN (2007). Human cerebrovascular and ventilatory CO2 reactivity to end-tidal, arterial and internal jugular vein PCO2. *J Physiol* **584**, 347-357.

Peebles KC, Richards AM, Celi L, McGrattan K, Murrell CJ & Ainslie PN (2008). Human cerebral arteriovenous vasoactive exchange during alterations in arterial blood gases. *J Appl Physiol* **105**, 1060-1068.

Pirnay F, Deroanne R & Petit JM (1970). Maximal oxygen consumption in a hot environment. *J Appl Physiol* **28**, 642-645.

Pott F, Knudsen L, Nowak M, Nielsen HB, Hanel B & Secher NH (1997). Middle cerebral artery blood velocity during rowing. *Acta Physiol Scand* **160**, 251-255.

Querido JS & Sheel AW (2007). Regulation of cerebral blood flow during exercise. *Sports Med* **37**, 765-782.

Quistorff B, Secher NH & Van Lieshout JJ (2008). Lactate fuels the human brain during exercise. *FASEB J* **22**, 3443-3449.

Rådegran G,(1997). Ultrasound Doppler estimates of femoral artery blood flow during dynamic knee extensor exercise in humans. *J Appl Physiol* **83**, 1383-1388.

Raichle ME, Grubb RL,Jr, Gado MH, Eichling JO & Ter-Pogossian MM (1976). Correlation between regional cerebral blood flow and oxidative metabolism. In vivo studies in man. *Arch Neurol* **33**, 523-526.

Ramanathan NL,(1964). A New Weighting System for Mean Surface Temperature of the Human Body. *J Appl Physiol* **19**, 531-533.

Rasmussen P, Dawson EA, Nybo L, van Lieshout JJ, Secher NH & Gjedde A (2007). Capillary-oxygenation-level-dependent near-infrared spectrometry in frontal lobe of humans. *J Cereb Blood Flow Metab* **27**, 1082-1093.

Rasmussen P, Nybo L, Volianitis S, Moller K, Secher NH & Gjedde A (2010). Cerebral oxygenation is reduced during hyperthermic exercise in humans. *Acta Physiologica* **199**, 63-70.

Rasmussen P, Secher NH & Petersen NT (2007*b*). Understanding central fatigue: where to go? *Exp Physiol* **92,** 369–370.

Rasmussen P, Stie H, Nielsen B & Nybo L (2006). Enhanced cerebral CO2 reactivity during strenuous exercise in man. *Eur J Appl Physiol* **96**, 299-304.

Raven PB,(2012). Neural control of the circulation: exercise. *Exp Physiol* **97**, 10-13.

Raven PB, Fadel PJ & Ogoh S (2006). Arterial baroreflex resetting during exercise: a current perspective. *Exp Physiol* **91**, 37-49.

Reese TS & Karnovsky MJ (1967). Fine structural localization of a blood-brain barrier to exogenous peroxidase. *J Cell Biol* **34**, 207-217.

Richardson RS, Harms CA, Grassi B & Hepple RT (2000). Skeletal muscle: master or slave of the cardiovascular system?. *Med Sci Sports Exerc* **32**, 89-93.

Richardson RS, Poole DC, Knight DR, Kurdak SS, Hogan MC, Grassi B, Johnson EC, Kendrick KF, Erickson BK & Wagner PD (1993). High muscle blood flow in man: is maximal O2 extraction compromised?. *J Appl Physiol* **75**, 1911-1916.

Roddie IC, Shepherd JT & Whelan RF (1956). Evidence from venous oxygen saturation measurements that the increase in forearm blood flow during body heating is confined to the skin. *J Physiol* **134**, 444-450.

Roelands B & Meeusen R (2010) Alterations in central fatigue by pharmacological manipulations of neurotransmitters in normal and high ambient temperature. *Sports Med* **40**, 229-246.

Roland PE, Eriksson L, Stone-Elander S & Widen L (1987). Does mental activity change the oxidative metabolism of the brain?. *J Neurosci* **7**, 2373-2389.

Romer LM & Polkey MI (2008). Exercise-induced respiratory muscle fatigue: implications for performance. *J Appl Physiol* **104**, 879-888.

Romero SA, Moralez G, Rickards CA, Ryan KL, Convertino VA, Fogt DL & Cooke WH (2011). Control of cerebral blood velocity with furosemide-induced hypovolemia and upright tilt. *J Appl Physiol* **110**, 492-498.

Rosenmeier JB, Hansen J & González-Alonso J (2004). Circulating ATP-induced vasodilatation overrides sympathetic vasoconstrictor activity in human skeletal muscle. *J Physiol* **558**, 351-365.

Ross EZ, Cotter JD, Wilson L, Fan JL, Lucas SJ & Ainslie PN (2012). Cerebrovascular and corticomotor function during progressive passive hyperthermia in humans. *J Appl Physiol* **112**, 748-758.

Rowell LB,(1992). Reflex control of the circulation during exercise. *Int J Sports Med* **13 Suppl 1**, S25-7.

Rowell LB,(1984). Reflex control of regional circulations in humans. *J Auton Nerv Syst* **11**, 101-114.

Rowell LB,(1974). Human cardiovascular adjustments to exercise and thermal stress. *Physiol Rev* **54**, 75-159.

Rowell LB,(1973). Regulation of splanchnic blood flow in man. *Physiologist* **16**, 127-142.

Rowell LB, Blackmon JR, Martin RH, Mazzarella JA & Bruce RA (1965). Hepatic clearance of indocyanine green in man under thermal and exercise stresses. *J Appl Physiol* **20**, 384-394.

Rowell LB, Brengelmann GL, Blackmon JR & Murray JA (1970). Redistribution of blood flow during sustained high skin temperature in resting man. *J Appl Physiol* **28**, 415-420.

Rowell LB, Brengelmann GL, Blackmon JR, Twiss RD & Kusumi F (1968). Splanchnic blood flow and metabolism in heat-stressed man. *J Appl Physiol* **24**, 475-484.

Rowell LB, Brengelmann GL & Murray JA (1969a). Cardiovascular responses to sustained high skin temperature in resting man. *J Appl Physiol* **27**, 673-680.

Rowell LB, Brengelmann GL, Murray JA, Kraning KK,2nd & Kusumi F (1969b). Human metabolic responses to hyperthermia during mild to maximal exercise. *J Appl Physiol* **26**, 395-402.

Rowell LB, Detry JR, Profant GR & Wyss C (1971). Splanchnic vasoconstriction in hyperthermic man--role of falling blood pressure. *J Appl Physiol* **31**, 864-869.

Rowell LB, Marx HJ, Bruce RA, Conn RD & 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**, 1801-1816.

Rowell LB, Murray JA, Brengelmann GL & Kraning KK,2nd (1969). Human cardiovascular adjustments to rapid changes in skin temperature during exercise. *Circ Res* **24**, 711-724.

Rowell LB, Saltin B, Kiens B & Christensen NJ (1986). Is peak quadriceps blood flow in humans even higher during exercise with hypoxemia?. *Am J Physiol* **251**, H1038-44.

Rowell, LB (1993). *Human cardiovascular control*. Oxford University Press, New York ; Oxford.

Roy CS & Sherrington CS (1890). On the Regulation of the Blood-supply of the Brain. *J Physiol* **11**, 85-158.17.

Saltin B,(1964). Aerobic and Anaerobic Work Capacity After Dehydration. *J Appl Physiol* **19**, 1114-1118.

Saltin B & Calbet JA (2006). Point: in health and in a normoxic environment, VO2 max is limited primarily by cardiac output and locomotor muscle blood flow. *J Appl Physiol* **100**, 744-745.

Saltin B, Rådegran G, Koskolou MD & Roach RC (1998). Skeletal muscle blood flow in humans and its regulation during exercise. *Acta Physiol Scand* **162**, 421-436.

Sato K, Ogoh S, Hirasawa A, Oue A & Sadamoto T (2011). The distribution of blood flow in the carotid and vertebral arteries during dynamic exercise in humans. *J Physiol* **589**, 2847-2856.

Sato K, Sadamoto T, Hirasawa A, Oue A, Subudhi AW, Miyazawa T & Ogoh S (2012). Differential blood flow responses to CO(2) in human internal and external carotid and vertebral arteries. *J Physiol* **590**, 3277-3290.

Sato K & Sadamoto T (2010). Different blood flow responses to dynamic exercise between internal carotid and vertebral arteries in women. *J Appl Physiol* **109**, 864-869.

Savard GK, Nielsen B, Laszczynska J, Larsen BE & Saltin B (1988). Muscle blood flow is not reduced in humans during moderate exercise and heat stress. *J Appl Physiol* **64**, 649-657.

Sawka MN,(1992). Physiological consequences of hypohydration: exercise performance and thermoregulation. *Med Sci Sports Exerc* **24**, 657-670.

Sawka MN, Cheuvront SN & Kenefick RW (2012a). High skin temperature and hypohydration impair aerobic performance. *Exp Physiol* **97**, 327-332.

Sawka MN, Cheuvront SN & Kenefick RW (2012b). High skin temperature and hypohydration impair aerobic performance. *Exp Physiol* **97**, 327-332.

Sawka MN, Knowlton RG & Critz JB (1979). Thermal and circulatory responses to repeated bouts of prolonged running. *Med Sci Sports* **11**, 177-180.

Sawka MN, Leon LR, Montain SJ & Sonna LA (2011). Integrated physiological mechanisms of exercise performance, adaptation, and maladaptation to heat stress. *Compr Physiol* **1**, 1883-1928.

Sawka MN, Young AJ, Cadarette BS, Levine L & Pandolf KB (1985a). Influence of heat stress and acclimation on maximal aerobic power. *Eur J Appl Physiol Occup Physiol* **53**, 294-298.

Sawka MN, Young AJ, Francesconi RP, Muza SR & Pandolf KB (1985b). Thermoregulatory and blood responses during exercise at graded hypohydration levels. *J Appl Physiol* **59**, 1394-1401.

Scheinberg P, Blackburn I, Saslaw M, Rich M & Baum G (1953). Cerebral circulation and metabolism in pulmonary emphysema and fibrosis with observations on the effects of mild exercise. *J Clin Invest* **32**, 720-728.

Scheinberg P, Blackburn LI, Rich M & Saslaw M (1954). Effects of vigorous physical exercise on cerebral circulation and metabolism. *Am J Med* **16**, 549-554.

Schlader ZJ, Prange HD, Mickleborough TD & Stager JM (2009). Characteristics of the control of human thermoregulatory behavior. *Physiol Behav* **98**, 557-562.

Schoning M & Scheel P (1996). Color duplex measurement of cerebral blood flow volume: intra- and interobserver reproducibility and habituation to serial measurements in normal subjects. *J Cereb Blood Flow Metab* **16**, 523-531.

Schoning M, Walter J & Scheel P (1994). Estimation of cerebral blood flow through color duplex sonography of the carotid and vertebral arteries in healthy adults. *Stroke* **25**, 17-22.

Secher NH, Clausen JP, Klausen K, Noer I & Trap-Jensen J (1977). Central and regional circulatory effects of adding arm exercise to leg exercise. *Acta Physiol Scand* **100**, 288-297.

Secher NH & Richardson RS (2009). A large slice of cardiac output or humble pie for the respiratory muscles?. *J Physiol* **587**, 3411.

Secher NH, Seifert T & Van Lieshout JJ (2008). Cerebral blood flow and metabolism during exercise: implications for fatigue. *J Appl Physiol* **104**, 306-314.

Seifert T, Rasmussen P, Secher NH & Nielsen HB (2009). Cerebral oxygenation decreases during exercise in humans with beta-adrenergic blockade. *Acta Physiologica* **196**, 295-302.

Seifert T & Secher NH (2011). Sympathetic influence on cerebral blood flow and metabolism during exercise in humans. *Prog Neurobiol* **95**, 406-426.

Serrador JM, Picot PA, Rutt BK, Shoemaker JK & Bondar RL (2000). MRI measures of middle cerebral artery diameter in conscious humans during simulated orthostasis. *Stroke* **31**, 1672-1678.

Shibasaki M, Aoki K, Morimoto K, Johnson JM & Takamata A (2009). Plasma hyperosmolality elevates the internal temperature threshold for active thermoregulatory vasodilation during heat stress in humans. *Am J Physiol Regul Integr Comp Physiol* **297**, R1706-12.

Shoemaker JK, Halliwill JR, Hughson RL & Joyner MJ (1997). Contributions of acetylcholine and nitric oxide to forearm blood flow at exercise onset and recovery. *Am J Physiol* **273**, H2388-95.

Smolander J & Louhevaara V (1992). Effect of heat stress on muscle blood flow during dynamic handgrip exercise. *Eur J Appl Physiol Occup Physiol* **65**, 215-220.

Sokoloff L,(1960). The effects of carbon dioxide on the cerebral circulation. *Anesthesiology* **21**, 664-673.

Stöhr EJ, González-Alonso J, Pearson J, Low DA, Ali L, Barker H & Shave R (2011a). Effects of graded heat stress on global left ventricular function and twist mechanics at rest and during exercise in healthy humans. *Exp Physiol* **96**, 114-124.

Stöhr EJ, González-Alonso J, Pearson J, Low DA, Ali L, Barker H & Shave RE (2011b). Dehydration Reduces Left Ventricular Filling at Rest and During Exercise Independent of Twist Mechanics. *J Appl Physiol* **111**, 891-897.

Stöhr EJ, González-Alonso J & Shave RE (2011c). Left ventricular mechanical limitations to stroke volume in healthy humans during incremental exercise. *Am J Physiol Heart Circ Physiol* **301**, H478-H487.

Strandgaard S & Sigurdsson ST (2008a). Last Word on Point:Counterpoint: Sympathetic nervous activity does/does not influence cerebral blood flow. *J Appl Physiol* **105**, 1375.

Strandgaard S & Sigurdsson ST (2008b). Point:Counterpoint: Sympathetic activity does/does not influence cerebral blood flow. Counterpoint: Sympathetic nerve activity does not influence cerebral blood flow. *J Appl Physiol* **105**, 1366-7; discussion 1367-8.

Subudhi AW, Lorenz MC, Fulco CS & Roach RC (2008). Cerebrovascular responses to incremental exercise during hypobaric hypoxia: effect of oxygenation on maximal performance. *Am J Physiol Heart Circ Physiol* **294,** H164–71.

Subudhi AW, Miramon BR, Granger ME & Roach RC (2009). Frontal and motor cortex oxygenation during maximal exercise in normoxia and hypoxia. *J Appl Physiol* **106**, 1153-1158.

Subudhi AW, Olin JT, Dimmen AC, Polaner DM, Kayser B & Roach RC (2011). Does cerebral oxygen delivery limit incremental exercise performance?. *J Appl Physiol* **111**, 1727-1734.

Sugawara J, Tanabe T, Miyachi M, Yamamoto K, Takahashi K, Iemitsu M, Otsuki T, Homma S, Maeda S, Ajisaka R & Matsuda M (2003). Non-invasive assessment of cardiac output during exercise in healthy young humans: comparison between Modelflow method and Doppler echocardiography method. *Acta Physiol Scand* **179**, 361-366.

Thrush, A & Hartshorne, T (2010). *Vascular ultrasound: how, why and when*. Churchill Livingstone/Elsvier, Edinburgh.

Todd G, Butler JE, Taylor JL & Gandevia SC (2005). Hyperthermia: a failure of the motor cortex and the muscle. *J Physiol* **563**, 621-631.

Trangmar SJ, Chiesa ST, Stock CG, Kalsi KK, Secher NH & Gonzalez-Alonso J (2014). Dehydration affects cerebral blood flow but not its metabolic rate for oxygen during maximal exercise in trained humans. *J Physiol* **592**, 3143-3160.

Trinity JD, Lee JF, Pahnke MD, Beck KC & Coyle EF (2012). Attenuated relationship between cardiac output and oxygen uptake during high-intensity exercise. *Acta Physiol* **204**, 362-370.

Tyler CJ & Sunderland C (2011). Cooling the neck region during exercise in the heat. *J Athl Train* **46**, 61-68.

Tzeng YC & Ainslie PN (2013). Blood pressure regulation IX: cerebral autoregulation under blood pressure challenges. *Eur J Appl Physiol*.

Valdueza JM, von Munster T, Hoffman O, Schreiber S & Einhaupl KM (2000). Postural dependency of the cerebral venous outflow. *Lancet* **355**, 200-201.

van Hall G, Stromstad M, Rasmussen P, Jans O, Zaar M, Gam C, Quistorff B, Secher NH & Nielsen HB (2009). Blood lactate is an important energy source for the human brain. *J Cereb Blood Flow Metab* **29**, 1121-1129.

van Lieshout JJ, Pott F, Madsen PL, van Goudoever J & Secher NH (2001). Muscle tensing during standing: effects on cerebral tissue oxygenation and cerebral artery blood velocity. *Stroke* **32**, 1546-1551.

van Lieshout JJ & Secher NH (2008a). Last Word on Point:Counterpoint: Sympathetic activity does/does not influence cerebral blood flow. *J Appl Physiol* **105**, 1374.

van Lieshout JJ & Secher NH (2008b). Point:Counterpoint: Sympathetic activity does/does not influence cerebral blood flow. Point: Sympathetic activity does influence cerebral blood flow. *J Appl Physiol* **105**, 1364-1366.

Van Lieshout JJ, Wieling W, Karemaker JM & Secher NH (2003). Syncope, cerebral perfusion, and oxygenation. *J Appl Physiol* **94**, 833-848.

Vogiatzis I, Athanasopoulos D, Habazettl H, Kuebler WM, Wagner H, Roussos C, Wagner PD & Zakynthinos S (2009). Intercostal muscle blood flow limitation in athletes during maximal exercise. *J Physiol* **587**, 3665-3677.

Vogiatzis I, Zakynthinos S, Boushel R, Athanasopoulos D, Guenette JA, Wagner H, Roussos C & Wagner PD (2008). The contribution of intrapulmonary shunts to the alveolar-to-arterial oxygen difference during exercise is very small. *J Physiol* **586**, 2381-2391.

Volianitis S & Secher NH (2009). Rowing, the ultimate challenge to the human body - implications for physiological variables. *Clin Physiol Funct Imaging* **29**, 241-244.

Wagner PD,(2011). Modeling O(2) transport as an integrated system limiting (.)V(O(2)MAX). *Comput Methods Programs Biomed* **101**, 109-114.

Wagner PD,(1996). Determinants of maximal oxygen transport and utilization. *Annu Rev Physiol* **58**, 21-50.

Wallin BG & Charkoudian N (2007). Sympathetic neural control of integrated cardiovascular function: insights from measurement of human sympathetic nerve activity. *Muscle Nerve* **36**, 595-614.

Watson G, Judelson DA, Armstrong LE, Yeargin SW, Casa DJ & Maresh CM (2005). Influence of diuretic-induced dehydration on competitive sprint and power performance. *Med Sci Sports Exerc* **37**, 1168-1174.

White MD,(2006). Components and mechanisms of thermal hyperpnea. *J Appl Physiol* **101**, 655-663.

White MD & Cabanac M (1996). Exercise hyperpnea and hyperthermia in humans. *J Appl Physiol* **81**, 1249-1254.

White MG, Saleh O, Nonner D, Barrett EF, Moraes CT & Barrett JN (2012). Mitochondrial dysfunction induced by heat stress in cultured rat CNS neurons. *J Neurophysiol* **108**, 2203-2214.

Willie CK, Colino FL, Bailey DM, Tzeng YC, Binsted G, Jones LW, Haykowsky MJ, Bellapart J, Ogoh S, Smith KJ, Smirl JD, Day TA, Lucas SJ, Eller LK & Ainslie PN (2011). Utility of transcranial Doppler ultrasound for the integrative assessment of cerebrovascular function. *J Neurosci Methods* **196**, 221-237.

Willie CK, Macleod DB, Shaw AD, Smith KJ, Tzeng YC, Eves ND, Ikeda K, Graham J, Lewis NC, Day TA & Ainslie PN (2012). Regional brain blood flow in man during acute changes in arterial blood gases. *J Physiol* **590**, 3261-3275.

Willie CK, Tzeng YC, Fisher JA & Ainslie PN (2014). Integrative regulation of human brain blood flow. *J Physiol* **592**, 841-859.

Wilson MH, Edsell ME, Davagnanam I, Hirani SP, Martin DS, Levett DZ, Thornton JS, Golay X, Strycharczuk L, Newman SP, Montgomery HE, Grocott MP, Imray CH & Caudwell Xtreme Everest Research Group (2011). Cerebral artery dilatation maintains cerebral oxygenation at extreme altitude and in acute hypoxia--an ultrasound and MRI study. *J Cereb Blood Flow Metab* **31**, 2019-2029.

Wilson TE, Cui J, Zhang R & Crandall CG (2006). Heat stress reduces cerebral blood velocity and markedly impairs orthostatic tolerance in humans. *Am J Physiol Regul Integr Comp Physiol* **291**, R1443-8.

Wilson TE, Cui J, Zhang R, Witkowski S & Crandall CG (2002). Skin cooling maintains cerebral blood flow velocity and orthostatic tolerance during tilting in heated humans. *J Appl Physiol* **93**, 85-91.

Wilson TE, Tollund C, Yoshiga CC, Dawson EA, Nissen P, Secher NH & Crandall CG (2007). Effects of heat and cold stress on central vascular pressure relationships during orthostasis in humans. *J Physiol* **585**, 279-285.

Yoon S, Zuccarello M & Rapoport RM (2012). pCO(2) and pH regulation of cerebral blood flow. *Front Physiol* **3**, 365.

Zauner, A & Muizelaar, JP (1997). Brain metabolism and cerebral blood flow. In *Head Injury: Pathophysiology and Management of Severe Closed Injury*, ed. Reilly P & Bullock R, pp. 89-99. CRC Press.

Zauner A, Doppenberg EM, Woodward JJ, Choi SC, Young HF & Bullock R (1997). Continuous monitoring of cerebral substrate delivery and clearance: initial experience in 24 patients with severe acute brain injuries. *Neurosurgery* **41**, 1082-91; discussion 1091-3.

Zhang R, Zuckerman JH, Iwasaki K, Wilson TE, Crandall CG & Levine BD (2002). Autonomic neural control of dynamic cerebral autoregulation in humans. *Circulation* **106**, 1814-1820.

Zhu M, Ackerman JJ, Sukstanskii AL & Yablonskiy DA (2006). How the body controls brain temperature: the temperature shielding effect of cerebral blood flow. *J Appl Physiol* **101**, 1481-1488.

Zhu M, Ackerman JJH & Yablonskiy DA (2009). Body and brain temperature coupling: the critical role of cerebral blood flow. *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology* **179**, 701-710.

APPENDICES

Appendix I - Ethical approval

Brunel
UNIVERSITY
WEST LONDON

University Research Ethics Committee

06 February 2012

Letter of Approval

Proposer: Mr. Steven Trangmar
School of Sport & Education

Title: **The effects of dehydration on brain circulation and oxygenation during prolonged sub-maximal and graded incremental exercise in the heat**

Dear Mr. Trangmar,

The University Research Ethics Committee has considered the amendments recently submitted by you in response to the Committee's earlier review of the above application.

The Chair, acting under delegated authority, is satisfied that the amendments accord with the decision of the Committee and has agreed that there is no objection on ethical grounds to the proposed study.

Any changes to the protocol contained in your application, and any unforseen ethical issues which arise during the project, must be notified to the Committee.

The Committee would appreciate a report on the project following its completion. This should include some indication of the success of the project, whether any adverse events occurred, and whether any participants withdrew from the research.

Kind regards,

Mary F. Liddell

Mary F. Liddell
Secretary, University Research Ethics Committee

for

David Anderson-Ford
Chair, Research Ethics Committee
Brunel University

Head of School of Sport & Education
Professor Susan Capel

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Mr Steven Trangmar
PhD (Sport Sciences) Student
School of Sport and Education
Brunel University

8th February 2012

Dear Steven

RE07-11 - The effects of dehydration on brain circulation and oxygenation during prolonged sub-maximal and graded incremental exercise in the heat

I am writing to confirm the Research Ethics Committee of the School of Sport and Education received your application connected to the above mentioned research study. Your application has been independently reviewed to ensure it complies with the University/School Research Ethics requirements and guidelines.

The Chair, acting under delegated authority, is satisfied with the decision reached by the independent reviewers and is pleased to confirm there is no objection on ethical grounds to grant ethics approval to the proposed study.

Any changes to the protocol contained within your application and any unforeseen ethical issues which arise during the conduct of your study must be notified to the Research Ethics Committee.

On behalf of the Research Ethics Committee for the School of Sport and Education, I wish you every success with your study.

Yours sincerely

pp Richard Godfrey

Dr Gary Armstrong
Chair of Research Ethics Committee
School Of Sport and Education

Brunel UNIVERSITY LONDON

University Research Ethics Committee

09 October 2013

Letter of approval

Proposer: Mr. Steven Trangmar
School of Sport & Education

Title: **The effect of heat stress on muscle, brain and systemic haemodynamics during incremental cycling exercise: partitioning the role of skin and internal temperature**

Dear Mr. Trangmar,

The University Research Ethics Committee has approved your research ethics application for the above-named project, which is to be undertaken in 2013/14.

Any changes to the protocol contained in your application, and any unforseen ethical issues which arise during the project, must be notified to the Committee.

The Committee would appreciate a report on the project following its completion. This should include some indication of the success of the project, whether any adverse events occurred, and whether any participants withdrew from the research.

Kind regards,

D. Anderson-Ford

David Anderson-Ford
Chair, Research Ethics Committee
Brunel University

Head of School of Sport & Education
Professor Ian Rivers

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Mr Steven Trangmar
PhD (Sport Sciences) Student
School of Sport and Education
Brunel University

6th September 2013

Dear Steve

RE54-12 The effect of heat stress on muscle, brain and systemic haemodynamics during incremental cycling exercise: partitioning the role of skin and internal temperature

I am writing to confirm the Research Ethics Committee of the School of Sport and Education received your application connected to the above mentioned research study. Your application has been independently reviewed to ensure it complies with the University/School Research Ethics requirements and guidelines.

The Chair, acting under delegated authority, is satisfied with the decision reached by the independent reviewers and is pleased to confirm there is no objection on ethical grounds to grant ethics approval to the proposed study.

Any changes to the protocol contained within your application and any unforeseen ethical issues which arise during the conduct of your study must be notified to the Research Ethics Committee for review.

On behalf of the Research Ethics Committee for the School of Sport and Education, I wish you every success with your study.

Yours sincerely

pp Dr Richard J Godfrey
Chair of Research Ethics Committee
School Of Sport and Education

Appendix II - Participant Information

Brunel
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Centre for Sports Medicine &
Human Performance
Sport Sciences, Heinz Wolff Building
School of Sport & Education
Uxbridge, Middlesex UB8 3PH, UK

Information for Research Participants – Part 1

Title of the study

The effects of dehydration on brain circulation and oxygenation during prolonged sub-maximal and graded incremental exercise in the heat.

Study background

When athletes exercise in a hot, humid environment for long durations they experience losses in body water through sweating and increases in core body temperature (dehydration and hyperthermia respectively). Research has shown that performing prolonged (~1-2hours) exercise in the heat, with dehydration, results in significant reductions in blood flow to the exercising muscles and brain. Intense exercise, with or without heat, also leads to similar reductions in blood flow to the brain that ultimately results in fatigue. Measuring these alterations will help our understanding of the body's response to exercise in the heat and help us to develop methods to help prevent the early onset of fatigue.

Objectives

To investigate whether dehydration impairs blood flow and the delivery of oxygen to the brain during exhaustive exercise in the heat, and whether rehydration restores these alterations.

Participation

Your participation in this study will be voluntary and you will have the right to withdraw from it, at any point, without providing reason and without penalty. This study will require 12 healthy, active males aged 18-35. Ideally those training regularly in cycling or triathlon will be required.

How long will the study last?

The study will require participants to visit the lab on five occasions. The first three visits will involve familiarisation to exercise in the heat (1-2 hours of cycling at 70% $\dot{V}O_2$peak; Temp, 35^{o}C; Humidity, 50%) and additional preliminary testing

(incremental exercise test to the limit of tolerance). The fourth visit will be the first experimental day lasting approximately six hours. During the experimental day you will perform the following;

1. A semi-recumbent, incremental cycling test (5x3minute stages) to exhaustion followed by 30 minutes of passive rest.
2. Two hours of semi-recumbent cycling in the heat at 70% max (Temp = 35^{o}C, Humidity = 50%; without fluid ingestion), followed by five minutes passive rest and body mass measurement.
3. A second semi-recumbent incremental test to exhaustion, followed by one hour of passive rest (which will include the ingestion of large volumes of carbohydrate-free fluid to restore hydration status).
4. A third and final semi-recumbent incremental test to exhaustion.

The fifth and final day will be the second experimental day and last approximately four hours. During this visit you will perform the same protocols as in visit 4, whilst maintaining hydration throughout. The two experimental days will be separated by a minimum of one week. For more information regarding the protocols involved in this study, please read; Information for Research Participants – Part 2.

Data collection methods

Measurements include the following;
Blood pressure
Blood samples
Cardiac output
Blood flow and velocity
Cerebral oxygenation
Core and skin temperature
For more information regarding the data collection methods involved in this study, please read; Information for Research Participants – Part 2.

Risks and hazards associated with the experiment

All measurement techniques used in the study have been deemed to be low risk and a health check questionnaire must be completed prior to participation in the study. Detailed risks and hazards will be explained to you prior to you agreeing to take part. For more information regarding the risks and hazards involved in this study, please read; Information for Research Participants – Part 2.
Benefit of participating in the study
You will gain information on how your body adapts to heavy exercise in the heat. You will have the opportunity to experience exercise in hot, humid conditions which may be relevant to future competitions or training you may take part in.

Will I be paid for my participation in the study?

You will be compensated for time and expenses related to the study such as transportation and time off from work (up to £400).

Data collected
Your personal information will remain confidential, and we will not disclose any of your personal information without your permission. For more information regarding data protection, please read part 2 of the information for research participants.

Who should you contact if you wish to make a complaint about the study?
You can contact the Chair of the School of Sport and Education Research Ethics Committee, Dr. Gary Armstrong (Gary.Armstrong@brunel.ac.uk).

What if I have questions?
If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision. If you have any additional questions about this research project, please contact Steven Trangmar;
Telephone: 07725358208; E-mail: steven.trangmar@brunel.ac.uk

This research project has been approved by the School of Sport & Education Ethics Committee and the Brunel University Research Ethics Committee.

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Information

for Research Participants – Part 2

Title of the study: The effects of dehydration on brain circulation and oxygenation during prolonged sub-maximal and graded incremental exercise.

Participant Requirements

Visit one:

You will become familiarised with the experimental set-up, perform an incremental cycling test to the limit of tolerance in the semi-recumbent (seated) position. This will be used to assess your maximal exercise performance and heart rate which will in turn be used to set the workloads for the remaining lab visits. You will then perform 1 hour of cycling, at 60% $\dot{V}O_{2peak,}$ in the heat (Temp, 35^{o}C, Humidity, 50%).

Visits two and three:

The second and third visit will be formed of two hours of semi-recumbent cycling in the heat at the same relative intensity and temperature conditions as visit one. A fan will be directed on you and you are permitted to listen to music and drink as much as you like throughout the familiarisation sessions. Core temperature will also be measured during these trials (see methods below).

Visit four:

This is the first experimental day. After arriving at the lab, catheters will be inserted into the jugular vein and brachial artery (see methods for full description) and you will rest in a seated position for 30 minutes where resting body mass and other measurements can be made. You will then move to the main laboratory and perform the following exercise protocol;

1. A semi-recumbent, incremental cycling test to exhaustion followed by 30 minutes of passive rest.

2. 2 hours of semi-recumbent cycling in the heat at 60% max (without fluid ingestion), followed by five minutes passive rest and body mass measurement.

3. A second semi-recumbent incremental test to exhaustion, followed by one hour of passive rest (which will include the ingestion of large volumes of carbohydrate-free fluid to restore hydration status).

4. A third and final semi-recumbent incremental test to exhaustion.

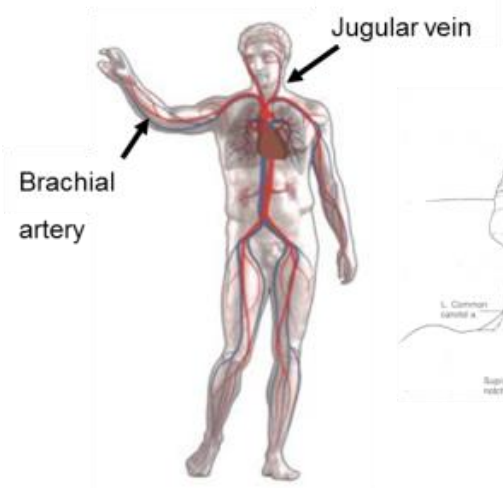
Visit 5:
The final visit will replicate the fourth visit with the exception of catheters and blood samples. During this visit you will remain hydrated throughout by consuming a carbohydrate-free electrolyte drink in proportion to your sweat rate.

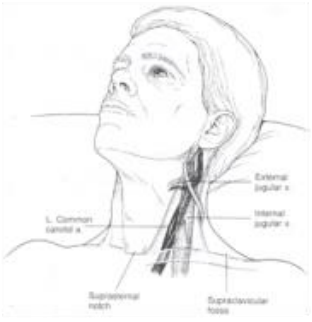
At the end of the trials, you will consume a small meal (a sandwich and fruit) and be provided with an isotonic sports drink. Trained clinicians who will place the catheter and withdraw samples will remain present with you for one hour after the end of the testing protocol to ensure you are ready to leave the laboratory.

Data collection methods
Blood pressure - measured in the brachial artery and jugular vein with transducers positioned at the level of the heart. The data are amplified and recorded on a data acquisition laptop.

Blood sampling – blood samples (a maximum of 350ml in total) will be taken at rest and at the end of each incremental stage and; every 30 minutes during semi-recumbent cycling exercise. These samples will be analysed for ATP, an important substance in the blood which may alter blood flow. Samples will also be analysed for blood gases (including oxygen saturation and acidity). Catheters will be placed in the internal jugular vein and the brachial artery (see diagrams below). A local anaesthetic gel (lidocaine) will be used at the site of catheter insertion to reduce sensations of pain.

Cardiac output – the amount of blood pumped by the heart will be calculated from the arterial pressure waves measured directly from the catheters.





Catheters to be used (not to scale)

Cerebral blood velocity – measured non-invasively using a trans-cranial Doppler (TCD). A small ultrasound probe, fixed in place by a headpiece, will measure the speed of blood travelling through the middle cerebral artery.

Cerebral oxygenation – measured non-invasively with two near-infrared saturation (NIRS) sensors attached to the forehead. The sensors emit an infrared light which, when reflected back to the probe, provide a measure of the oxygen saturation of the brain.

Core temperature – measured throughout the protocol using an ingestible temperature sensor. You will consume the sensor orally, with a meal on the evening prior to the experimental day and it will normally be expelled within 24-36 hours after consumption.

Cerebral blood flow (CBF) – measured non-invasively using Doppler ultrasound. A sound emitting probe will be used to measure vessel diameter at rest, and blood velocity at rest and during exercise in the internal (ICA) and common carotid artery (CCA).

Heart rate - measured with short-wave telemetry. A heart rate monitor (Polar) will be attached to the torso throughout the study, which will measure the electrical activity of the heart.

Skin temperature – measured using skin thermisters attached to the surface of the skin and secured in place using an adhesive spray and medical tape.

Risks and hazards associated with the experiment

A thorough risk assessment has been conducted for this study. All measurement techniques used in the study have been deemed to be low risk. A health check

questionnaire must be completed prior to participation in the study. Any risks associated with maximal exercise will be limited by a thorough warm up and completion of the health check questionnaire. Arterial and venous catheterisation may incur the following risks and/or hazards:

- **Haematoma:** a blue mark around the catheterised vessels occurs when blood escapes from the vessel and accumulates in the surrounding tissues. This happens in less than 8% of cases. Pressure will be applied by experienced clinicians after removal of the catheters to constrain the blood flow within the vessel and therefore minimise the chances of developing a haematoma. Moreover, you should refrain from performing intense exercise for 24 hours after the experiment.

- **Bleeding:** occurs in less than 1% of cases and can be prevented by pressure application.

- **Infection:** The risk of infection is present, as in all procedures which require puncture or cutting of the skin however, this risk is minimal when strict sterile procedures are followed.

Additional risks include;

- **Dehydration and exercise in the heat:** exercise in these conditions may increase the sensations of effort that are usually felt during heavy exercise. We will closely monitor your core temperature to ensure your safety throughout.

Several members of the research team have extensive experience with the procedures used in this experiment as demonstrated by numerous publications in sports medicine and physiology journals. Moreover, clinicians, who are medically qualified anaesthetists, will perform all catheterisation procedures.

Exclusion criteria

- Current or chronic history of lower limb muscle, tendon, ligament or knee joint injury
- Known cardiac diseases and / or cardiovascular risk factors
- Smokers
- Aversion to blood and needles
- Under treatment for any disease
- Known allergy to local anaesthetic drug

Requirements or abstentions imposed upon the participants prior to and after the main experiment.

You will be asked to refrain from:

1. Strenuous physical activity 24 hours prior to the experiment
2. alcohol ingestion 24 hours prior to the experiment
3. caffeine intake at least 12 hours before the experiment

4. eating within 2 hours prior to the experiment (food intake will be assessed with a food diary for the 24hrs prior to the study).
5. strenuous physical activity for 24-48 hours after the experiment
6. Blood donations (NHS or other studies) six-eight weeks prior to and, eight weeks post participation.

Pre-participation meals.

On the day before the visits four and five we aim to standardise the meals you will consume. Below is a simple guide to what you should eat prior to the study;

Evening meal (standard adult portions):
Lean meat (e.g chicken, turkey)
Carbohydrate (Pasta, potato, rice, couscous)
Vegetables (any, mixed)

Breakfast
Cereal (e.g. porridge, weetabix)
Juice (NO tea/coffee)
Fruit (e.g. Banana, apple)

Appreciate that you will have no opportunity to eat for the duration of the protocol and your choice of food should reflect this. Ensuring that you are well hydrated before the main experimental day is also important. We would suggest the approximate consumption of fluid to be in the region of 2-4 litres (inclusive of fluid in food) on the day before visit four and five.

Pre-participation health check questionnaire.

Health and safety within this investigation is of paramount importance. For this reason we need to be aware of your current health status before you begin any testing procedures. To identify whether you are eligible to participate in this investigation, we will ask you to fill in the pre-participation health check questionnaire included below.

Data collected

An identification code will be ascribed to each participant and all data collected will be electronically compiled anonymously. Your personal information will remain confidential, and we will not disclose any of your personal information without your permission. The data will be stored at the School of Sport and Education, Brunel University, London, for a maximum of 5 years. Results will be presented anonymously in scientific conferences and research articles.

Benefit of participating in the study

You will gain an insight into the limitations to maximal exercise and the regulation of brain blood flow and oxygenation during prolonged sub-maximal and graded incremental exercise. You will also benefit by gaining important information about your own individual maximal exercise capacity. You will also experience intense

exercise in the heat which may be of relevance for your training and athletic performance.

Will I be paid for my participation in the study?
You will be compensated for time and expenses related to the study such as transportation (£400). Partial or non-completion will result in a lower compensation amount. Payment will be made through the Payroll Department, Brunel University, and this requires you to complete a non-staff expenses form with your contact and bank account details.

How can I get information about the study findings?
You will get all information about your results and the study findings by contacting Steven Trangmar.

Compensation arrangements for negligent and non-negligent harm
Brunel University has an insurance policy (NHE-01CA29-0013) with public and products liabilities of £30m. In the case of clinical trials the University maintains a comprehensive policy to cover negligent and no fault harm up to a maximum of £10 m.

What if I have questions?
If you have any questions about this research project, please contact:
Steven Trangmar; telephone: 07725358208; email: steven.trangmar@brunel.ac.uk

How is this research project funded?
This study will be supported by a grant from PEPSICO, owners of the Gatorade Sport Science Institute.

This research project has been approved by the School of Sport & Education Ethics Committee and the Brunel University Research Ethics Committee.

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Information for Research Participants – Part 1

Title of the study

The role of skin and internal temperature on blood flow to the brain and muscles during incremental cycling exercise.

Study background

The limiting factors to maximal exercise have interested researchers for many years. Research has shown that reductions in blood returning to, and ejected from the heart, and blood flow to the brain and legs are reduced prior to fatigue during maximal exercise. Heat stress and subsequent exercise provides a major challenge to the body to ensure a stable internal temperature and exercise performance. Measuring the impact of heat stress during incremental cycling exercise will help our understanding of the maximal limits of human exercise performance.

Objectives

To investigate whether heat stress impairs muscle and brain blood flow and the delivery of oxygen to the exercising muscles during exhaustive exercise compared to control (normal) exercise conditions.

Participation

Your participation in this study will be voluntary and you will have the right to withdraw from it, at any point, without providing reason, without penalty and, without negative impact on your university grade (where applicable). This study will require 10 healthy, competitive active males aged 18-40. Ideally those who are motivated to achieve their best athletic performance that train regularly in cycling or triathlon will be required.

How long will the study last?

The study will require participants to visit the lab on three occasions. The first visit will last approximately 90 min and involve familiarisation to the exercise tests and additional preliminary testing ($\dot{V}O_{2max}$ testing; a test to determine the maximum

amount of oxygen your body can consume and utilise during exercise). The second visit will be the control trial and involve the performance of 3 incremental exercise tests, separated by ~90 min of rest. This visit will last ~4 hours. The third and final visit will be the main experimental day and will last approximately six hours. During the experimental day you will perform the following;

1. Passive rest with whole body heating. You will wear a tubed-lined suit whilst 50°C water will be pumped through the tubing to increase core temperature by 1°C.

2. An incremental cycling test (5 x 2.5 minute stages) to exhaustion, followed by 1 hour of passive rest.

3. Approximately 10 minutes of passive rest with whole body heating, to increase skin temperature only, followed by a second incremental exercise text.

4. One hour of passive rest followed by a third and final incremental test to exhaustion

You will be permitted to drink as much as you want throughout the day (water only). For more information regarding the protocols involved in this study, please read; Information for Research Participants – Part 2.

Data collection methods

The following measurements will be obtained at rest and at the end of every exercise stage during each incremental test;
Blood flow and velocity
Blood pressure
Blood samples
Cardiac output
Cerebral and muscle oxygenation
Core and skin temperature
Muscle activity

Blood samples will be obtained using some invasive procedures; however these are deemed to carry limited risks. For more information regarding the data collection methods involved in this study, please read; Information for Research Participants – Part 2.

Risks and hazards associated with the experiment

All measurement techniques used in the study have been deemed to be low risk and a health check questionnaire must be completed prior to participation in the study. Detailed risks and hazards will be explained to you prior to you agreeing to take part. For more information regarding the risks and hazards involved in this study, please read; Information for Research Participants – Part 2.

Benefit of participating in the study
You will gain information on how your body adapts to maximal incremental exercise in a normal environment and in the heat. You will have the opportunity to receive maximal power outputs, $\dot{V}O_{2max}$ and, blood lactate levels which will aid training for future competitions.

Will I be paid for my participation in the study?
You will be compensated for time and expenses related to the study such as transportation and time off from work (up to £250). This remuneration is additional to receiving a consultancy report with your test data (normally priced at £199).

Data collected
Your personal information will remain confidential, and we will not disclose any of your personal information without your permission. For more information regarding data protection, please read part 2 of the information for research participants.

Who should you contact if you wish to make a complaint about the study?
You can contact the Chair of the School of Sport and Education Research Ethics Committee, Dr. Richard Godfrey (Richard.Godfrey@brunel.ac.uk).

What if I have questions?
If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision. If you have any additional questions about this research project, please contact: Steven Trangmar; Telephone: 07725358208; E-mail: steven.trangmar@brunel.ac.uk

This research project has been approved by the School of Sport & Education Ethics Committee and the Brunel University Research Ethics Committee.

Brunel
UNIVERSITY
LONDON
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Sport Sciences, Heinz Wolff Building
School of Sport & Education
Uxbridge, Middlesex UB8 3PH, UK

Information for Research Participants – Part 2

Title of the study: The role of skin and internal temperature on blood flow to the brain and muscles during incremental cycling exercise.

Participant Requirements

Visit 1:

You will become familiarised with the experimental set-up and perform an incremental cycling test to the limit of tolerance on a cycle ergometer. This will be used to assess your maximal exercise performance, heart rate, and $\dot{V}O_{2max}$ (a test to determine the maximum amount of oxygen your body can consume and utilise during exercise) which will in turn be used to set the workloads for the remaining lab visits. After a 30 minute recovery period you will then perform 8-10 short bouts of intense cycling exercise (90-110% of your peak power output), in the heat (Temp, 35^{o}C, Humidity, 50%), to experience exercise whilst heated. This visit will take approximately 90 min to complete.

Visit 2 (Control day):

The second visit will be formed of three incremental cycling exercise tests, each separated by ~90 min of passive rest, at 20, 40, 60, 80 and 100% of WR_{max} from visit 1. This will take place in normal laboratory conditions (~20°C). This visit will take approximately 4 hr to complete.

Visit 3 (Experimental day):

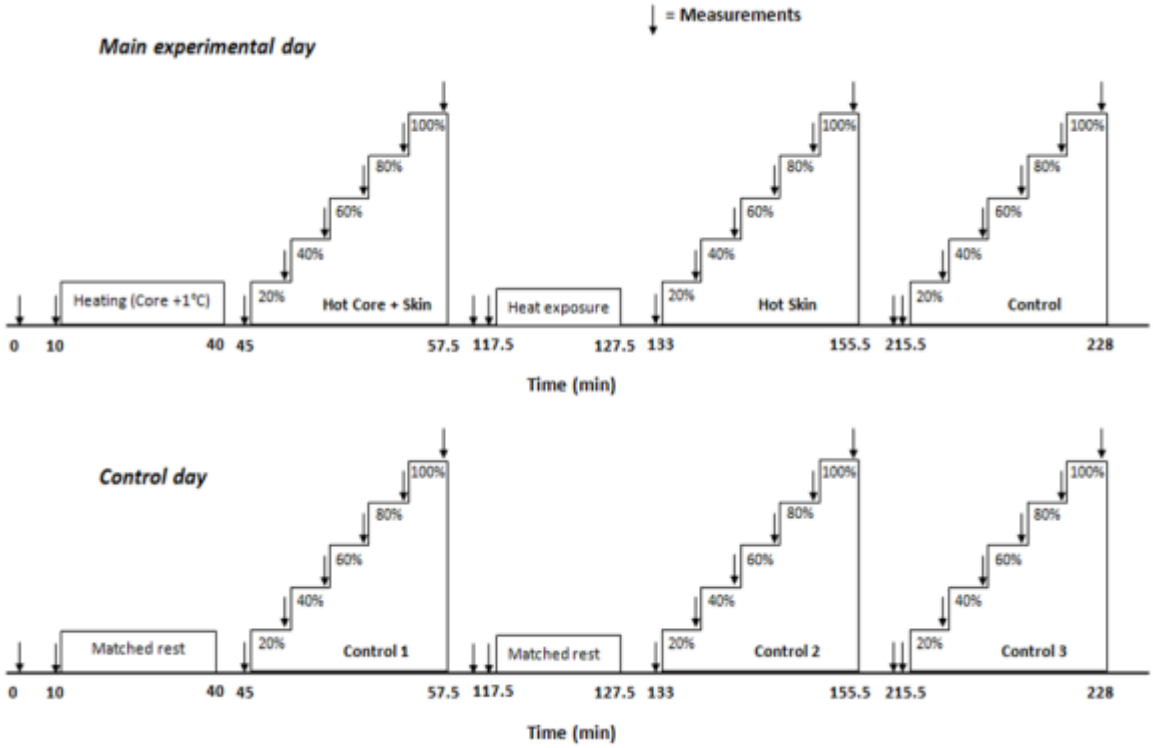
This is the main experimental day. After arriving at the lab you will take your own measurement of nude body mass in a closed room. Subsequently, catheters will be inserted into the radial artery and the femoral vein in one leg (see methods for full description). You will rest in a seated position for 30 minutes before moving to the main laboratory and perform the following exercise protocols;

1. Passive rest with whole body heating. You will wear a tubed-lined suit whilst 50°C water will be pumped through the tubing to increase core temperature by 1°C.

2. An incremental cycling test (5 x 2.5 minute stages at 20, 40, 60, 80 and 100% of maximal power from visit 1) to exhaustion.

3. Approximately 10 minutes of passive rest wearing a tube-lined suit with a circulating water temperature of 50°C, to increase skin temperature only, followed by a second incremental exercise text.

4. One hour of passive rest followed by a third and final incremental test to exhaustion



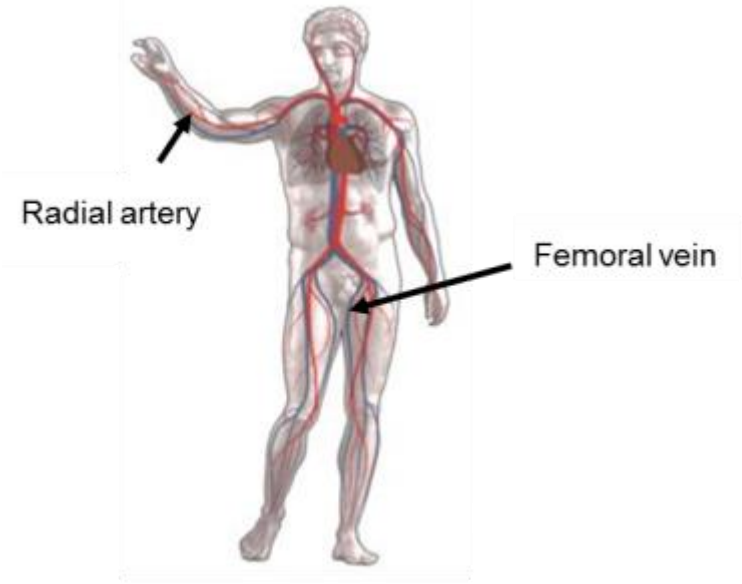
At the end of the trials, you will consume a small meal (a sandwich and fruit) and be provided with an isotonic sports drink. Trained clinicians who will place the catheters and withdraw samples will remain present with you for one hour after the end of the testing protocol to ensure you are ready to leave the laboratory. This visit will take approximately 6 hr.

Data collection methods

Blood pressure - measured in the radial artery and femoral vein. The data are amplified and recorded on a data acquisition laptop.

Blood sampling – blood samples (a maximum of 350 millilitres in total) will be taken at rest and at the end of each incremental stage. These samples will be analysed for ATP (an important energy source for muscle contraction), an important substance in the blood which may alter blood flow. Samples will also be analysed for blood gases (including oxygen saturation and acidity) and Catecholamines. Catheters will be placed in the femoral vein and, the radial artery of the non-dominant arm (see diagrams below). A local anaesthetic gel (lidocaine)

will be used at the site of catheter insertion to reduce sensations of pain. During the control trial, a small venous catheter will be placed in the median antecubital vein (small vein in the elbow crease).



Catheters to be used (not to scale)

Cardiac output – the amount of blood pumped by the heart will be calculated from the arterial pressure waves measured directly from the catheters.

Cerebral blood velocity – measured non-invasively using a trans-cranial Doppler (TCD). A small ultrasound probe, fixed in place by a headpiece, will measure the speed of blood travelling through the middle cerebral artery.

Cerebral and muscle oxygenation – measured non-invasively with two near-infrared saturation (NIRS) sensors attached to the forehead and thigh. The sensors emit an infrared light which, when reflected back to the probe, provide a measure of the oxygen saturation of the brain and muscle.

Core temperature – measured throughout the protocol using an ingestible temperature sensor. You will consume the sensor orally, with a meal on the evening prior to the experimental day and it will normally be expelled within 24-36

hours after consumption. The consumed pill is disposable and you are not expected to retrieve it after use.

Heart rate - measured with ECG (measurements of the electrical activity of the heart). Electrodes will be placed slightly below your collar bones and below the ribcage that will measure the electrical activity of the heart.

Leg blood flow – measured using the constant-infusion thermodilution technique. Briefly, cold saline (water and salt) will be infused into the femoral venous catheter and the rate of change in temperature, from the point of infusion to the tip of the catheter, will be measured for the calculation of blood flow.

Muscle activity – measured using EMG (measurements of the electrical activity of muscle contraction). Electrodes will be placed on your thigh muscles to measure the electrical activity of the muscle during exercise.

Oxygen uptake – measured using a face mask covering the mouth and nose and attached to an analyser unit. Breathing through the mask, whilst unusual, should not impact your exercise performance.

Skin temperature – measured using wireless data recorders (iButton®) attached to the surface of the skin and secured in place using medical tape.

Risks and hazards associated with the experiment

A thorough risk assessment has been conducted for this study. All measurement techniques used in the study have been deemed to be low risk. A health check questionnaire must be completed prior to participation in the study. Any risks associated with maximal exercise will be limited by a thorough warm up and completion of the health check questionnaire. Arterial and venous catheterisation may incur the following risks and/or hazards:

- **Haematoma:** a blue mark around the catheterised vessels occurs when blood escapes from the vessel and accumulates in the surrounding tissues. This happens in less than 8% of cases. Pressure will be applied by experienced clinicians after removal of the catheters to constrain the blood flow within the vessel and therefore minimise the chances of developing a

haematoma. Moreover, you should refrain from performing intense exercise for 24 hours after the experiment.

- Complications associated with arterial catheterisation: as with all invasive procedures, there is a risk associated with placement of an arterial catheter. These risks, though not exhaustive, include clotting of the blood (thrombus), restriction of blood supply and, bleeding. We have appointed a medically trained clinician with many years of experience in the placement of these catheters. Clinicians within our laboratory have placed arterial catheters in 60 participants with no complications.

- **Bleeding:** occurs in less than 1% of cases and can be prevented by pressure application.

- **Infection:** the risk of infection is present, as in all procedures which require puncture or cutting of the skin however, this risk is minimal when strict sterile procedures are followed.

Additional risks include;

- **Exercise in the heat:** exercise in these conditions may increase the sensations of effort that are usually felt during heavy exercise. We will closely monitor your core temperature to ensure your safety throughout.

Several members of the research team have extensive experience with the procedures used in this experiment as demonstrated by numerous publications in sports medicine and physiology journals. Moreover, clinicians, who are medically qualified anaesthetists, will perform all catheterisation procedures.

Exclusion criteria

- Current or chronic history of lower limb muscle, tendon, ligament or knee joint injury
- Known cardiac diseases and / or cardiovascular risk factors
- Smokers
- Aversion to blood and needles
- Under treatment for any disease
- Known allergy to local anaesthetic drug

Requirements or abstentions imposed upon the participants prior to and after the main experiment.

You will be asked to refrain from:

1. **<u>Strenuous physical activity 24 to 48 hours prior to the experiment</u>** – it is essential that you refrain from your normal training routine for at least one day prior to the two preliminary visits and two days prior to the main experimental visit as this could impact your performance in the trials.
2. Cycling to, and from, the laboratory on the main experimental day.
3. Alcohol ingestion 24 hours prior to the experiment

4. Caffeine intake at least 12 hours before the experiment.
5. Eating within 2 hours prior to the experiment (food intake will be assessed with a food diary for the 24hrs prior to the study).
6. Strenuous physical activity for 24-48 hours after the experiment.
7. Manual (and ideally all) work in the hours after the experimental day.
8. Blood donations (NHS or other studies) eight weeks prior to and, eight weeks post participation.

Pre-participation meals.

On the day before the main experimental visit we aim to standardise the meals you will consume. Below is a simple guide to what you should eat prior to the study;

Evening meal (standard adult portions):

- Lean meat (e.g chicken, turkey)
- Carbohydrate (Pasta, potato, rice, couscous)
- Vegetables (any, mixed)

Breakfast

- Cereal (e.g. porridge, weetabix)
- Juice (NO tea/coffee or caffeine containing beverages)
- Fruit (e.g. Banana, apple)

Appreciate that you will have no opportunity to eat for the duration of the protocol and your choice of food should reflect this. Ensuring that you are well hydrated before the main experimental day is also important. We would suggest the approximate consumption of fluid to be in the region of 2-4 litres (inclusive of fluid in food) on the day before most visits. You will be supplied with three food diary's to record your food intake prior to all sessions.

Pre-participation health check questionnaire

Health and safety within this investigation is of paramount importance. For this reason we need to be aware of your current health status before you begin any testing procedures. To identify whether you are eligible to participate in this investigation, we will ask you to fill in the pre-participation health check questionnaire included below.

Data collected

An identification code will be ascribed to each participant and all data collected will be electronically compiled anonymously. Your personal information will remain confidential, and we will not disclose any of your personal information without your permission. The data will be stored at the School of Sport and Education, Brunel University, London, for a maximum of 5 years. Results will be presented anonymously in scientific conferences and research articles.

Benefit of participating in the study
You will gain information on how your body adapts to maximal incremental exercise in a normal environment and in the heat. You will have the opportunity to receive maximal power outputs, $\dot{V}O_{2max}$ and, blood lactate levels which will aid training for future competitions.

Will I be paid for my participation in the study?
You will be compensated for time and expenses related to the study such as transportation and time off from work (up to £250). This remuneration is additional to receiving a consultancy report with your test data (normally priced at £199). Partial or non-completion will result in a lower compensation amount. Payment will be made through the Payroll Department, Brunel University, and this requires you to complete a non-staff expenses form with your contact and bank account details.

How can I get information about the study findings?
You will get all information about your results and the study findings by contacting Steven Trangmar.

Compensation arrangements for negligent and non-negligent harm
Brunel University has an insurance policy (NHE-01CA29-0013) with public and products liabilities of £30m. In the case of clinical trials the University maintains a comprehensive policy to cover negligent and no fault harm up to a maximum of £10 m.

What if I have questions?
If you have any questions about this research project, please contact:
Steven Trangmar; telephone: 07725358208; email: steven.trangmar@brunel.ac.uk

How is this research project funded?
This study will be supported by a grant from PEPSICO, owners of the Gatorade Sport Science Institute.

This research project has been approved by the School of Sport & Education Ethics Committee and the Brunel University Research Ethics Committee.

Appendix III - Health Questionnaire

PRE-PARTICIPATION HEALTH CHECK QUESTIONNAIRE

Health and safety within this investigation is of paramount importance. For this reason we need to be aware of your current health status before you begin any testing procedures. The questions below are designed to identify whether you are able to participate now or should obtain medical advice before undertaking this investigation, Whilst every care will be given to the best of the investigators ability, an individual must know his/her limitations.

Subject name……

Date of birth…..……..…….

Emergency contact name and number

……....

Please answer the following questions:

	YES	NO
1. Has your doctor ever diagnosed a heart condition or recommend only medically supervised exercise?	☐	☐
2. Do you suffer from chest pains, heart palpitations or tightness of the chest?	☐	☐
3. Do you have known high blood pressure? If yes, please give details (i.e. medication)	☐	☐
4. Do you have low blood pressure or often feel faint or have dizzy spells?	☐	☐
5. Do you have known hypercholesteremia?	☐	☐
6. Have you ever had any bone or joint problems, which could be aggravated by physical activity?	☐	☐
7. Do you suffer from diabetes? If yes, are you insulin dependent?	☐	☐
8. Do you suffer from any lung/chest problem, i.e. Asthma, bronchitis, emphysema?	☐	☐
9. Do you suffer from epilepsy? If yes, when was the last incident?	☐	☐
10. Are you taking any medication?	☐	☐
11. Have you had any injuries in the past? E.g. back problems or muscle, tendon or ligament strains, etc…	☐	☐
12. Are you currently enrolled in any other studies?	☐	☐
13. **I have already participated in a recent blood donation program**	☐	☐
14. Are you a smoker?	☐	☐
15. Do you consider yourself to be a cyclist/triathlete of good club level standard?	☐	☐

16. Describe your exercise routines (mode, frequency, intensity/speed, race times):

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

I have read and fully understand this questionnaire. I confirm that to the best of my knowledge, the answers are correct and accurate. I know of no reasons why I should not participate in physical activity and this investigation and I understand I will be taking part at my own risk.

Appendix IV - Consent form

Consent Form

Participant's name & signature:_____Date:

Investigator's name & signature:_____Date:

The participant should complete the whole of this sheet him/herself

Please tick the appropriate box

	YES	NO
Have you read the Information for Research Participants (Part 1 & 2)?	☐	☐
Have you had an opportunity to ask questions and discuss this study?	☐	☐
Have you received satisfactory answers to all your questions?	☐	☐
Who have you spoken to? ……		
Do you understand that you will not be referred to by name in any report concerning the study?	☐	☐
Do you agree for your blood samples to be analysed and used in future studies that may not be detailed in the information provided?	☐	☐
Do you provide consent for us to inform your GP of information Obtained from the study if deemed necessary by our clinicians?	☐	☐
Do you understand that you are free to withdraw from the study:		
at any time	☐	☐
without having to give a reason for withdrawing?	☐	☐
without affecting your future care	☐	☐
Do you agree to take part in this study?	☐	☐

Signature of Research Participant: **Date:**

Name in capitals:

<u>Witness statement</u>

'I am satisfied that the above-named has given informed consent.'

Signature of Witness: Date:

Name in capitals:

Appendix V - Publications and related commentaries

The Journal of Physiology

J Physiol 592.14 (2014) pp 3143–3160

Dehydration affects cerebral blood flow but not its metabolic rate for oxygen during maximal exercise in trained humans

Steven J. Trangmar[1], Scott T. Chiesa[1], Christopher G. Stock[1], Kameljit K. Kalsi[1], Niels H. Secher[1,2] and José González-Alonso[1]

[1]*Centre for Sports Medicine and Human Performance, Brunel University, London, UK*
[2]*Department of Anaesthesia, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark*

Key points

- Dehydration accrued during exercise in the heat challenges systemic and locomotor muscle blood flow, but its impact on cerebral blood flow (CBF) and metabolism remains unknown.
- This study assessed whether dehydration compromises CBF and the cerebral metabolic rate for oxygen ($CMRO_2$) during incremental exercise to exhaustion in trained males.
- Dehydration induced an early reduction in CBF during progressive exercise, but increased O_2 extraction secured $CMRO_2$.
- In all hydration conditions declining CBF at high exercise intensities was correlated to decreasing arterial carbon dioxide tension and increasing jugular venous plasma noradrenaline.
- These results suggest that dehydration impairs CBF at high exercise intensities, but this circulatory strain on the human brain does not compromise $CMRO_2$.

Abstract Intense exercise is associated with a reduction in cerebral blood flow (CBF), but regulation of CBF during strenuous exercise in the heat with dehydration is unclear. We assessed internal (ICA) and common carotid artery (CCA) haemodynamics (indicative of CBF and extra-cranial blood flow), middle cerebral artery velocity (MCA V_{mean}), arterial–venous differences and blood temperature in 10 trained males during incremental cycling to exhaustion in the heat (35°C) in control, dehydrated and rehydrated states. Dehydration reduced body mass (75.8 ± 3 *vs.* 78.2 ± 3 kg), increased internal temperature (38.3 ± 0.1 *vs.* 36.8 ± 0.1°C), impaired exercise capacity (269 ± 11 *vs.* 336 ± 14 W), and lowered ICA and MCA V_{mean} by 12–23% without compromising CCA blood flow. During euhydrated incremental exercise on a separate day, however, exercise capacity and ICA, MCA V_{mean} and CCA dynamics were preserved. The fast decline in cerebral perfusion with dehydration was accompanied by increased O_2 extraction ($P < 0.05$), resulting in a maintained cerebral metabolic rate for oxygen ($CMRO_2$). In all conditions, reductions in ICA and MCA V_{mean} were associated with declining cerebral vascular conductance, increasing jugular venous noradrenaline, and falling arterial carbon dioxide tension (P_{aCO_2}) ($R^2 \geq 0.41$, $P \leq 0.01$) whereas CCA flow and conductance were related to elevated blood temperature. In conclusion, dehydration accelerated the decline in CBF by decreasing P_{aCO_2} and enhancing vasoconstrictor activity. However, the circulatory strain on the human brain during maximal exercise does not compromise $CMRO_2$ because of compensatory increases in O_2 extraction.

 DOI: 10.1113/jphysiol.2014.272104

Editor's choice (15th July 2014)

Perspective – Anthony R. Bain and Philip N. Ainslie (2014). On the limits of cerebral oxygen extraction. *The Journal of Physiology* **592,** 2917-2918.

Appendix VI - Conference abstracts

Are restrictions in blood flow and oxygen supply to the human brain a mechanism by which dehydration impairs maximal exercise capacity?

Trangmar SJ.[1], Chiesa ST. [1], Stock CG. [1], Kalsi KK. [1], Secher NH. [1,2], González-Alonso J. [1]

[1]Centre for Sports Medicine and Human Performance, Brunel University, London (UK), [2]Copenhagen Muscle Research Centre, Department of Anaesthesia, Rigshospitalet, University of Copenhagen, Denmark (Denmark).

Introduction: During maximal exercise, a reduction in blood flow to the human brain may contribute to the development of fatigue by lowering O2 supply and diminishing cerebral O2 availability (1, 3). It is presently unknown if dehydration exacerbates the influence of circulatory strain upon the human brain sufficiently to compromise cerebral $\dot{V}O_2$. We tested the hypothesis that dehydration accelerates the reductions in cerebral blood flow during incremental exercise, but without impairing brain $\dot{V}O_2$. **Methods**: Ten cyclists ($\dot{V}O_{2peak}$ 59 ± 2 mL/kg/min) performed 3 incremental cycle ergometer exercise tests in a warm environment (35^{o}C) in the following conditions: 1) *euhydrated* (control; maximal work rate (WRmax) 336 ± 14 W), 2) *dehydrated* after 2 hours of sub-maximal cycling without fluid ingestion (DEH; 3.1 ± 0.3 % body mass loss; 222 ± 10 W), and 3) *rehydrated* after 1 h passive recovery with full fluid replacement (REH; 294 ± 15 W). Cerebral blood flow and velocity were assessed in the internal carotid artery (CBF) and middle cerebral artery (MCA V_{mean}). Blood samples were obtained from the brachial artery and left internal jugular vein to measure a-vO2 differences and for the calculation of $\dot{V}O_2$. **Results:** During control, CBF and MCA V_{mean} increased from rest to 40% WRmax (17±2%; $P < 0.01$) and declined gradually thereafter to baseline values. During DEH, CBF and MCA V_{mean} declined earlier and were 12-23% lower than at the same workload in control; however, the a-v O2 diff and O2 extraction were higher ($P < 0.05$), resulting in a similar brain $\dot{V}O_2$ between conditions. The flow and oxygenation responses during REH were similar to control. In all trials, the declines in CBF and MCA V_{mean} and vascular conductance during intense and maximal exercise were strongly correlated to reductions in P_aCO2 ($R^2 \geq 0.74$, $P \leq 0.01$). This suggests P_aCO2 has a role in the observed cerebral vasoconstriction (2,4). **Discussion:** The present findings demonstrate that dehydration accelerates the decline in blood flow and O2 supply to the human brain during incremental cycling exercise to the limit of tolerance. However, $\dot{V}O_2$ was not compromised because of compensatory increases in cerebral O2 extraction. A compromised brain $\dot{V}O_2$ is therefore an unlikely mechanism underpinning the impaired exercise capacity in dehydrated individuals. However, it remains possible that dehydration impairs maximal exercise capacity independently of disturbances in aerobic metabolism.

References

1) González-Alonso et al. (2004). *J Physiol 557,* 331-342; **2)** Willie et al. (2012). *J Physiol 590,* 3261-3275; **3)** Nybo et al. (2001). *J Physiol 534,* 279-286; **4)** Sato et al. (2011). *J Physiol 589,* 2847-2856.

Presented at the European College of Sports Science (ECSS) conference, Barcelona (June 2013).

Dehydration reduces blood flow to the human brain and increases oxygen extraction during prolonged exercise in humans

Trangmar, SJ.[1], Chiesa, ST.[1], Kalsi, KK.[1], Secher, NH.[1, 2], González-Alonso, J.[1]

[1]Centre for Sports Medicine and Human Performance, Brunel University, London (UK), [2]Copenhagen Muscle Research Centre, Department of Anaesthesia, Rigshospitalet, University of Copenhagen, Denmark (Denmark).

Background: Dehydration accrued during prolonged exercise in the heat induces significant cardiovascular strain on the human body characterised by reductions in cardiac output, active muscle and skin blood flow, arterial blood pressure, vascular conductance and an overall impaired exercise capacity (1). However, it is presently unknown whether progressive dehydration during exercise in the heat reduces blood flow to the brain, thereby impairing aerobic metabolism. A hyperthermic-hyperventilation induced lowering of the P_aCO_2 may reduce blood flow to the brain, assuming that reductions in middle cerebral artery velocity (MCA V_{mean}) reflect reductions in cerebral blood flow (CBF) (2,3,4,5). This study tested the hypothesis that progressive dehydration reduces CBF during prolonged exercise in the heat, in part through mechanisms associated with P_aCO_2, but without impairing brain $\dot{V}O_2$. **Methods:** We assessed blood flow in the internal carotid artery (CBF) using Doppler ultrasonography and middle cerebral artery velocity (MCA V_{mean}) in ten cyclists ($\dot{V}O_{2PEAK}$: 59 ± 2 ml/kg/min), who performed two hours of prolonged cycling exercise in a warm environment (182 ± 6W; 35^{o}C), without fluids to induce moderate dehydration (DEH; 3.1 ± 0.3 % body mass loss). Subjects returned one week later to repeat the protocol, but with regular fluid ingestion to maintain hydration status (Control). Blood samples were obtained from the brachial artery and left internal jugular vein (DEH only) to measure a-vO_2 differences and for the calculation of brain $\dot{V}O_2$. All data are mean ± SEM and were compared with ANOVA and Pearson correlation (SPSS).**Results:** During dehydration CBF and MCA V_{mean} increased by 13% from rest to 30 min ($P < 0.05$). Thereafter CBF declined to resting values with flow at 120 min significantly lower than at 30, 60 and 90 min ($P < 0.001$). During control, CBF and MCA V_{mean} increased from rest to 30 min and were subsequently maintained throughout exercise (Increase ≥ 25%, $P < 0.05$). Reductions in CBF and MCA V_{mean} during DEH were accompanied with significant increases ($P < 0.05$) in a-vO_2 diff resulting in an unchanged brain $\dot{V}O_2$. P_aCO_2 declined in accordance with flow and velocity ($P < 0.05$) with changes in flow correlated to changes in P_aCO_2 ($R^2 = 0.75$, $P < 0.001$), suggesting a role for P_aCO_2 in cerebral vasoconstriction. **Discussion:** The present findings show that progressive dehydration during prolonged exercise results in a marked reduction in CBF, whereas in control CBF did not decline. Compensatory increases in cerebral oxygen extraction allow for the maintenance of brain $\dot{V}O_2$ throughout exhaustive exercise. These findings suggest that a reduction in brain $\dot{V}O_2$ is an unlikely mechanism underpinning exercise capacity during prolonged, exhaustive exercise with dehydration.

This work was support by the Gatorade Sports Science Institute, PepsiCo, USA.

Presented at the Internal Union of Physiological Sciences (IUPS) conference, Birmingham, UK (July 2013).

Brain oxygen consumption is maintained during prolonged exercise in the heat despite reductions in cerebral blood flow

Trangmar, SJ.[1], Chiesa, ST.[1], Kalsi, KK.[1], Secher, NH.[1, 2], González-Alonso, J.[1]

[1]Centre for Sports Medicine and Human Performance, Brunel University, London (UK), [2]Copenhagen Muscle Research Centre, Department of Anaesthesia, Rigshospitalet, University of Copenhagen, Denmark (Denmark).

Introduction: Exercise in the heat with concomitant dehydration induces a significant cardiovascular strain on the human body. Blood flow to the brain (CBF) and extra-cranial tissues including the skin may also be compromised, potentially challenging brain oxygen uptake (brain $\dot{V}O_2$) and local heat dissipation. Whether dehydration reduces regional blood flow across the head and reduces brain $\dot{V}O_2$ during strenuous exercise in the heat remains unknown. **Methods:** We assessed CBF and extra-cranial blood flow in the internal, external and common carotid arteries (ICA, ECA and CCA) using Doppler ultrasonography in ten cyclists ($\dot{V}O_{2PEAK}$: 59 ± 2 ml/kg/min), who performed two hours of prolonged cycling exercise in a warm environment (182±6 W; 35 ^{o}C), without fluids, to induce moderate dehydration (DEH; 3.1 ± 0.3 % body mass loss). Blood samples were obtained from the brachial artery and left internal jugular vein for a-vO_2 differences and calculation of brain $\dot{V}O_2$ using the Fick principle. Participants returned one week later to repeat the protocol whilst hydration was maintained with regular ingestions of a carbohydrate/electrolyte drink (Gatorade®). **Results:** In the dehydration trial, CBF was elevated after 30 min of strenuous exercise in the heat (+13% from rest to 30 min; $P < 0.05$), before declining to baseline values at 120 min ($P < 0.001$). Extra-cranial blood flow increased from rest to 60 min before declining prior to exhaustion; overall an indication of a reduced total blood flow to the head. Reductions in CBF were accompanied by increases ($P < 0.05$) in a-vO_2 diff, resulting in a stable brain $\dot{V}O_2$. Fluid ingestion sufficient to prevent dehydration maintained CBF (≥ 25% increase *vs.* rest; $P < 0.05$) and extra-cranial blood flow throughout exercise. **Conclusions:** The present findings demonstrate that progressive dehydration during prolonged exercise in the heat results in a distinct circulatory strain on the brain. However, brain $\dot{V}O_2$ appears not to be compromised due to compensatory increases in cerebral oxygen extraction. Regular fluid ingestion precludes the reductions in blood flow to regional vascular beds in proximity to the brain.

Supported by the Gatorade Sports Science Institute, PepsiCo Inc., USA.

Presented at Training and Competing in the Heat, Aspetar, Doha, Qatar (March 2014).

Winner of an Aspetar travel and accommodation grant.

Physiological mechanisms impairing cardiovascular function and exercise capacity in the heat stressed human: role of skin *versus* body temperature

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Introduction: Cardiovascular strain and hyperthermia are thought to be important factors limiting exercise capacity in heat-stressed humans, but the contribution of elevations in skin ($\overline{T}_{sk}$) *versus* body temperature remains unknown. Here we tested the hypothesis that an increased body temperature would accelerate the attenuation in leg, brain and systemic perfusion leading to impaired exercise performance, but the sole increase in $\overline{T}_{sk}$ would not. **Methods:** Nine cyclists completed 3 incremental cycling tests after (a) ~30 min whole-body heating (H_{30}), (b) ~10 min whole-body heating (H_{10}), and (c) in control conditions. $\overline{T}_{sk}$ and core temperature (T_c), heart rate (HR) and $\dot{V}O_2$ were measured continuously; whereas leg, brain and systemic haemodynamics and haematological parameters were assessed at the end of each exercise stage. To eliminate the effects of repeated exercise, the incremental tests were repeated, on a separate day, with each test performed in control conditions. **Results:** Prior to exercise in H_{30}, $\overline{T}_{sk}$, T_c and cardiac output were elevated by 6.2 ± 0.2 °C, 0.9 ± 0.1 °C and 4.8 L/min ($P < 0.05$) compared to control, whereas only $\overline{T}_{sk}$ was elevated prior to exercise in H_{10} (6.0 ± 0.2°C). During incremental exercise, $\overline{T}_{sk}$ was maintained, yet T_c rose gradually to a similar peak value in the 3 conditions (39.2 ± 0.1 °C). Exercise capacity and $\dot{V}O_{2max}$ were reduced in H_{30} by 13 ± 1% and 6 ± 2% ($P < 0.05$), but remained unchanged in H_{10}. On the transition from rest to sub-maximal exercise, $\dot{V}O_2$, cardiac output and leg blood flow increased at a similar rate across conditions. In contrast, mean arterial pressure and brain blood velocity increased but were lower, whereas HR and leg a-vO_2 difference were higher in H_{30} *vs.* H_{10} and control. At exhaustion, HR_{max} (~186 ± 3 beats/min) and leg a-vO_2 difference (~182 ± 5 ml/L) were similar in the 3 conditions, whereas mean arterial pressure (-14 ± 1%), brain blood velocity (-16 ± 6%), leg blood flow (-11 ± 3 %) and cardiac output (-9 ± 3%; all $P< 0.05$) were lower in H_{30} compared to H_{10} and control. In the 3 control incremental tests, exercise capacity, $\dot{V}O_{2max}$, HR_{max} and T_c were similar. **Discussion:** These findings demonstrate that skin hyperthermia *per se* does not compromise cardiovascular capacity or incremental exercise performance. Rather, combined skin and internal body hyperthermia reduces $\dot{V}O_{2max}$ and exercise capacity through the early attenuation of leg, brain and systemic blood flow. Our findings have important implications for understanding why athletic performance in warm environments is not universally impaired across all sports and exercise modalities.

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