

**HAEMODYNAMIC RESPONSES TO HEAT STRESS AND HYPOHYDRATION
IN RESTING AND EXERCISING HUMANS: IMPLICATIONS FOR THE
REGULATION OF SKELETAL MUSCLE BLOOD FLOW**

A thesis submitted for the degree of Doctor of Philosophy

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Abstract

Heat stress-induced hyperthermia and exercise-induced hypohydration are associated with marked alterations in limb and systemic haemodynamics in humans. However, the mechanisms underlying these alterations their effects on muscle blood flow are not well understood. The present thesis examined whether whole body and local heat stresses increased limb skin and muscle blood flow (Study 1) and whether hypohydration and hyperthermia compromised leg muscle, skin and systemic haemodynamics (Study 2). The effects of heat stress and combined hypohydration and hyperthermia were examined at rest and during mild small muscle mass exercise in humans. The results from Study 1 suggested that heat stress was accompanied by vasodilation in both skeletal muscle and skin vasculatures. Therefore in line with concomitant elevations in blood flow, skeletal muscle and skin vasodilation contribute to increases in leg blood flow and vascular conductance with whole body heat stress. Furthermore, increases in leg muscle and skin blood flow with isolated elevations in leg tissue temperature accounted for at least one half of the total increase in leg blood flow with whole body heat stress. Enhanced leg blood flow owed to a net vasodilation as explained by an elevation in vasodilator activity that exceeded increases in vasoconstrictor activity. This phenomenon was closely related to increases in muscle temperature and intravascular adenosine triphosphate (ATP). The results from Study 2 demonstrated that mild and moderate hypohydration and hyperthermia do not compromise leg muscle and skin blood flow or cardiac output at rest or during mild exercise in humans. Furthermore, acute rehydration did not alter leg muscle and skin blood flow or cardiac output compared to hypohydration and hyperthermia despite large alterations in blood volume and haematological variables and the restoration of core temperature. Taken together, the findings of this thesis indicate that: 1) heat stress induces vasodilation in both skeletal muscle and cutaneous vasculature, 2) elevations in muscle temperature and intravascular ATP play a role in heat stress- and exercise-induced hyperaemia, and 3) moderate hypohydration-induced hypovolemia and haemoconcentration and rehydration-induced hypervolaemia and haemodilution do not alter leg blood flow or cardiac output at rest and during low intensity exercise in humans when a large cardiovascular reserve is available.

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Table of contents

	Page
CHAPTER 1. General Introduction	1
1.1. Study Context	2
1.2. Thesis Overview	2
CHAPTER 2. Literature Review	4
2.1. Introduction	5
2.2. Control of limb muscle and skin blood flow in humans	7
2.2.1. Regulation of muscle blood flow	7
2.2.2. Regulation of skin blood flow	9
<i>Summary</i>	12
2.3. Limb and systemic haemodynamics with heat stress and hypohydration and hyperthermia	13
2.3.1. Limb and systemic haemodynamics	13
2.3.2. Central and local limitations to leg blood flow and cardiac output	15
2.3.3. Muscle and skin blood flow with heat stress and hypohydration and hyperthermia	19
2.4. Summary	23
2.5. Aims and hypotheses	24
2.5.1. Thesis aims	24
<i>Study 1</i>	24
<i>Study 2</i>	24
2.5.2. Hypotheses	24
<i>Study 1</i>	24
<i>Study 2</i>	25

CHAPTER 3. General Methodology	26
3.1. Pre-test procedures	27
3.1.1. Ethical Approval	27
3.1.2. Participants	27
3.1.3. Anthropometry	27
3.1.4. Peak power test	27
3.1.5. Familiarisation	28
Study 1 – Heat stress	28
Study 2 – Combined hypohydration and hyperthermia	28
3.1.6. Pre experimental ultrasound scanning	29
3.2. Test procedures	29
3.2.1. Leg blood flow measurement using ultrasound	29
Background	30
Two-dimensional diameter measurement	30
Pulse wave Doppler measurements of blood velocity	31
Common femoral artery blood flow measurement	32
Measurement error: validity and reliability	33
Strengths and weaknesses of using ultrasound to assess leg blood flow	34
3.2.2. Skin blood flow	34
3.2.3. Temperature measurements	35
3.2.4. Assessment of cardiovascular haemodynamics	35
Overview	35
Arterial catheter	35
Venous catheter	36
Modelflow method for estimation of systemic haemodynamics	36

3.2.5	Leg and systemic haemodynamics: calculated variables	37
3.2.6	Muscle oxygenation	37
3.2.7	Oxygen uptake	37
3.2.8	Blood and plasma parameters	37
3.3.	Statistical analysis	39
CHAPTER 4.		
Heat stress increases skeletal muscle blood flow in the resting and exercising leg		
		40
4.0.	Summary	41
4.1.	Introduction	42
4.2.	Methods	45
4.2.1.	Participants	45
4.2.2.	Design	45
4.2.3.	Instrumentation of participants	46
4.2.4.	Temperature measurements	49
4.2.5.	Systemic haemodynamics and muscle oxygenation	50
4.2.6.	Leg and skin haemodynamics	51
4.2.7.	Statistics	51
4.3.	Results	52
4.3.1.	Hydration and temperature during whole body heat stress	52
4.3.2.	Leg and systemic haemodynamics during whole body heat stress	55
4.3.3.	Effect of whole body heat stress and rest and during exercise	59
4.3.4.	Effect of local and whole body heat stress on resting leg haemodynamics	59

4.3.5	Circulating plasma ATP and catecholamines during whole body heat stress	62
4.3.6	Hydration, temperature and haemodynamics during isolated leg heat stress	63
4.4.	Discussion	64
4.5.	Conclusion	71
CHAPTER 5.		
Hypohydration and hyperthermia do not compromise limb muscle and skin blood flow or cardiac output in resting and mildly exercising humans		
		72
5.0.	Abstract	73
5.1.	Introduction	74
5.2.	Methods	77
5.2.1	Participants	77
5.2.2	Design	77
5.2.3	Instrumentation of participants	78
5.2.4	Temperature and blood volume measurements	78
5.2.5	Systemic haemodynamics	78
5.2.6	Statistics	79
5.3.	Results	80
5.3.1.	Hydration and temperature changes with hypohydration and rehydration	80
5.3.2.	Resting haemodynamic responses	84
5.3.3.	Exercising haemodynamic responses	88
5.4.	Discussion	89

5.5. Conclusion	93
CHAPTER 6. General Discussion	94
6.1. Introduction	95
6.2. Summary of main findings	95
6.3. Leg muscle and skin blood flow with heat stress and combined hypohydration and hyperthermia	97
6.3.1. Influence of temperature elevations and hypohydration upon skeletal muscle and skin blood flow and cardiac output in resting and mildly exercising humans	97
6.3.2. Limitations	110
6.4. Future directions	113
6.4.1. Hypotheses	116
<i>Study 1</i>	116
<i>Study 2</i>	116
6.5. Summary	111
REFERENCES	121
APPENDICES	
I. Informed consent form	147
II. Health questionnaire	149
III. Letter of ethical approval – study 1	151
IV. Letter of ethical approval – study 2	153
V. Letter of ethical approval – study 1 – muscle temperature measurements	155
VI. Conference abstracts	157

List of Tables

- 3.0.** Coefficient of variation for measurements of common femoral artery diameter and femoral artery blood velocity and blood flow at rest and during exercise. **33**
- 4.0.** Summary of haematological changes, noradrenaline, and plasma ATP during whole body heat stress at rest and during exercise. **53**
- 4.1.** Blood oxygenation data from arterial, mixed femoral venous and deep femoral venous blood samples obtained from three participants during whole body heat stress at rest and during exercise. **57**
- 5.0.** Summary of haematological changes, plasma catecholamines, and plasma ATP during combined hypohydration and hyperthermia at rest and during exercise. **83**

List of Figures

3.0.	Vivid 7 ultrasound system	30
3.1.	A typical 2D image obtained for the purposes of measuring common femoral artery diameter.	31
3.2.	A typical blood velocity profile taken from the common femoral artery for the purposes of calculating leg blood flow.	32
4.0.	A schematically presented view of the experimental protocol for Study 1 – whole body heat stress.	45
4.1.	A schematically presented view of the experimental protocol for Study 1 – isolated leg heat stress.	46
4.2.	A picture of the primary veins of the leg highlighting the deep portion of the femoral vein.	47
4.3.	A picture of the experimental set-up in the laboratory during the heat stress study, protocol 1.	49
4.4.	Body temperature changes during whole body heat stress.	54
4.5.	Leg haemodynamics during whole body heat stress at rest and during exercise.	55
4.6.	Systemic haemodynamics during whole body heat stress at rest and during exercise.	58
4.7.	Systemic and local haemodynamic responses to isolate leg heat stress.	60
4.8.	Leg blood flow and cardiac output during one legged <i>versus</i> whole body heat stress.	61
4.9.	Relationship between heat stress induced elevations in leg vascular conductance and plasma arterial ATP at rest and during exercise.	62
5.0.	A schematically represented view of the experimental protocol for Study 2 – combined hypohydration and hyperthermia.	77
5.1.	Body mass, blood volume, arterial oxygen content and body temperature during hypohydration and hyperthermia and following rehydration.	81

5.2.	Leg haemodynamics during hypohydration and hyperthermia and following rehydration at rest and during exercise	84
5.3.	Systemic haemodynamics during hypohydration and hyperthermia and following rehydration at rest and during exercise.	86
5.4.	Plasma arterial and venous catecholamines and ATP at rest and during exercise with hypohydration and hyperthermia and following rehydration.	87

Definition of Terms

Arterial oxygen content (CaO_2 , $\text{ml}\cdot\text{l}^{-1}$): The total amount of oxygen molecules in arterial blood.

Blood velocity ($\text{cm}\cdot\text{s}^{-1}$): A measurement of the speed at which blood is flowing through a blood vessel and expressed as a function of time. In the context of this thesis the velocity of blood was measured in the common femoral artery. A typical blood velocity profile obtained from the femoral artery has two distinct phases; anterograde where blood flows down the leg away from the heart and retrograde where blood flow becomes temporarily turbulent creating a negative flow.

Cardiac output (\dot{Q} , $\text{l}\cdot\text{min}^{-1}$): The amount of blood which leaves the heart via the aorta in one minute.

Cardiovascular reserve: The degree to which cardiac output, heart rate and stroke volume can change from baseline values without resulting in a compromised cardiac output.

Cardiovascular strain: Referring to the degree to which systemic haemodynamics, i.e. \dot{Q} , HR, SV and MAP are altered beyond baseline conditions in order to meet the demands of a given intervention, i.e. heat stress or combined hypohydration and hyperthermia.

Dehydration (DE): The progressive loss of body water which in the case of the Study 2 was achieved by prolonged cycling in a hot environment. This process of becoming dehydrated results in hypohydration (see below for definition).

Diastole: The relaxation phase of the cardiac cycle where blood flows into its chambers.

Femoral venous pressure (mm Hg): The force the blood exerts onto the walls of the femoral vein as it returns from the leg to the heart.

Finometer: A device to non-invasively monitor arterial blood pressure and which is used to calculate stroke volume beat by beat.

Haemoconcentration: An elevated proportion of red blood cells compared to plasma. This can be achieved either through the reduction of blood volume and re-infusion of red blood cells or, as in the present study, through continuous sweating during exercise in a hot environment.

Haemodilution: A reduced proportion of red blood cells compared to plasma. This can be achieved through intravenous infusion of saline or, as in the present thesis, by acute oral rehydration.

Heat Stress: The exposure to high external temperatures exceeding those of the skin and core in normal environmental and physiological conditions. Classically heat stress is induced by a drastic elevation in skin temperature either through water immersion, exposure to high environmental ambient temperatures or a water-perfused suit. During heat stress skin temperature becomes elevated rapidly and if exposure is prolonged core temperature also increases.

Hyperthermia: An elevation in core body temperature, typically of at least 1°C in magnitude. This can be due to the separate or combined effects of exercise (termed exercise hyperthermia) and heat stress or exercise induced hypohydration.

Hypervolaemia: An increased blood volume above normovolaemic levels (~ 80 ml kg⁻¹).

Hypohydration: Hypohydration is a consequence of the process of dehydration. It is measured by a reduction in body mass, calculated blood volume and elevations in blood osmolality.

Hypovolaemia: A reduction in blood volume below normal, normovolaemic, levels.

Leg a-vO₂ difference (leg a-vO₂ diff): The difference in oxygen content between the arterial and femoral venous blood. Oxygen is extracted from blood as it runs through the arterial to the venous circulation.

Leg blood flow (LBF, l·min⁻¹): The amount of blood flowing through the common femoral artery in either leg in one minute.

Leg haemodynamics: The movement of blood through the tissues of the leg.

Leg oxygen uptake (Leg $\dot{V}O_2$, l·min⁻¹): A measure of the metabolism of the tissues within the leg.

Leg vascular conductance (LVC, ml·min⁻¹·mm Hg⁻¹): A measure of the pressure of blood travelling through leg relative to the blood flow.

Mean arterial pressure (MAP, mm Hg): The average force that blood exerts on the walls of the arteries, which in this thesis was measured in the radial artery.

Muscle Hyperthermia: An elevation in skeletal muscle temperature.

Modelflow analysis: The use of this analysis allows the estimation of stroke volume, from arterial pressure waveforms. These waveforms were obtained from either invasive blood pressure monitoring or non-invasively using the finometer.

Muscle blood flow: A reference to the amount of blood flowing through the vasculature within the skeletal muscle tissue, in this case the leg.

Perfusion pressure (mm Hg): The difference between arterial and venous pressure. In the case of this thesis, mean arterial and femoral venous pressure.

Pulse wave Doppler: An ultrasound mode which enables the assessment of the velocity and quantity of red blood cells travelling through a specific site of a vessel, i.e., the femoral artery.

Shear Stress: an elevated mechanical force exerted onto a given vessel wall by the blood as flows, typically seen with whole body exercise.

Skin blood flow (SkBF, AU): A reference to the amount of blood flowing through the vasculature of the skin. Skin blood flow is measured in arbitrary units (AU).

Skin Hyperthermia: An elevation in skin temperature above normal levels. Normal skin temperature in humans is approximately 33°C.

Stroke volume (SV, ml): The amount of blood leaving the left ventricle per heart beat.

Systemic haemodynamics: The movement of blood through the systemic circulation.

Systemic vascular conductance (SVC, ml·min⁻¹·mm Hg⁻¹): An index of the blood flow travelling through the systemic circulation relative to the pressure gradient between the arterial circulation and the central venous circulation (right atrium or central venous pressure).

Systole: The contraction phase of the cardiac cycle where blood is ejected through the left and right ventricles of the heart.

List of Abbreviations

ATP – Adenosine triphosphate
a-vO₂ diff – Arterial – venous oxygen difference
BM – Body mass
CaO₂ – Arterial oxygen content
Cl⁻ - Serum chloride concentration in blood
FVP – Femoral venous pressure
Hb – Haemoglobin concentration in blood
HCT – Blood haematocrit concentration
HR – Heart rate
K⁺ - Serum potassium concentration in blood
LBF – Leg blood flow
LVC – Leg vascular conductance
MAP – Mean arterial pressure
mOSM – Osmolality as expressed as milliosmoles (mOSM·l⁻¹).
Na⁺ - Serum sodium concentration in blood
OSM - Osmolality
 \dot{Q} – Cardiac output
SV – Stroke volume
SVC – Systemic vascular conductance
T_c – Core temperature
T_{sk} – Mean whole body skin temperature
T_{leg} – Mean leg skin temperature
 $\dot{V}O_2$ – Oxygen uptake

CHAPTER 1

General Introduction

1.1. Study Context

Hyperthermia and hypohydration are stresses commonly experienced by athletes during training and athletic competition. These two conditions present a regulatory challenge to the cardiovascular system resulting in altered limb and systemic haemodynamics (González-Alonso, Calbet & Nielsen, 1998; González-Alonso, et al., 2004; González-Alonso, Mora-Rodriguez, Below & Coyle, 1997; González-Alonso, Mora-Rodriguez & Coyle, 2000; González-Alonso, et al., 1999c; Horstman & Horvath, 1972; Kenney, et al., 1990; Montain & Coyle, 1992b; Nielsen, Savard, Richter, Hargreaves & Saltin, 1990; Rowell, 1974; Rowell, Brengelmann & Murray, 1969a; Rowell, Marx, Bruce, Conn & Kusumi, 1966; Rowell, Murray, Brengelmann & Kraning, 1969b; Savard, Nielsen, Laszczynska, Larsen & Saltin, 1988).

However, it is unclear how limb muscle and skin blood flow are influenced by heat stress and combined hypohydration and hyperthermia in resting and mildly exercising humans. Investigating the partition of blood flow between limb muscle and skin vasculatures under conditions of heat stress and combined hypohydration and hyperthermia is important for two reasons. First this investigation will help us to further understand the thermoregulatory influences upon limb and systemic haemodynamics and secondly, to examine their influence upon exercise hyperaemia. The examination of exercise hyperaemia is important in itself because during exercise with hyperthermia and hypohydration reductions in leg blood flow and cardiac output are associated with increased fatigue (González-Alonso, Calbet & Nielsen, 1999a).

1.2. Thesis Overview

It remains unclear whether skeletal muscle vasodilation contributes to heat stress induced increases in limb blood flow and cardiac output. Furthermore, with combined hypohydration and hyperthermia it is unknown whether limb muscle and skin blood flow are reduced in humans at rest and during mild exercise when the cardiovascular strain is minimised. When a large cardiovascular reserve is available and the centrally and locally mediated restrictions to blood flow are reduced it is unknown how limb muscle and skin blood flow are influenced by heat stress and combined hypohydration and hyperthermia. Two comprehensive invasive studies that included various protocols were completed to address the aims of this thesis in resting and mildly exercising humans. The first study examined whether whole body and local heat stress increases both skin and

muscle blood flow. The second study examined whether combined hypohydration and hyperthermia compromises leg muscle and skin blood flow and cardiac output. Both studies were completed at the Centre for Sports Medicine and Human Performance, Brunel University between February 2008 and December 2009.

The present thesis begins with a review of literature (Chapter 2) which provides an overview of the regulation of limb muscle and skin blood flow. The literature review introduces what is known about limb muscle and skin blood flow and cardiac output with heat stress and combined hypohydration and hyperthermia whilst also identifying what is currently unknown. This chapter is followed by an overview of the general methods used (Chapter 3) whilst Chapters 4 and 5 are the empirical research studies that the present thesis is based upon. Finally, Chapter 6 provides a general discussion of the main findings of this thesis.

CHAPTER 2
Literature Review

2.1. Introduction

Elevations in body temperature and reductions in body water accompanied by a concomitant haemoconcentration are commonly experienced in athletic training and competition. These two conditions can be classified under the umbrella terms of hyperthermia and hypohydration. Hyperthermia and hypohydration represent a significant regulatory challenge to the cardiovascular system (Gonzalez-Alonso, Crandall & Johnson, 2008) and are typically manipulated using different interventions. Classically, elevations in body temperature *per se* are created experimentally by either dressing participants in a suit perfused with hot water (Rowell, et al., 1969b), or immersing them in a warm water bath (Barcroft & Edholm, 1943). In both scenarios, skin temperature is elevated quickly and if heating is maintained the ensuing thermal gradient between the skin and internal core results in an eventual increase in internal body temperature (Rowell, et al., 1969b). However, the degree to which skin and core temperatures are elevated can vary depending on the efficiency of the heating method and both the magnitude and length of exposure to heat stress.

Hypohydration and concomitant reductions in blood volume are commonly achieved through exercise in a hot, compensable environment. In such an environment evaporative cooling is favoured by the use of a fan while fluid replacement is restricted resulting in a loss of body water. This is the process of becoming dehydrated *ergo* hypohydration develops. When fluid replenishment is adequate to replace the fluid lost through sweating, although core temperatures increase they remain stable at approximately 38°C. However if hypohydration develops, the dissipation of heat formed in the metabolically active muscles is reduced. The impaired heat dissipation is at least in part a consequence of a reduced skin blood flow and blunted sweating rate that classically accompanies hypohydration (Nadel, Fortney & Wenger, 1980; Sawka, Knowlton & Critz, 1979). This intervention classically results in the combination of hypohydration and hyperthermia (Montain & Coyle, 1992b). Other methods of inducing hypohydration include sauna exposure and diuretic use, which generally result in a similar cardiovascular strain (Sawka, et al., 1979).

The magnitude of skin temperature increase is generally greater during heat stress than exercise-induced hypohydration. This difference is physiologically important

because the magnitude of skin temperature increase can influence the degree to which leg blood flow and cardiac output increase at rest and during exercise (Barcroft & Edholm, 1943). Nevertheless, it is well established that the strain imposed by heat stress and combined hypohydration and hyperthermia pose a significant cardiovascular challenge particularly during intense whole body exercise (González-Alonso & Calbet, 2003; González-Alonso, et al., 1998).

The cardiovascular strain produced during heat stress is primarily due to the profound increase in thermoregulatory drive for blood flow. This augmented thermoregulatory drive is associated with a redistribution of blood flow away from central regions to the peripheral vasculature throughout the whole body (Crandall, et al., 2008; Rowell, Detry, Profant & Wyss, 1971). Blood flow redistribution is accompanied by a large increase in cardiac output at rest and during light exercise (Rowell, et al., 1966). At rest the increase in cardiac output occurs despite decreases in venous return to the heart (Crandall, et al., 2008). With combined hypohydration and hyperthermia, a significant reduction in blood volume compounds the challenge of promoting heat dissipation in humans exercising in hot environments. In this regard, a major problem concerns the regulation of limb and systemic haemodynamics whilst satisfying the drive for thermoregulation with a lower blood volume (González-Alonso, et al., 2000). These challenges are further compounded during whole body intense exercise owing to the markedly increased metabolic drive for blood flow in the active skeletal muscle. This results in a competition for the available blood flow between skeletal muscle and the skin (González-Alonso, Crandall & Johnson, 2008; Rowell, 1974). However, in resting humans it is unclear whether skeletal muscle vasodilation contributes to any of the increase in limb blood flow with heat stress. It is also unclear whether hypohydration causes reductions in muscle blood flow in resting humans. Furthermore, it is unknown how limb muscle and skin blood flow are affected with heat stress and hypohydration and hyperthermia in mildly exercising humans.

2.2. Control of limb muscle and skin blood flow in humans

In humans, the adequate control of skeletal muscle and skin blood flow is crucial for the maintenance of muscle metabolism and the appropriate regulation of body temperature at rest and during exercise. Thermoregulatory control is particularly crucial in humans for the maintenance of homeostasis in conditions of environmental heat stress and exercise. During exercise the peripheral demands for blood flow are normally met by proportional adjustments in the systemic circulation through increases in stroke volume, heart rate and blood flow pressure (Higginbotham, et al., 1986). Similar adjustments occur with heat stress-induced elevations in body core and skin temperatures (Rowell, et al., 1969a). However it is the combination of heat stress and exercise that can most likely lead to a compromised cardiovascular regulation. Skeletal muscle and skin blood flow is controlled by various mechanisms which will be discussed throughout this section.

2.2.1. Regulation of muscle blood flow

Gaskell's seminal discovery more than 120 years ago of an augmented skeletal muscle blood flow in response to contractions set the stage for an intense search for the precise mechanisms underpinning muscle blood flow regulation (Gaskell, 1877). Since this discovery a large body of research has focused upon factors that could mediate contraction or exercise-induced hyperaemia. This focus initially included the identification of potential metabolites involved in the regulation of vasodilation (Lewis and Grant, 1925 cited in Rowell, 2004). However more recently research has focused upon the identification of an obligatory role of the endothelium for normal vascular functioning (Furchgott & Zawadzki, 1980).

Many potential avenues have been investigated in the search for regulating factors of vasodilation which have led to the theory that skeletal muscle blood flow is essentially controlled through a combination of neural, hormonal and local mechanisms (Rowell, 1993). These different mechanisms are ultimately collated and regulated by the central nervous system via various feedback and feed forward pathways (Rowell, 1993). To date, however, central mechanisms have only received minimal support (Joyner & Halliwill, 2000a, 2000b). This is due to the results of research that directly examined the influence of neural blockade upon exercise hyperaemia showing minimal direct effects upon human and animal limb blood flow (Buckwalter & Clifford, 1999; Buckwalter, Ruble, Mueller & Clifford,

1998; Joyner, Nauss, Warner & Warner, 1992; Williams, Mudd & Lind, 1985). In this light, support for a role of acetylcholine spill over from motor nerves in vascular control is highly debatable (Buckwalter, et al., 1998; Edwards, et al., 1993; Shoemaker, Halliwill, Hughson & Joyner, 1997). Furthermore, the role of hormonal factors appears to be negligible (Delp & Laughlin, 1998) while the local influence of myogenic factors (which are intrinsic to the smooth muscle blood vessels, particularly in small arteries and arterioles, and are responsive to changes in blood pressure) have also been found to have minimal or no effect upon skeletal muscle hyperaemia (Delp & Laughlin, 1998; Lind & Williams, 1979; Sheriff, Rowell & Scher, 1993; Tschakovsky, Shoemaker & Hughson, 1996). However, a well-established principle is that muscle blood flow is tightly regulated to match oxygen supply to the metabolic demand (Andersen & Saltin, 1985; Delp & Laughlin, 1998; González-Alonso, Olsen & Saltin, 2002; Koskolou, Calbet, Radegran & Roach, 1997; Mortensen, Damsgaard, Dawson, Secher & Gonzalez-Alonso, 2008; Rowell, Saltin, Kiens & Christensen, 1986). In an attempt to explain this principle, one theory that has received substantial attention in recent years postulates a primary role of the erythrocyte as an oxygen sensor and blood flow controller.

More specifically, the erythrocyte has been proposed to play a key role in the regulation of the matching of oxygen delivery to the muscle metabolic demand. This regulation has been proposed to occur through the release of vasodilatory substances in proportion to the level of haemoglobin deoxygenation (Ellsworth, Forrester, Ellis & Dietrich, 1995; González-Alonso, et al., 2002; Stamler, et al., 1997). This concept is supported by studies outlining that: 1) alterations in blood oxygen content are inversely related to exercising muscle blood flow (González-Alonso, et al., 2002; González-Alonso, Richardson & Saltin, 2001; Roach, Koskolou, Calbet & Saltin, 1999; Rowell, et al., 1986; Welch, Bonde-Petersen, Graham, Klausen & Secher, 1977), 2) a reduced blood oxygen content increases the release of vasodilatory substances including nitric oxide (NO) and ATP (Bergfeld & Forrester, 1992; Ellsworth, et al., 1995; Jia, Bonaventura, Bonaventura & Stamler, 1996; McMahon, et al., 2002; Stamler, et al., 1997), 3) muscle blood flow, sympathetic vasoconstrictor activity and plasma ATP are more closely related to arterial oxygen content than oxygen dissolved in plasma (González-Alonso, et al., 2002; González-Alonso, et al., 2001; Hanada, Sander & Gonzalez-Alonso, 2003; Jagger, Bateman, Ellsworth & Ellis, 2001) and 4) blood flow supply

and demand is tightly linked to the oxygenation state of haemoglobin (González-Alonso, Mortensen, Dawson, Secher & Damsgaard, 2006).

While it has been shown that an elevated sympathetic vasoconstrictor activity can be negated during exercise in a manner termed functional sympatholysis (Remensnyder, Mitchell & Sarnoff, 1962), it was only recently discovered that this phenomenon could involve the sympatholytic effects of adenosine triphosphate (ATP) (Kirby, Voyles, Carlson & Dinunno, 2008; Rosenmeier, Hansen & Gonzalez-Alonso, 2004). Thus, the release and action of the many different vasodilator substances, including intravascular ATP, are crucial for regulation of blood flow to the exercising muscles.

ATP is released from the erythrocyte in proportion to oxygen offloading from haemoglobin molecules (Ellsworth, 2004). Upon its release, ATP binds to purinergic P_{2y} receptors located on the vascular endothelium (Ralevic & Burnstock, 1998) and also smooth muscle cells in the skeletal muscle (Mortensen, Nyberg, Thaning, Saltin & Hellsten, 2009). In turn purinergic receptor activation triggers the release of endothelial NO, endothelium-derived hyperpolarizing factor (EDHF), and/or prostaglandins into the blood stream (Huang, et al., 2001; Jia, et al., 1996; Messina, Weiner & Kaley, 1977; Mortensen, Gonzalez-Alonso, Damsgaard, Saltin & Hellsten, 2007; Mortensen, et al., 2009; Rubanyi, Romero & Vanhoutte, 1986; Stamler, et al., 1997). Furthermore ATP (Burnstock, 1999) and endothelial derived NO (Green, et al., 2005; Green, Maiorana, O'Driscoll & Taylor, 2004; Pohl, Holtz, Busse & Bassenge, 1986) can also be released in response to an elevated shear stress. The release of these substances results in an upstream vasodilator response within the smooth muscle tissue of the vessel and ultimately an increase in blood flow (Allen & Piantadosi, 2006; Ellsworth, et al., 1995; Gladwin, et al., 2000; González-Alonso, et al., 2006; Huang, et al., 2001; Mortensen, et al., 2007; Singel & Stamler, 2005). Whether skeletal muscle blood flow is elevated during heat stress or not is subject to debate. If vasodilatation does occur in the skeletal muscle in response to heat stress, the governing regulatory mechanisms are unknown. Despite the implications for the role of the erythrocyte in skeletal muscle blood flow, it is unknown how skeletal muscle blood flow is affected by combined hypovolaemia and haemoconcentration which accompany hypohydration.

2.2.2. Regulation of skin blood flow

Our knowledge and understanding of the regulation of human skin blood flow is generally based upon studies measuring skin or whole limb blood flow in the resting forearm. Whether skin blood flow responses and regulation in active limbs differ from that of the resting limbs is presently unknown. Generally, skin blood flow is thought to be influenced by thermoregulatory and non-thermoregulatory factors which are controlled by both active vasoconstrictor and active vasodilator systems (Johnson & Proppe, 1996).

Thermoregulatory factors influencing skin blood flow include elevations in both skin and core body temperatures which act via augmentation of active vasodilator activity (Shibasaki, Rasmussen, Secher & Crandall, 2009). In response to such elevations in temperature, approximately 80% of the cutaneous vasodilatory response is due to the active vasodilator system (Charkoudian, 2003; Johnson & Proppe, 1996). Approximately 30% of the elevated vasodilatory response to increases in temperature is governed by an augmented NO release (Kellogg, Crandall, Liu, Charkoudian & Johnson, 1998; Shastry, Dietz, Halliwill, Reed & Joyner, 1998). While elevations in body temperature are associated with cutaneous vasodilation it is also important to consider the effects of elevations in core and regional body temperatures. Increases in local limb temperatures *per se* are associated with a progressive increase in skin blood flow (Barcroft & Edholm, 1943; Johnson, Brengelmann & Rowell, 1976), while increases in core temperature *per se* are also influential (Wyss, Brengelmann, Johnson, Rowell & Niederberger, 1974). Furthermore it has recently been shown that in situations where body temperature becomes elevated, brain temperature and central command contribute to the regulation of cutaneous circulation (Shibasaki, Secher, Johnson & Crandall, 2005).

Elevations in skin blood flow are more sensitive to elevations in skin temperature *per se* when basal skin temperature is low. The removal of the augmented vasoconstrictor drives associated with cold skin might be a mechanism that helps mediate this phenomenon (Johnson, 1986; Shepherd, 1963). Furthermore, an elevated skin temperature is of greater importance to cutaneous vasodilation when core temperatures are already elevated above control. This is evident during exercise when internal temperature is augmented in contrast to imposing a

thermal stress upon conditions of normothermic rest (Johnson, 1977; Johnson & Park, 1979; Wyss, et al., 1974; Wyss, Brengelmann, Johnson, Rowell & Silverstein, 1975). Increases in local skin temperature induce a neurally mediated vasodilator response in the skin vasculature (Stephens, Charkoudian, Benevento, Johnson & Saumet, 2001). This vasodilator response appears to be governed by the release of calcitonin gene-related peptide, substance P and neurokinin A release (Holzer, 1998), triggered via heat sensitive nerve receptors (Caterina, et al., 1997; Xu, et al., 2002), as well as the release of NO (Kellogg, Liu, Kosiba & O'Donnell, 1999).

Non-thermoregulatory factors involved in the regulation of skin blood flow include the baro, mechano, and metabo reflexes as well as central command (Shibasaki, et al., 2005). Both arterial (Beiser, Zelis, Epstein, Mason & Braunwald, 1970) and cardiopulmonary baroreceptors (Abboud, Eckberg, Johannsen & Mark, 1979) influence the control of skin blood flow leading to increases in cutaneous vasoconstriction when arterial and/or central venous pressure declines (Johnson, 1986). This was evidenced by reductions in skin blood flow during lower body negative pressure and orthostasis in normothermic subjects (Beiser, et al., 1970; McNamara, Sikorski & Clavin, 1969; Rowell, 1974) which persisted despite a significant heat stress (Crossley, Greenfield, Plassaras & Stephens, 1966; Johnson, et al., 1976; Lind, Leithead & McNicol, 1968; Mosley, 1969; Stephenson, Wenger, O'Donovan & Nadel, 1984).

During exercise, studies have shown evidence of an initial increase in cutaneous vasoconstrictor activity and thus a reduced skin blood flow (Bevegard & Shepherd, 1966; Hirata, Nagasaka, Hirai, Hirashita & Takahata, 1983; Johnson, 1992; Kenney & Johnson, 1992). This effect is greater during combined heat stress and exercise (González-Alonso, et al., 2008; Johnson, 1986). The initial cutaneous vasoconstrictor response to exercise is abolished in a cold environment (Johnson, 1979; Wenger, Roberts, Nadel & Stolwijk, 1975; Wenger, Roberts, Stolwijk & Nadel, 1975) possibly because baseline skin vasodilator activity is minimal (Johnson, 1986). However during prolonged exercise internal body temperature becomes elevated and skin blood flow increases (Johnson, 1977, 1986; Johnson & Park, 1982; Kamon & Belding, 1969; Shaffrath & Adams, 1984). This well researched phenomenon describes the delayed threshold of cutaneous

vasodilation relative to core temperature evidenced during exercise compared to rest (González-Alonso, et al., 2008; Johnson, 1992; Kellogg, Johnson & Kosiba, 1991; Kenney & Johnson, 1992; Smolander, Saalo & Korhonen, 1991).

The delayed threshold of increases in skin blood flow during exercise is common to both hyperthermic and normothermic conditions (Kellogg, Johnson & Kosiba, 1990) and is thought to be due to the inhibition of the active vasodilator system (Kellogg, et al., 1991). However, this threshold can be altered by athletic training and heat acclimation (Roberts, Wenger, Stolwijk & Nadel, 1977), circadian rhythms (Aoki, Stephens & Johnson, 2001) and, in females, hormonal levels (Brooks, et al., 1997). During submaximal exercise in normothermic conditions this phenomenon serves to limit skin blood flow to ~50% compared to the resting value which is reached at a core temperature of approximately 38°C (Bregelmann, Johnson, Hermansen & Rowell, 1977; Gonzalez-Alonso, et al., 2008; González-Alonso, et al., 1999c).

While it is known that elevations in temperature are associated with an increased skin blood flow, reductions in blood volume are associated with decrements in skin blood flow during exercise (Nadel, et al., 1980). It is possible that in reducing cutaneous blood flow, further reductions in central blood volume and venous pressure that occur with heat stress and combined hypohydration and hyperthermia are limited (Nadel, et al., 1980). However, in resting humans it is unknown how skin blood flow is affected by the combination of hypohydration and hyperthermia. Furthermore, in classical studies, the measurement of skin blood flow during exercise has been made in the forearm, which declines during leg exercise (Bevegard & Shepherd, 1966; Hirata, et al., 1983; Johnson & Park, 1982). Therefore it is unclear whether skin blood flow is compromised during exercise, heat stress and/or combined hypohydration and hyperthermia when skin blood flow is measured in the exercising limb.

Summary

In general, the literature clearly demonstrates that skin blood flow increases with elevations in body temperature. Recent evidence, which is discussed below in further detail, suggests that vasodilation may also occur in the skeletal muscle (Abraham, Leftheriotis, Desvaux, Saumet & Saumet, 1994; Ogura, Takayasu &

Dacey, 1991; Unthank, 1992). This phenomenon remains unclear. When hyperthermia is combined with reductions in blood volume the effect upon cutaneous vasodilation in resting humans is also unknown. Finally, while it is clear that the control of muscle blood flow has been shown to be sensitive to changes in blood oxygen content and blood volume (González-Alonso, et al., 2000; González-Alonso, et al., 2006) this has never been examined in hypohydrated and hyperthermic resting and mildly exercising humans.

2.3. Limb and systemic haemodynamics at rest and during exercise with heat stress and hypohydration and hyperthermia.

In conditions of heat stress and combined hypohydration and hyperthermia, both leg blood flow and cardiac output are altered as a result of centrally and locally mediated limitations to blood flow. These alterations in blood flow are distinct between conditions of heat stress and combined hypohydration and hyperthermia and manifest in changes in skeletal muscle and skin blood flow. These issues are discussed in the forthcoming sections and areas where there is currently no or little scientific knowledge are identified.

2.3.1. Limb and Systemic Haemodynamics

In resting humans, whole body heat stress is accompanied by an increased thermoregulatory drive which is associated with an augmented limb blood flow (Abraham, et al., 1994; Barcroft, Bonnar & Edholm, 1947; Detry, Brengelmann, Rowell & Wyss, 1972; Edholm, Fox & Macpherson, 1956; Johnson, et al., 1976; Roddie, Shepherd & Whelan, 1956; Rowell, et al., 1969a; Wenger, Bailey, Roberts & Nadel, 1985) and cardiac output (\dot{Q}) (Rowell, 1974; Rowell, et al., 1969a). During whole body exercise and heat stress the increases in leg blood flow and cardiac output observed at rest are either attenuated (Nadel, Cafarelli, Roberts & Wenger, 1979; Nielsen, et al., 1990; Savard, et al., 1988; Smolander & Louhevaara, 1992) or abolished resulting in a reduced blood flow (González-Alonso & Calbet, 2003). Furthermore, while previous studies suggest that limb blood flow increases with isolated limb heat stress at rest (Barcroft & Edholm, 1943; Barcroft & Edholm, 1946; Johnson, et al., 1976) during exercise the findings are somewhat equivocal (Ferguson, et al., 2006; Williams & Lind, 1979). However the effect of local limb heat stress and whole body skin temperature elevations upon cardiac output and heat stress exercise hyperaemia is currently unknown. While the effects of whole body heat stress upon exercise hyperaemia have been measured before (Savard, et al., 1988), the potentially confounding effects of hypohydration were not controlled for. As such, it is unknown how whole body heat

stress influences leg and systemic haemodynamics during small muscle mass exercise.

Increases in leg blood flow and cardiac output during heat stress are due to either a direct or indirect effect of temperature upon peripheral vasodilation throughout the whole body. This is evidenced by the elevations in limb and systemic vascular conductance while whole body oxygen consumption and systemic a-vO₂ differences remained unaltered (Rowell, et al., 1969a). In line with increases in vascular conductance, blood flow is redistributed away from central organs and tissues to help meet this demand (Rowell, et al., 1966). Using a variety of techniques including *p*-aminohippurate clearance, indocyanine green dye clearance, electrical impedance and more recently the gamma camera imaging of technetium-99^m labelled red blood cells it has been established that blood flow through renal, hepatic, splanchnic, thoracic and visceral regions declines during heat stress (Cai, Jenstrup, Ide, Perko & Secher, 2000; Crandall, et al., 2008; Minson, Wladkowski, Cardell, Pawelczyk & Kenney, 1998; Radigan & Robinson, 1949; Rowell, Blackmon, Martin, Mazzarella & Bruce, 1965; Rowell, Brengelmann, Blackmon & Murray, 1970; Rowell, et al., 1971; Rowell, et al., 1966). The redistribution of blood flow away from these regions is due to an elevated vascular resistance that is due to an augmented sympathetic activity (Minson, et al., 1998; Rowell, 1974; Rowell, et al., 1971). Blood flow redistribution is estimated to account for ~1 l·min⁻¹ of the increase in whole body skin blood flow during whole body heat stress (Minson, et al., 1998) while increases in cardiac output are widely believed to be exclusively confined to the cutaneous circulatory system (Johnson, et al., 1976).

The combined effects of hypohydration and hyperthermia upon leg blood flow and cardiac output have received little attention in resting humans despite their deleterious effects upon the ability to exercise in hot environments (Adolph, 1947; Adolph & Dill, 1938; Pitts, Johnson & Consolazio, 1944). In resting humans the available literature suggests that \dot{Q} is unchanged whereas limb blood flow is either increased (Fan, et al., 2008; Lynn, Minson & Halliwill, 2009) or unaltered (Horstman & Horvath, 1972; Kenney, et al., 1990). These discrepancies could be due to the varying experimental methodologies, i.e., whole body uncompensable heat stress (Fan, et al., 2008) *versus* environmental heat exposure (Kenney, et al.,

1990). In light of these discrepancies, the effects of hypohydration and hyperthermia upon metabolism across the human leg and systemic circulations remain unclear. However, during intense whole body exercise with combined hypohydration and hyperthermia it is well documented that both leg blood flow and cardiac output are compromised (González-Alonso, et al., 1998; González-Alonso, et al., 1997; González-Alonso, Mora-Rodriguez & Coyle, 1999b; González-Alonso, et al., 2000; González-Alonso, et al., 1999c; Montain & Coyle, 1992b; Nadel, et al., 1980; Saltin, 1964; Sawka, et al., 1979). However, the mechanisms underlying these deleterious responses have not been directly assessed which will be discussed in further detail later.

2.3.2. Central and local limitations to leg blood flow and cardiac output

It was first identified in the 1920's that whole body oxygen uptake has an upper limit in humans exercising at high intensities until exhaustion (Hill, Long & Lupton, 1924). This is classically referred to as $\dot{V}O_2$ Max (Hill, et al., 1924). Subsequent to this discovery it has been argued that either central (Mitchell, Sproule & Chapman, 1958) or local (Holloszy, 1967) factors were responsible for this upper limit in oxygen uptake. Ensuing research demonstrated that after superimposing arm onto leg exercise, i.e., whole body exercise, the maximal pumping capacity of the heart was unable to optimally perfuse the working skeletal muscle (Secher, Clausen, Klausen, Noer & Trap-Jensen, 1977). In contrast to whole body exercise, it has been shown that when the exercising muscle is confined to an isolated group, i.e., the quadriceps, muscle blood flow can rise to a much higher level (Andersen & Saltin, 1985; Richardson, et al., 1993). This indicates that, centrally, blood flow is limited during whole body exercise. In this regard, it is unclear whether the aforementioned limitations to blood flow with heat stress and combined hypohydration and hyperthermia reported during exercise are due to elevations in temperature and/or hypovolaemia or whether they are exacerbated as a result of the limitations to blood flow accompanying whole body exercise.

The attenuation and reduction in exercise hyperaemia during heat stress and combined hypohydration and hyperthermia respectively are better understood when considered in terms of local and central limitations to blood flow. At the level of the leg, blood flow does not appear to be reduced or attenuated by local factors

during heat stress or combined hypohydration and hyperthermia. In this regard, vascular conductance in the exercising limb was maintained with hypohydration and hyperthermia despite elevations in sympathetic vasoconstrictor activity (Charkoudian, Eisenach, Joyner, Roberts & Wick, 2005) and reductions in cardiac output (González-Alonso, et al., 1998). Despite increases in muscle sympathetic nerve activity, whole body and local limb heat stresses are associated with either an increased or maintained leg blood flow, cardiac output and vascular conductance at rest and during exercise respectively (Bini, Hagbarth, Hynninen & Wallin, 1980; Niimi, et al., 1997; Ray & Gracey, 1997). Therefore, it appears that at the level of the human leg a net limb vasodilation prevails during heat stress and combined hypohydration and hyperthermia. This net limb vasodilation occurs in a manner resembling functional sympatholysis, which describes that sympathetic vasoconstrictor activity can be negated in the active muscles (Hanada, et al., 2003; Remensnyder, et al., 1962; Rosenmeier, et al., 2004). However, in contrast to heat stress, it appears that at the level of the systemic circulation the local drive for blood flow cannot be maintained during combined hypohydration and hyperthermia (González-Alonso, et al., 1998). This suggests that centrally, the systemic circulation limits blood flow, yet this has only been shown once and thus further investigation into the effects of hypohydration and hyperthermia upon local muscle and skin blood flow is warranted.

During exercise with combined hypohydration and hyperthermia blood flow to the exercising limb becomes compromised despite a maintained leg vascular conductance (González-Alonso, et al., 1998). Consequently, this finding suggests that the maintenance of blood flow through the exercising limb is largely dependent upon the maintenance of \dot{Q} rather than a reduction in local drive for blood flow. However \dot{Q} declines during exercise with hypohydration and hyperthermia owing to large reductions in stroke volume (González-Alonso, 1998; González-Alonso, Mora-Rodriguez, Below & Coyle, 1995; Montain & Coyle, 1992b). Individually both reductions in blood volume and increases in heart rate that occur with hypohydration and hyperthermia are associated with reductions in stroke volume (González-Alonso, et al., 2000).

During exercise in the cold, when hyperthermia was prevented, reductions in blood volume were solely accountable for the small decline in stroke volume (González-

Alonso *et al.*, 1997). Reductions in blood volume were responsible for approximately one-half of the decline in stroke volume during exercise in the heat with combined hypohydration and hyperthermia but an artificially expanded blood volume (González-Alonso, *et al.*, 1997; Montain & Coyle, 1992a). In separation, the reductions in stroke volume with both hypohydration and hyperthermia are compensated for by elevations in heart rate, thus \dot{Q} is largely maintained (González-Alonso, *et al.*, 1997; González-Alonso, *et al.*, 2000). In contrast to their separated effects, in combination, hypohydration and hyperthermia, cause \dot{Q} to become compromised during exercise (González-Alonso, *et al.*, 1997). Thus in combination the isolated deleterious effects of hypohydration and hyperthermia upon systemic haemodynamics become exacerbated (González-Alonso, 1998; González-Alonso, *et al.*, 1997). Therefore reductions in \dot{Q} with combined hypohydration and hyperthermia are likely due to the combination of hypovolaemia mediated reductions in stroke volume and hyperthermia induced increases in heart rate (González-Alonso, *et al.*, 2000).

The reductions in stroke volume during exercise with combined hypohydration and hyperthermia cannot be explained by a large skin blood flow. This was suggested by a small decline in stroke volume during sub-maximal exercise in a cold environment where skin blood flow was minimal (González-Alonso, *et al.*, 2000). Although stroke volume was significantly compromised during exercise with combined hypohydration and hyperthermia, skin blood flow was also compromised despite significant hyperthermia (González-Alonso, *et al.*, 1998). This attenuation in skin blood flow during exercise with combined hypohydration and hyperthermia is perhaps due to the baroreceptors sensing changes in central blood volume and mean arterial pressure, thus serving to defend the systemic circulation (González-Alonso, *et al.*, 1995). Despite this the reductions in stroke volume occurring with combined hypohydration and hyperthermia have been found without any change in skin blood flow.

At rest and during exercise with heat stress, stroke volume and cardiac output are either maintained or increased (Rowell, 1974; Rowell, *et al.*, 1969a; Savard, *et al.*, 1988). It has more recently been described that this maintenance in cardiac output at rest is due to an increased cardiac contractility as evident in an elevated ejection fraction (Crandall, *et al.*, 2008). The maintenance of stroke volume during

heat stress occurs despite reductions in central blood volume which accompany blood flow redistribution, peripheral vasodilation and decreases in mean arterial and central venous pressures (Crandall, 2008; Rowell, et al., 1970). Therefore when stroke volume is maintained and heart rate becomes elevated, \dot{Q} increases at rest with heat stress. Conversely, during maximal exercise with heat stress, leg blood flow and cardiac output decline due to large increases in heart rate that compromise stroke volume, possibly due to a reduction cardiac filling time (González-Alonso & Calbet, 2003). In light of the literature discussed here, the level of heart rate response to either heat stress or hypohydration and hyperthermia can largely determine whether increases in vascular conductance owing to an augmented thermoregulatory drive can be met with elevations in blood flow.

However, the effects of heart rate upon blood flow with heat stress and combined hypohydration and hyperthermia at rest are exacerbated during exercise as heart rate increases accompanying the elevated metabolic demand. Sympathetic vasoconstrictor activity increases as a function of both exercise intensity and with large compared to small muscle mass exercise modes (Richardson, et al., 1999; Richardson, Kennedy, Knight & Wagner, 1995; Richter, Kiens, Hargreaves & Kjaer, 1992; Rosenmeier, et al., 2004; Saito, Tsukanaka, Yanagihara & Mano, 1993; Savard, et al., 1989). Hence with whole body exercise protocols the sympathetic vasoconstrictor stimulus and therefore the opposition to local and systemic vasodilation and drive to increase heart rate is augmented (Pawelczyk, Hanel, Pawelczyk, Warberg & Secher, 1992; Richardson, et al., 1999). Thus it is possible that the elevations in heart rate that accompany whole body exercise, at least in part, attenuate the heat stress mediated increase in cardiac output at rest. Similarly elevations in heart rate could contribute the tachycardia-mediated reductions in cardiac output with hypohydration and hyperthermia.

In the exercising muscle, the metaboreflex and baroreflex may limit hyperaemia during whole body exercise in order to protect systemic arterial blood pressure (Gonzalez-Alonso, et al., 2008; Rowell, 2004). A small muscle mass exercise model could reduce the influence of centrally mediated limitations to leg blood flow and cardiac output. These limitations to blood flow could be reduced due to the availability of a large cardiovascular reserve during exercise (Andersen & Saltin,

1985; González-Alonso, et al., 2008; Mortensen, et al., 2008). While this has been investigated previously during heat stress (Savard, et al., 1988), the potentially confounding effects of hypohydration upon blood flow were not explicitly controlled for and could have influenced the results. As such a lower local and systemic vasoconstrictor drive may help explain why limb blood flow increased during small muscle mass exercise with isolated forearm heating (Williams & Lind, 1979).

In humans it has been documented that heat stress and combined hypohydration and hyperthermia can attenuate and/or reduce the hyperaemic response to exercise. However, it is unknown if this phenomenon is due to the potential development and confounding influences of hypohydration and/or the reflexes which underpin the circulatory limitations associated with whole body exercise. It is also unknown how limb muscle and skin blood flow are influenced under conditions of low cardiovascular strain with heat stress and hypohydration and hyperthermia.

2.3.3. Muscle and skin blood flow with heat stress and hypohydration and hyperthermia

During passive heat stress cutaneous blood flow increases in response to elevations in skin (Ahmad, 1956; Barcroft & Edholm, 1943; Barcroft & Edholm, 1946) and core temperatures (Johnson, et al., 1976; Wyss, et al., 1974; Wyss, et al., 1975). Despite the well documented cutaneous vascular response, it is unclear whether vasodilation within the skeletal muscle vasculature contributes to the well-documented increase in limb blood flow observed during heat stress.

Consequently, the degree to which heat stress mediated skeletal muscle vasodilation contributes to combined heat stress and exercise hyperaemia is also unclear. Early studies investigating the partition of limb blood flow between skin and skeletal muscle compartments during heat stress reported equivocal findings (Barcroft, et al., 1947; Barcroft & Edholm, 1943; Barcroft & Edholm, 1946; Roddie, et al., 1956). However, later studies suggested that any elevations in cardiac output were confined entirely to the cutaneous vasculature (Detry, et al., 1972; Johnson, et al., 1976).

In an attempt to determine whether heat stress induces vasodilation within the skeletal muscle, a variety of experimental approaches have been used. These approaches included the assessment of whole limb blood flow during the abolition of skin blood flow using adrenaline iontophoresis (Barcroft, et al., 1947; Edholm, et al., 1956), the measurement of skeletal muscle blood flow using isotope clearance methods (Detry, et al., 1972; Johnson, et al., 1976) and/or the assessment of deep and superficial forearm venous blood oxygen saturation (Detry, et al., 1972; Roddie, et al., 1956). At the time of investigation these were novel techniques, but it is now recognised that the use of isotope clearance techniques and adrenaline iontophoresis are potentially unreliable as an index of muscle blood flow (Heymann, Payne, Hoffman & Rudolph, 1977; Rowell, 1993). Specifically the limitations to the isotope clearance method include the exchange of isotopes between arterioles and veins and also the solubility of isotopes in different tissues within skeletal muscle, making derived values too low or the methodology insensitive to accurately reflect muscle blood flow (Rowell, 1993). A general concern in measuring changes in skeletal muscle blood flow in the forearm is that the absolute changes in flow are small compared to the leg where muscle mass is larger.

In the most recent studies to investigate muscle blood flow during heat stress, despite elevations in local and whole body temperatures, forearm muscle blood flow was unchanged (Detry, et al., 1972; Johnson, et al., 1976). Accordingly, the classic dogma was shaped that heat stress induced elevations in limb blood flow and cardiac output were confined entirely to the cutaneous circulation. This perception was shaped further by research which estimated maximal skin blood flow to be approximately $6-8 \text{ l}\cdot\text{min}^{-1}$, based upon measures of \dot{Q} and visceral blood flow during passive heat stress (Detry, et al., 1972; Minson, et al., 1998; Rowell, 1974, 1986; Rowell, et al., 1969a). However, more recent evidence suggests that increases in skin blood flow cannot account for all of the increase in whole limb blood flow with heat stress (Abraham, et al., 1994). In this regard it has also been speculated that increases in cardiac output occurring with whole body heat stress are too large to be solely accommodated by the cutaneous circulation (Greenfield, 1963; Hertzman, 1959; Rowell, 1974; Rowell, et al., 1969a). In light of these discrepancies it remains unknown whether heat stress induces vasodilation in both skeletal muscle and skin.

Despite early research indicating that muscle blood flow was not elevated with heat stress, more recently it has been shown that skin blood flow, as measured in the saphenous vein, cannot account for the entire increase in whole leg blood flow through the femoral vein during heat stress (Abraham, et al., 1994). The disparity in findings could be due to the differing methodologies used. Prior to the work of Abraham and colleagues, blood flow through the saphenous vein had never been measured during heat stress. These more recent findings raise the possibility that heat stress may be associated with increases in muscle as well as skin blood flow. Research conducted on rat arterioles, both *in vivo* and *in vitro*, has shown that increases in temperature *per se* are associated with vasodilation (Ogura, et al., 1991; Unthank, 1992). Specifically, *in vitro*, rat arteriole diameter increased following immersion into a hot water bath (Ogura, et al., 1991). *In situ*, rat abdominal small arterioles exhibited vasodilation in direct response to elevations in temperature (Unthank, 1992). These findings are especially pertinent given that the elevations in temperature were similar to local tissue temperatures reported in human experiments where a net limb vasodilation and elevations in blood flow occurred with whole body and isolated limb heating (Barcroft & Edholm, 1943; Barcroft & Edholm, 1946; Johnson, et al., 1976). Thus it is possible that arterioles may vasodilate in direct response to elevations in temperature, and where muscle tissue temperature becomes elevated, vasodilation may occur within the skeletal muscle as well as the skin. In extension of this elevations in local tissue temperature, induced via isolated limb heat stress, are associated with increases in limb blood flow (Johnson, et al., 1976). In this regard it is possible that regulatory pathways exist in the microvasculature linking increases in local temperature and vessel dilatation. However, it is unclear as to the extent elevations in tissue temperature contribute, if at all, to skeletal muscle blood flow regulation. Furthermore, given the aforementioned attenuation in limb blood flow with heat stress exercise it is unknown if increases in local tissue temperature contribute to exercise hyperaemia and the systemic response.

While it is clear that during whole body exercise with hypohydration and hyperthermia there is a large thermoregulatory drive for skin blood flow, cutaneous blood flow declines (González-Alonso, et al., 1998; González-Alonso, et al., 1995). It is thought that this phenomenon occurs to prevent further reductions in central

blood volume and \dot{Q} which can reach $\sim 3\text{-}4 \text{ l min}^{-1}$ (González-Alonso, et al., 1998; González-Alonso, et al., 1995). Muscle blood flow also declines, as indicated by the $\sim 1 \text{ l min}^{-1}$ reduction in leg blood flow and concomitant elevations in leg a- $\dot{V}O_2$ difference but for the most part a maintained leg $\dot{V}O_2$ (González-Alonso, et al., 1998). These reductions in muscle blood flow were reported to occur in response to the fall in cardiac output and therefore perfusion pressure rather than local muscle vasoconstriction as evident in the maintenance of leg vascular conductance (González-Alonso, et al., 1998). However, it is unknown how limb muscle blood flow is affected by the combination of hypohydration and hyperthermia both at rest and during mild exercise in humans where the central limitations to blood flow are reduced.

Despite a lack of investigation the haemoconcentration which accompanies hypohydration and hyperthermia, may influence local blood flow responses. This has been suggested by experiments demonstrating the influence of blood oxygenation, particularly at the level of haemoglobin oxygenation, on limb muscle blood flow regulation (González-Alonso, et al., 2006). Furthermore, in analysis of data obtained from González-Alonso and colleagues (1998), it has been suggested that elevations in arterial oxygen content concomitant to hypohydration and hyperthermia are strongly correlated ($r=0.89$, $p<0.01$) with reductions in blood flow to the exercising limb (Calbet, 2000). While this correlation does not necessarily imply a cause and effect relationship, it may indicate the changes in blood oxygen content are involved in the reductions in blood flow to the exercising limb with combined dehydration and hyperthermia. This is at least possible given the coupling between erythrocyte-derived ATP release into plasma and muscle blood flow during exercise (González-Alonso, et al., 2002). Thus it is possible that hypohydration and hyperthermia, and concomitant haemoconcentration, might alter limb blood flow in part via alterations in intravascular ATP. Hypovolaemia has also been shown to be partly responsible for the reductions in \dot{Q} with combined hypohydration and hyperthermia during exercise (González-Alonso, et al., 2000). As such reductions in blood volume could reduce limb muscle and skin blood flow. However, the influence of blood volume, body temperature and haematological changes upon limb muscle and skin blood flow in resting and mildly exercising humans remains unknown.

2.4. Summary

In resting and exercising humans limb and systemic haemodynamics are influenced by heat stress and combined hypohydration and hyperthermia in a contrasting manner. Whole body heat stress is accompanied by elevations in leg blood flow and cardiac output at rest, which become attenuated or reversed during exercise. Recent evidence suggests that the increases in vascular conductance which accompany elevations in body temperature may be due, at least in part, to increases in local limb temperature *per se*. These increases in local limb temperature may induce local vasodilation in both the skeletal muscle and skin. However, little is known about the responses of leg and skin blood flow and cardiac output to hypohydration and hyperthermia at rest. During whole body exercise, muscle and skin blood flow and cardiac output are reduced with hypohydration and hyperthermia. Leg blood flow is attenuated compared to rest during whole body exercise and heat stress. However, it is unknown how skeletal muscle and skin blood flow are influenced during exercise with hypohydration and hyperthermia and heat stress when the cardiovascular strain is low and centrally and locally mediated signals restricting blood flow are minimised. As such it is unknown how hypovolaemia and haemoconcentration influence local blood flow when combined with hyperthermia and in the presence of a large cardiovascular reserve at rest and during mild exercise. The focus of the present thesis was to investigate the role of heat stress induced elevations in local and whole body temperatures and hypohydration induced alterations in blood volume and haematology combined with hyperthermia upon leg muscle and skin blood flow and cardiac output in resting and mildly exercising humans.

2.5. Aims and Hypotheses

2.5.1 Thesis Aims

The aim of the present thesis was to examine the effects of heat stress and hypohydration and hyperthermia upon leg muscle, skin and systemic haemodynamics at rest and during mild exercise in humans. In extension of this a further aim was to gain a particular insight into the effects of local temperature and changes in blood oxygen content and haemoglobin concentration in the regulation of skeletal muscle blood flow. Therefore two separate studies were completed. The primary aims of each study are outlined below:

Study 1, Aim a: To examine whether heat stress induces vasodilation within skeletal muscle vasculature and thus increases leg muscle blood flow at rest and during mild exercise. *Aim b:* To examine whether isolated increases in limb temperature could account for all the increases in leg blood flow evoked by whole body heat stress. *Aim c:* to gain insight into the role of plasma ATP in heat stress mediated limb vasodilation.

Study 2, Aim a: To examine whether graded hypohydration and hyperthermia impair leg muscle, skin and systemic haemodynamics at rest and during mild exercise. *Aim b:* To determine whether restoring blood volume and blood oxygen and haemoglobin concentrations along with internal body temperature through oral rehydration would restore any alterations in blood flow associated with mild and moderate hypohydration and hyperthermia.

2.5.2. Hypotheses

Study 1

1. *Research hypothesis:* local hyperthermia induces vasodilation in resting and exercising human skeletal muscle, thereby contributing to heat stress and exercise hyperaemia.
2. *Research Hypothesis:* the whole leg and systemic hyperaemic response to heat stress is attenuated during exercise.

3. *Research Hypothesis*: local hyperthermia accounts for a large portion of leg hyperaemia during whole body heat stress.

Study 2

1. *Research Hypothesis*: hypohydration and hyperthermia reduces leg muscle, skin and systemic haemodynamics at rest and during exercise.
2. *Research Hypothesis*: rehydration restores leg muscle, skin and systemic haemodynamics to control levels at rest and during exercise.

CHAPTER 3

General Methodology

3.1 Pre-test procedures

3.1.1. Ethical Approval

Prior to the start of each study, ethical approval was obtained from both the Research Ethics Committees of the School of Sport and Education and Brunel University. All of the procedures employed within studies contained in this thesis conformed to the standards set by the declaration of Helsinki. Please see appendices for letters of ethical approval.

3.1.2. Participants

Participation in the studies comprising this thesis was entirely voluntary. Participants were informed both in writing and verbally about all of the procedures and risks involved in each study. All participants provided both verbal and written consent (Appendix I) on the morning of the study after which they completed a health questionnaire (Appendix II). Subject to successful completion of both documents participants proceeded onto the experimental trial.

3.1.3. Anthropometry

Prior to the start of each experimental trial participant's stature and body mass were recorded using standard procedures. Height was recorded using a stadiometer (SECA model 798, Germany) and recorded to the nearest 1 mm. Body mass was recorded using electronic scales (SECA model 798, Germany) and recorded to the nearest 0.1 kg. Each measurement of body mass was recorded immediately post voiding and in similar conditions to baseline measurements.

3.1.4. Peak power test

To ascertain peak power output participants completed an incremental one-legged maximal knee-extensor exercise test to exhaustion in both studies. This occurred ~7 days prior to the experimental trial. Knee extensor exercise began at 5 W increasing every minute thereafter in 5 W increments. Participants exercised until either volitional fatigue or the examiner observed kicking frequency to have dropped below 60 rpm.

Prior to the hypohydration study participants also completed an incremental peak power test on an electromagnetically braked cycle ergometer (Excalibur; Lode, Groningen, The Netherlands) until volitional fatigue using a RAMP protocol. The rate of increase in work was determined using 'Hansen's Rule' (Hansen, Casaburi, Cooper & Wasserman, 1988) and lasted between 8 and 12 minutes. Oxygen consumption was measured continuously using an online gas analysis system (Quark b², Cosmed, Italy).

3.1.5. Familiarisation

In both studies, prior to knee-extensor peak power tests and experimental trials, participants were familiarised with the custom-built knee-extensor ergometer. Participants completed three bouts of exercise at approximately 20 W, or until the participant was able to start and maintain one-legged kicking on the ergometer at 60 rpm. The purpose of the familiarisation sessions was to minimise the involvement of the gluteal and hamstring muscles during exercise thereby isolating the workload to the knee-extensors.

Study 1 - Heat Stress

Prior to the experimental trial, participants reported to the laboratory on two separate occasions separated by 2 days. Participants cycled at ~150 W on an electro magnetically braked cycle ergometer in a heat chamber at 37 °C and 60% humidity for 60 min. The purpose of these sessions was to mentally prepare the participants for the elevations in temperature that they would experience during heat stress. Fluids were available *ad-libitum* as the purpose of the study was to induce elevations in body temperature via heat stress, not hypohydration.

Study 2 – Combined Hypohydration and Hyperthermia

Prior to the experimental trial participants reported to the laboratory on three separate occasions separated by two days. Participants cycled at 50% of their predetermined peak power output at 36 °C and 60% humidity.

Fluid ingestion was not permitted on the second and third visits. The purpose of these sessions was to familiarise participants with the process of becoming dehydrated and the experiences associated with elevations in body temperature and levels of hypohydration that they would experience during experimental trials.

In both studies, core temperature was monitored throughout and never increased above 39.5 °C in compliance with ethical guidelines. The last visit occurred 2 days prior to the experimental trial.

Prior to all familiarisation visits participants were instructed to drink at least 2 litres of fluid in the preceding day. On all familiarisation visits participants body mass was recorded immediately post-voiding and served as an indication of euhydrated body mass. Upon reporting to the laboratory on the experimental day a lower body mass was taken to be indicative of hypohydration and would have resulted in exclusion from both study 1 and 2.

3.1.6. *Pre experimental ultrasound scanning*

Prior to the experimental day the common femoral artery of the left leg was identified on all participants via ultrasound during the aforementioned familiarisation sessions (Vivid 7 Dimension, GE Medical, Horton, Norway). The purpose of this was to allow a fast and efficient location of the common femoral artery and hence blood flow measurements on experimental days in all conditions.

3.2. Test Procedures

3.2.1. *Leg blood flow (LBF) measurement using ultrasound*

In all studies LBF was measured from the common femoral artery in the left leg at a site 2-3 cm proximal to the bifurcation of the common femoral artery into the profunda femoral artery and superficial femoral artery. LBF was measured using an ultrasound equipped with Doppler mode (Vivid 7 Dimension, GE Medical, Horton, Norway), using a 10MHz linear probe (GE medical systems, UK Fig. 3.0).



Fig. 3.0. The Vivid 7 Ultrasound used to measure leg blood flow.

A 3-lead electrocardiogram, inherent to the ultrasound system was used to identify systolic and diastolic phases of the cardiac cycle and enable appropriate measurements of leg blood flow.

Background

The ultrasound system is able to detect leg blood flow using the Doppler mode that determines the frequency shift in Doppler signals emitted. Sound waves are transmitted, in this case, by a longitudinal probe and are reflected by red blood cells within the blood. The change in signal as the sound waves return is called the Doppler frequency shift (Δf), which tells us the magnitude and direction of the flow. Depending on the direction of the blood flow within a blood vessel the Doppler frequency shift becomes either positive or negative and is used to calculate the velocity of blood flow.

Two-dimensional common femoral artery diameter measurement

Femoral artery vessel diameter was determined after obtaining three 2D images in the longitudinal view at approximately 48 frames per second depending on artery depth at an imaging frequency of 10 MHz. A sample image is presented in Fig. 3.1.

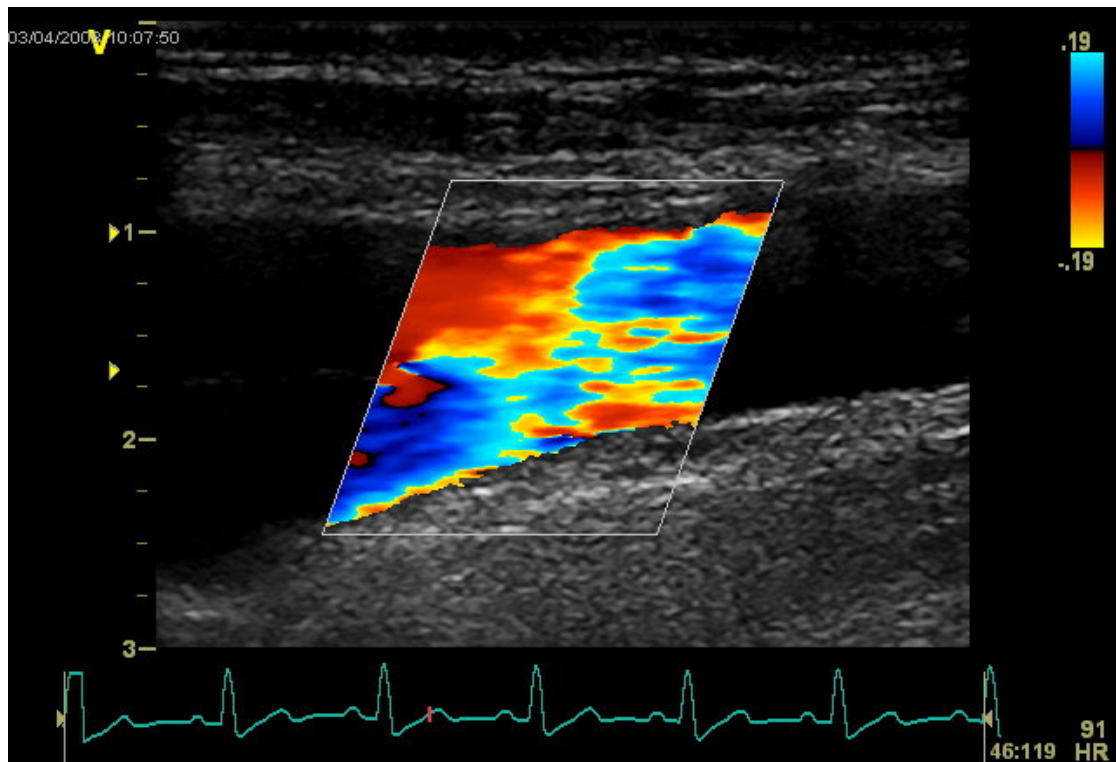


Fig. 3.1. A sample image of the common femoral artery used to calculate vessel diameter and ultimately leg blood flow.

Vessel diameter was calculated 6 times for each condition under a perpendicular insonation angle using measurements obtained from systolic and diastolic phases, as indicated by ECG. Systolic and diastolic vessel diameters accounted for 1/3 and 2/3 of the each vessel diameter calculation, respectively (Rådegran, 1997).

Pulse wave Doppler measurements of common femoral artery blood velocity

Mean blood velocity (V_{mean}) was calculated from an insonation angle that was consistently below 60° (Rådegran, 1997) at a sampling frequency of 4.4 MHz's and approximately 22 frames per second depending on artery depth. The sample volume was positioned in the centre of the femoral artery. This process was aided by real-time 2D imaging of the femoral artery. This is illustrated in Fig. 3.2.

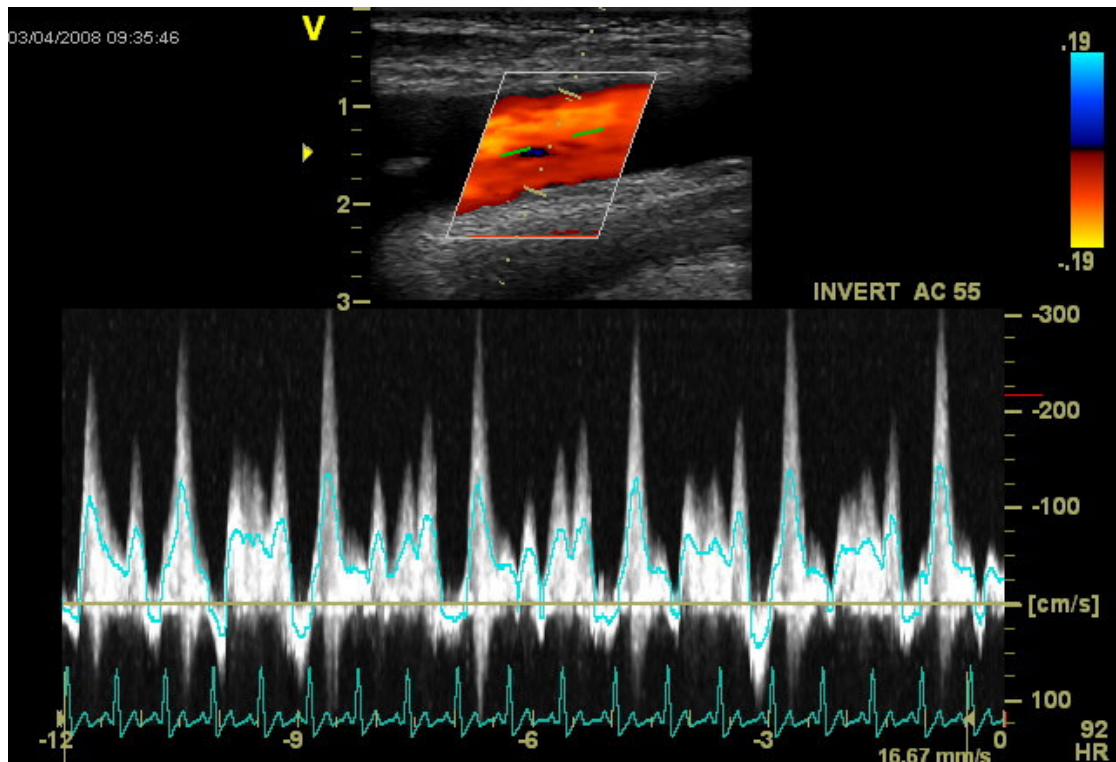


Figure 3.2. An example of a blood velocity profile obtained from the common femoral artery during exercise. Blood velocity is used to calculate leg blood flow at rest during exercise.

Mean blood velocity was calculated from the average net velocity of three separate measurements each lasting 12 s. The contribution of turbulence occurring at the vascular wall to blood flow measurement was reduced by using a low velocity rejection filter.

Blood velocity was determined from the following equation:

$$\Delta f = 2 \times f \times v \times \cos\theta / c$$

Where: f = frequency of sound waves; v = blood flow velocity; θ = insonation angle; and c = velocity of sound in tissue (~ 1540 m/s).

Common femoral artery blood flow measurement

Whole leg blood flow was comprised of vessel diameter and mean blood velocity and was calculated using the following equation:

$$V_{\text{mean}} \times \pi \left(\text{vessel diameter}/2 \right)^2 \times (6 \times 10^4).$$

Where V_{mean} is mean blood velocity and 6×10^4 changes metres per second to litres per minute. All leg blood flow values reported within this thesis are the mean of 3 consecutive measurements.

Measurement error: validity and reliability

In order to appropriately assess changes in leg blood flow, it is good practice to acknowledge the coefficient of variation of each measurement obtained. While it is important to know the variation within each measurement period, in line with the design of studies within this thesis, it is also important to acknowledge the variation between different time points. Accordingly, five participants were studied on separate occasions at four non-consecutive time points on the same day to simulate experimental trials. Participants were studied at rest and during one-legged knee-extensor exercise at 20W and 60 RPM. The results are displayed in Table 3.0.

Table 3.0. Coefficient of variation for measurements of leg blood flow (LBF).

Measure	Situation	CV (%)
Femoral Artery Diameter	Rest	2.7
	Exercise	1.5
Blood Velocity	Rest	9.1
	Exercise	5.1
Blood Flow	Rest	8.1
	Exercise	4.9

Values are means from 5 participants. Coefficient of variation is reported for measurements of common femoral artery; diameter, blood velocity and blood flow at rest and during mile knee-extensor exercise.

The values reported in Table 3.0 are well within widely accepted standards for the measurements of blood flow (Rådegran, 1999; Shoemaker, Pozeg & Hughson, 1996).

Advantages and limitations of using ultrasound to assess leg blood flow

The ultrasound method to measure leg blood flow has advantages and limitations. The advantage of this technique is primarily that blood flow can be measured in continuous real time and can, therefore, be used to observe rapid changes in blood flow to a given intervention. In addition, the technique is non-invasive and, given the low reported measurement error, is accurate in detecting even small changes in blood flow.

Limitations include the expense of buying the equipment and that it cannot be used to measure femoral artery blood flow during exercise modalities such as running and cycling. With such exercise modalities, the angle of the hip at certain points of the associated movements would cause contact between the probe and the skin to be lost along with measurements of diameter and blood velocity. Accordingly it cannot be used to measure leg blood flow during maximal whole body exercise. However, this latter limitation does not apply to this thesis given that one-legged knee-extensor exercise was used wherein the angle of the hip remains fixed throughout the entire range of motion.

3.2.2. Skin blood flow

In all studies, skin blood flow was measured in the exercising leg and right forearm using a single point laser Doppler probe (PROBE 408, Periflux, Jarfalla, Sweden) via laser-Doppler flowmetry (Periflux™ Flowmetry System, Jarfalla, Sweden). Each laser Doppler probe emits a single continuous 780 nMm laser beam at a maximum of 1 milliwatt from the probe tip. Skin blood flow was measured at a depth of 1mm. The probe was secured to the skin of the thigh (i.e., above the vastus lateralis). In study 1 the probe was not in contact with the water-perfused suit. In both studies, care was taken to position the probe at a site free from any prominent underlying blood vessels. In both studies the probe was positioned on the right leg above the vastus lateralis muscle at a site where underlying muscle tissue and skin movement would be minimised. This served to reduce the amount of 'noise' in reflected laser Doppler signal.

3.2.3. Temperature measurements

In all studies, rectal temperature was measured 10 cm past the sphincter muscle using a commercially available rectal probe (Physitemp, Clifton, New Jersey, USA). Mean skin temperatures were also measured, although the specific sites of measurements were different between studies 1 and 2 and so are discussed in chapters 4 and 5 respectively.

3.2.4. Assessment of cardiovascular haemodynamics

Overview

Blood pressure was assessed invasively at rest and during one-legged knee-extensor exercise using arterial and venous catheters, which also allow the continuous estimate of systemic haemodynamics. Please see specific methodology sections of each study for study. All catheters were inserted in sterile conditions using the Seldinger technique under local anaesthesia (1% lidocaine) by a group of experienced anaesthetists from Ealing Hospital, United Kingdom. All catheters were stitched onto the skin to avoid displacement during exercise.

While both catheters were used for the purposes of blood sampling, the arterial catheter was used to continuously record arterial pressure, from which the pressure wave forms were used to estimate stroke volume using the Modelflow method, incorporating age, gender, height and weight (BeatScope version 1.1, Finapres Medical Systems BV, Amsterdam, Netherlands) (Wesseling, Jansen, Settels & Schreuder, 1993). The venous catheter was also used to record femoral venous pressure.

Arterial catheter

The arterial catheter was inserted in the radial artery of the right arm using a needle and a guide wire under local anaesthesia. Successful placement of the catheter into the artery was indicated by a pulsatile flow of blood out from the cannula. The catheter was connected to a pressure transducer via a commercially available tubing system (Pressure Monitoring Kit, Baxter) that was in turn connected to an amplifier (BP amp, ADInstruments, Bella Vista, NSW, Australia) and monitored online via a data acquisition system (Powerlab 16/30 ML 880/P, ADInstruments, Bella Vista, NSW, Australia). The data was analysed offline using

the same data acquisition software. Catheters were regularly flushed with saline to prevent the formation of blood clots within the sample lines.

Venous catheter

The venous catheter was inserted in the femoral vein under local anaesthesia 1-2 cm proximal to the inguinal ligament of the exercising left leg. Successful placement of the catheter into the vein was indicated by; a steady flow of blood from the cannula, the easy drawing of blood and a resting venous pressure of approximately 10 mmHg. Femoral venous pressure was continuously recorded at the level of the heart using the same equipment, software and analysis as described previously for the arterial pressure.

Modelflow method for estimation of systemic haemodynamics

In all studies, the Modelflow method was used to estimate stroke volume from aortic flow incorporating age, gender, height and weight (BeatScope version 1.1, Finapres Medical Systems BV, Amsterdam, Netherlands) (Wesseling, et al., 1993). This allowed the calculation of cardiac output by multiplying heart rate and stroke volume. Aortic flow is calculated using arterial pressure waveforms that are used to estimate aortic input impedance (Wesseling, et al., 1993). Arterial pressure waveforms were obtained via either invasive or non-invasive methods which are detailed in the specific study chapters. The three elements which represent aortic haemodynamics are arterial compliance, peripheral vascular resistance and the characteristic impedance of the aorta (Wesseling, et al., 1993). Arterial compliance and aortic characteristic impedance are influenced by the elasticity of the aorta. The aortic characteristic impedance can be expressed as aortic pressure divided by blood flow into the aorta from the left ventricle (Bogert & van Lieshout, 2005). As blood is ejected from the left ventricle into the aorta, the pressure within the aorta produces a resistance to the ventricular outflow and hence the elasticity of the aorta influences the pressure and thus the impedance to pulsatile flow while arterial compliance refers to the resistance of the aorta to an increase in volume (Bogert & van Lieshout, 2005). Finally peripheral vascular resistance refers to the constant flow of blood out of the aorta to vascular beds in the periphery and is expressed as mean arterial pressure divided by \dot{Q} (Jansen, et al., 2001). The model flow method calculates the aortic flow wave and is

expressed over time and is used to estimate stroke volume from which cardiovascular haemodynamics can be calculated.

The estimation of systemic haemodynamics using the model flow method has been shown to be accurate when compared to measures of cardiac output derived using ultrasound (van Lieshout, et al., 2003) and thermodilution (Wesseling, et al., 1993) in patients undergoing cardiac surgery. Systemic haemodynamics at rest and during one-legged knee-extensor exercise including heart rate and cardiac output. All values were representative of mean data collected and averaged over 1 minute after being carefully checked for signal variations.

3.2.5. Leg and systemic haemodynamics: calculated variables

Blood flow pressure at the level of the leg was calculated as MAP minus femoral venous pressure obtained directly from the exercising or experimental leg. Systemic and leg vascular conductance were calculated as cardiac output divided by mean arterial pressure and LBF divided by blood flow pressure respectively. Leg a-vO₂ difference was the difference in arterial and femoral venous blood O₂ content while leg O₂ delivery was the product of arterial O₂ content and LBF. Leg O₂ extraction was the ratio between leg a-vO₂ difference and arterial O₂ content. Finally, leg $\dot{V}O_2$ was calculated by multiplying LBF by leg a-vO₂ difference.

3.2.6. Muscle oxygenation

Muscle oxygenation of the vastus lateralis muscle was measured using near-infrared spectroscopy (NIRS; INVOS Cerebral Oximeter, Somanetics, Troy, MI, USA). The optode was placed over the vastus lateralis muscle and securely taped to the skin ensuring no light interfered with the reading.

3.2.7. Oxygen uptake

Systemic oxygen uptake was continuously measured and recorded online (Quark b², Cosmed, Italy). Breath-by-breath data was analysed over a period of one minute coinciding with measurements of leg and systemic haemodynamics.

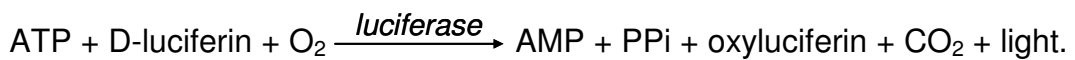
3.2.8. Blood and plasma parameters

In both study 1 and 2 blood samples were drawn from arterial and venous catheters. However for a sub-protocol of study 1 catheters were not placed and

therefore no blood samples were taken. Specific information regarding blood sampling for each protocol is located within each study chapter.

Blood gas variables, haemoglobin concentration, electrolytes and osmolality were measured using an automated analyser (ABL 825, Radiometer, Copenhagen, Denmark).

Plasma ATP was determined with the luciferin–luciferase technique. The luciferin-luciferase technique involves the addition of D-luciferin and O₂ to a plasma sample, in the context of this thesis, obtained from the radial artery or femoral vein. This causes a reaction which is catalyzed by the addition of luciferase. The reaction can be seen below:



The principle of ATP measurements from this reaction involves the release of light. Light is emitted from the plasma sample in proportion to the amount of ATP present in the sample. This assay is optimized so that it can emit a stable light up to 10⁻⁶ mol·l⁻¹ and is optimized to allow a detection of ATP throughout a range of 10⁻¹² to 10⁻⁶ mol·l⁻¹. This procedure was determined using a luminometer (Orion Microplate Luminometer, Berthold Detection System GmbH, Pforzheim, Germany) with three automatic injectors. The luminometer was sensitive enough to detect ATP as low as 10 attomole. Blood samples (2.0 ml) were drawn into a stop solution (2.7 ml) 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) (Gorman *et al.*, 2003). Immediately thereafter, the samples were centrifuged for 3 min at 4000 g in plastic tubes containing a gel for plasma separation (BD, Franklin Lakes, NJ, USA) and measured in duplicates at room temperature (20-22 °C) using an ATP kit (ATP Kit SL; BioTherma AB, Dalarö, Sweden) with an internal ATP standard procedure. As an indicator of haemolysis, plasma haemoglobin was measured spectrophotometrically (Jenway 3500, Essex, England). For the measurement of ATP the within day coefficient of variation was <3% and between day coefficient of variation was <4%.

Catecholamines were measured from plasma which was rapidly frozen at -80°C . Analysis was conducted once the samples had fully thawed and samples were kept fully on ice until extraction. Plasma was extracted with activated alumina and eluted with 0.5 mmol/L acetic acid. Samples were analysed for catecholamines by liquid chromatography/mass spectrometry. Chromatographic separation was achieved with a 5 x 0.2 cm column packed with 1.8 μm Eclipse Plus C18 (Agilent). Elution for noradrenaline, adrenaline and 3,4-Dihydroxybenzylamine (DHBA) (internal standard) were carried out at 0.2 mL/min in gradient mode from 100% buffer A (2.5 mmol/L nonafluoropentanoic acid) to 70% buffer B (100% acetonitrile). Mass spectrometry was achieved using positive ionisation mode with fragmentation by tandem MS mode (QQQ Agilent 6140 triple quad). This procedure was verified positively for reproducibility and linearity with regard to detection of noradrenaline and adrenaline in more than 40 different human plasma samples (data not shown). The within day coefficient of variation was <5% for measures of noradrenaline and <10% for adrenaline. The between day coefficient of variation was <10% for noradrenaline and <15% for adrenaline. The liquid chromatography/mass spectrometry method was sensitive enough to detect changes in noradrenaline and adrenaline as low as 0.2 and 0.3 $\text{nmol}\cdot\text{l}^{-1}$ respectively.

3.3. Statistical Analysis

All of the data used to comprise this thesis were statistically examined using computerised data analysis software (SPSS Inc, Chicago, Illinois). As a general rule, the alpha level was set at 0.05 for the rejection of the null hypothesis, indicating a 95% confidence level. However specific information to each study can be found in their appropriate chapters.

CHAPTER 4

Study 1

4.0. Summary

Heat stress increases cardiac output (\dot{Q}) in humans, presumably in sole response to an augmented thermoregulatory demand of the skin circulation, rather than a shared contribution from hyperthermia mediated skeletal muscle and skin vasodilation. Increases in local temperature, comparable to those occurring during heat stress or exercise, have been shown to induce vasodilation of arterioles *in vitro* and *in situ*. However the *in vivo* effects of elevations in temperature remain unclear. This study was designed to test the hypothesis that local hyperthermia induces vasodilation in resting and exercising human limb muscle thereby contributing to heat stress and exercise hyperaemia. Leg and systemic haemodynamics and oxygenation were measured at rest and during one-legged knee-extensor exercise in 7 males across 4 conditions of whole body heating and at rest during isolated leg heating, while hydration status was maintained. During whole body heating, leg blood flow (LBF), \dot{Q} and leg and systemic vascular conductance increased in line with body temperature at rest and during exercise, although the rate of increase was attenuated during exercise compared to rest (LBF = 0.26 ± 0.08 vs. 0.47 ± 0.07 l·min⁻¹ °C⁻¹ and \dot{Q} = 1.50 ± 0.14 vs. 1.97 ± 0.12 l·min⁻¹ °C⁻¹; $P < 0.05$). Enhanced leg blood flow due to a net vasodilation was paralleled by reductions in leg a-vO₂ difference reflecting elevations in muscle and skin oxygenation and blood flow and was associated with increases in arterial plasma ATP (rest; $r = 0.94$; $P = 0.03$; exercise; $r = 0.68$; $P = 0.18$). At rest, isolated leg hyperthermia was accompanied by elevations in LBF that accounted for $52 \pm 9\%$ of the peak increase in LBF associated with whole body heating, 1.0 ± 0.1 l·min⁻¹, without any increase in \dot{Q} ($P = 0.90$). The findings from this study suggest that local hyperthermia induces leg muscle and skin vasodilation and that skeletal muscle vasodilation contributes to heat stress-mediated leg hyperaemia. However, the magnitude of increase in resting and exercising limb blood flow with severe heat stress and the blunted hyperaemic response during exercise suggest that local hyperthermia can only account for a small fraction of exercising limb muscle hyperaemia in humans.

4.1. Introduction

Heat stress augments limb blood flow and cardiac output (\dot{Q}) in resting humans (Abraham, et al., 1994; Barcroft, et al., 1947; Detry, et al., 1972; Edholm, et al., 1956; Johnson, et al., 1976; Roddie, et al., 1956; Rowell, et al., 1969a; Wenger, et al., 1985). An unresolved question is whether muscle vasodilation contributes to this process. Early investigation into the partition of limb blood flow between the skin and skeletal muscle during heat stress produced conflicting results (Barcroft, et al., 1947; Barcroft & Edholm, 1943; Barcroft & Edholm, 1946; Edholm, et al., 1956; Roddie, et al., 1956). Later research utilizing the 4-iodoantipyrine-¹²⁵I clearance method found no evidence for elevations in muscle blood flow (Detry, et al., 1972; Johnson, et al., 1976), which along with the $\sim 6-8 \text{ l}\cdot\text{min}^{-1}$ estimate of the maximal cutaneous blood flow based on the indirect measures of increased \dot{Q} (Detry, et al., 1972; Minson, et al., 1998; Rowell, 1974; Rowell, et al., 1969a) and splanchnic and renal blood flow (Minson, et al., 1998) helped shape the view that heat stress-induced hyperaemia is confined to the skin circulation. Despite this view, more recent evidence shows that blood flow elevations in the saphenous vein, which drains the skin of the leg, cannot fully account for all of the increases in whole leg blood flow evoked by whole body heat stress (Abraham, et al., 1994). This suggests that muscle vasodilation might contribute to heat stress-induced hyperaemia, possibly via direct or indirect effects of temperature on the vasculature.

Elevations in the temperature of rat cerebral and abdominal arterioles, both *in vitro* in isolated vessel preparations (Ogura, et al., 1991) and *in situ* during local abdominal heating (Unthank, 1992) are associated with arteriolar vasodilation. This vasodilation is noteworthy given that the increases in temperature are comparable to that occurring during heat stress or exercise in the human forearm where increases in blood flow are observed (Barcroft & Edholm, 1943; Barcroft & Edholm, 1946; Johnson, et al., 1976) and. These findings collectively support the existence of regulatory pathways in the microvasculature linking increases in local temperature to vessel dilatation. This possibility provides a rationale to investigate whether elevations in skeletal muscle blood flow contribute to the increases in blood flow that accompany heat stress in humans.

In contrast to resting responses, the effects of heat stress during exercise remain equivocal with some reports showing elevations (Smolander & Louhevaara, 1992; Williams & Lind, 1979) and others unchanged or reduced limb blood flow and cardiac output (González-Alonso & Calbet, 2003; Nadel, et al., 1979; Nielsen, et al., 1990; Savard, et al., 1988). Differences in the mode and intensity of exercise, magnitude of heat stress and possibly hypohydration may account for these discrepancies. In this context, limb blood flow is elevated during isolated forearm or leg exercise with exposure to a moderate degree of either local or whole body heat stress when hypohydration is negligible (Smolander & Louhevaara, 1992; Williams & Lind, 1979). On the other end of the spectrum, limb blood flow is reduced in association with the haemoconcentration and the declines in blood flow pressure and cardiac output that accompany severe heat stress and hypohydration during short high intensity and prolonged moderate intensity whole body exercise (González-Alonso & Calbet, 2003; González-Alonso, et al., 1998). Hence, while it is clear that exercise can attenuate the effects of heat stress upon limb blood flow compared to rest, it remains uncertain whether the blunted response is due to the confounding influences of hypohydration and/or the reflexes underpinning the circulatory limitations to whole body exercise.

The effects of local limb compared to whole body heat stress upon elevations in limb blood flow and cardiac output have only been partially examined in the forearm (Johnson, et al., 1976). Local temperatures were found to account for approximately half of the increase in forearm blood flow with whole body heating. However, because \dot{Q} was not measured, it is not possible to determine whether the enhanced limb blood flow with local heat stress was associated with increases in \dot{Q} and/or a redistribution of blood flow from other territories such as the visceral organs. It is clear, however, that increases in limb blood flow with passive whole body heat stress are associated with both enhanced \dot{Q} and reduced visceral blood flow (Minson, et al., 1998).

Limb blood flow increases with heat stress in the presence of enhanced muscle and skin sympathetic nerve activity (Bini, et al., 1980; Niimi, et al., 1997; Ray & Gracey, 1997) indicating that heat stress directly or indirectly modulates sympathetic vasoconstrictor activity such that vasodilation prevails over vasoconstriction. The observation that the plasma concentration of the potent

vasodilator and sympatholytic molecule adenosine triphosphate (ATP) increases during exercise with severe heat stress (González-Alonso, et al., 2004; Rosenmeier, et al., 2004) raises the possibility that ATP may be involved in the prevailing heat stress induced limb vasodilation. Alternatively, while other vasodilatory mechanisms may also be involved in the mediation of heat stress hyperaemia, an insight into the involvement of intravascular ATP remains largely unexplored.

Accordingly, the main aim of this study was to determine whether skeletal muscle vasodilation contributes to the generally observed increases in leg blood flow and cardiac output with whole body heat stress in resting humans. A second aim was to determine whether increases in blood flow during whole body heat stress at rest are attenuated during mild exercise in euhydrated individuals. A third aim was to determine the contribution of isolated leg heat stress to the increases in leg blood flow with whole-body heat stress. A third aim was to gain insight into the role of plasma ATP in heat stress mediated limb vasodilation.

Consistent to the aims of this study, leg and systemic haemodynamics, blood oxygenation, plasma ATP and both quadriceps muscle oxygenation and temperature were measured at rest and during moderate one-legged knee-extensor exercise in healthy male volunteers during control conditions and 3 graded levels of whole body skin and core hyperthermia as well as during isolated leg tissue hyperthermia. It was hypothesised that: 1) heat stress will locally induce vasodilation in resting and exercising human limb muscle, thereby contributing to whole body heat stress and exercise hyperaemia, 2) the leg and systemic hyperaemic response to whole body heat stress will be attenuated during exercise, 3) leg hyperaemia will be associated with increases in plasma ATP, and 4) Isolated leg heat stress will account for a large portion of the leg hyperaemia associated with whole body heating.

4.2. Methods

4.2.1. Participants

Seven healthy recreationally active males (mean \pm SD age 21 ± 2 years, body mass 76.3 ± 10.4 kg and height 178 ± 6 cm) participated in this study involving two different protocols. This study conformed to the code of Ethics of the World Medical Association (Declaration of Helsinki) and was conducted after ethical approval from the Brunel University Research Ethics Committee. Informed written and verbal consent was obtained from all participants before participation.

4.2.2. Design

In protocol 1 leg and systemic haemodynamics and quadriceps muscle oxygenation were examined at rest and during moderate one-legged knee-extensor exercise (mean \pm SEM 20.8 ± 0.9 W at 65 ± 0.7 rpm for 6 min) in four consecutive and increasing thermal conditions (Fig. 4.0). Thermal conditions were manipulated by dressing participants in a water-perfused suit whilst in the supine position: 1) *Control*, with normal skin (~ 32 C) and core temperatures (~ 37), 2) *Skin Hyperthermia*, whole body skin temperature was increased (~ 37 C) while rectal temperature remained at control (~ 37), 3) *Skin and Mild Core Hyperthermia*, where whole body skin temperature remained elevated (~ 37 C) and rectal temperature increased (~ 38 C) and 4) *Skin and Core Hyperthermia*, where skin temperature remained elevated (~ 37 C) and core temperature increased further from the previous condition (~ 38.5) (Fig. 4.0).

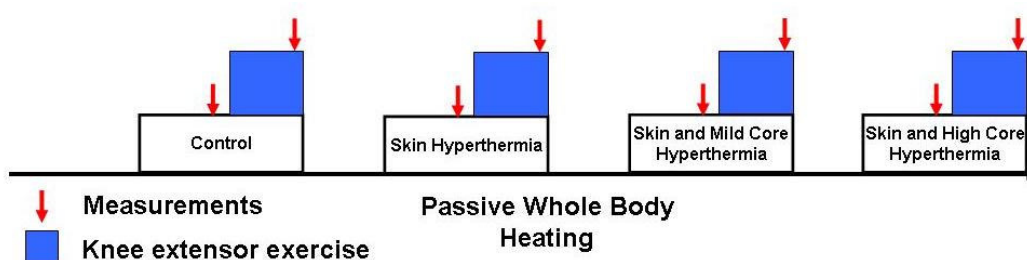


Figure 4.0. The experimental protocol for inducing whole body heat stress in study 1, protocol 1.

In protocol 2 leg and systemic haemodynamics were examined at rest over 60 min of isolated leg heating using non-invasive methods in the supine position (Fig. 4.1). Between whole body heating (protocol 1) and isolated leg heating (protocol 2)

the temperature of water perfusing the water perfused suit or leg respectively was kept constant to ensure similar mean leg skin temperatures between protocols.

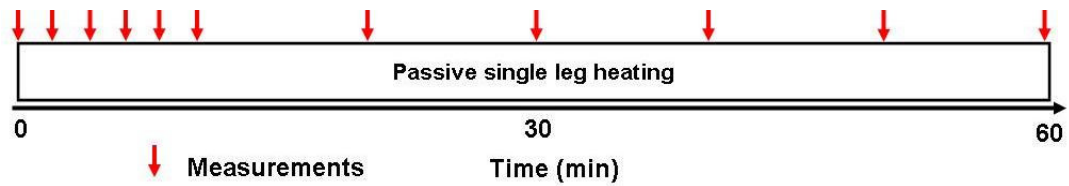


Figure 4.1. The experimental protocol for isolated leg heat stress in study 1, protocol 2.

Protocols 1 and 2 were separated by at least two weeks. Participants ingested a carbohydrate-electrolyte beverage (Gatorade®) throughout the heating protocol to maintain their hydration status. The temperature of the beverage was 35-40 °C in order to avoid any decreases in internal temperature caused by its consumption. Hydration status was maintained throughout. In both protocols a maintenance of hydration status was indicated by both an unchanged pre and post heat stress body weight and, in protocol 1, an increase in blood osmolality of no more than 5 mOsm·kg⁻¹ from control.

4.2.3. Instrumentation of participants

On the morning of the protocol participants arrived in the laboratory after eating a light breakfast. After insertion of the rectal thermister, participants rested in the supine position while catheters were placed under local anaesthesia into the femoral vein of the exercising leg (left leg) and in the radial artery (right forearm) as described previously. In 3 additional participants, a 'retrograde' catheter was inserted in the femoral vein of the left (exercising) leg that ran in the opposite direction to the aforementioned catheter in the femoral vein i.e., running distally. The 'retrograde' venous catheter was 12 cm in length and was inserted into the femoral vein at a similar location to the existing venous catheter drawing blood from a deep portion of the femoral vein (fig. 4.2).

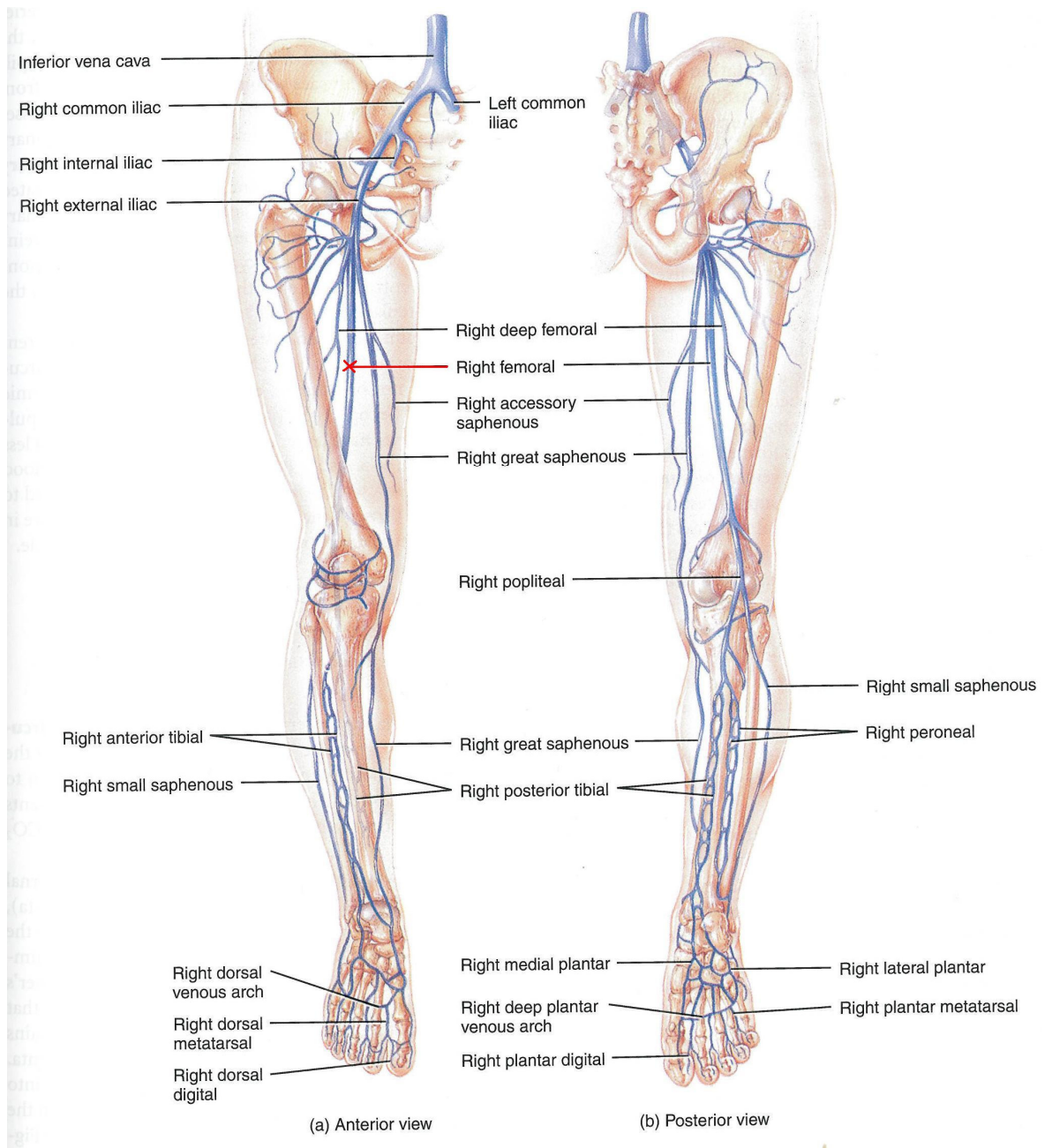


Figure 4.2: Illustration of the principle veins within the human leg (Tortora & Grabowski, 2000).

Figure 4.2 illustrates that blood withdrawn from the ‘retrograde’ venous catheter in the deep portion of the femoral vein would not sample blood from the saphenous vein. The blood in the saphenous vein has a high oxygenation because it primarily drains the vasculature of the skin (Tayefeh, et al., 1997). In these 3 additional subjects catheters were placed in the radial artery as previously described. Blood samples taken from the ‘retrograde’ femoral vein catheter were analysed for blood oxygenation as described in the general methods. Successful placement of the catheter was indicated as described previously but also by lower values of blood oxygenation compared to the other femoral venous catheter. For the purposes of

this thesis, the femoral venous catheter running on the retrograde direction will be referred to as the deep femoral venous catheter.

Following placement of the catheters, participants walked to the experimental room and sat on the knee-extensor ergometer where they were dressed in a custom built water-perfused suit that was interwoven with silicone tubing and connected to a water circulator (Julabo F34, Seelbach, Germany). The water circulator was fitted with an auxiliary pump and temperature control unit capable of controlling the temperature of the water in the suit, which covered the participant's entire body except their head, hands and feet. Whole-body heating was induced by perfusing 47°C water through the suit.

Once the specific skin and/or core temperatures were attained at each heating stage the temperature of the water perfusing the suit was decreased to ~43°C to limit further increases in skin and/or core temperature during baseline and exercise data collection. To minimise heat loss during heating, a thermal foil blanket covered the torso and was wrapped around the lower body of the participants, socks covered both feet and a woolly hat was also worn. After the participants were dressed in the suit they lay supine on a reclining chair that was part of the knee-extensor ergometer (Ergometer LE220, FBJ Engineering, Denmark) while the left foot and ankle were inserted into the boot of the ergometer. Both of the participant's lower legs were supported during resting conditions (Fig. 4.3).



Figure 4.3. The experimental set up for study 1 – whole body heat stress.

In protocol 2 participants only wore the left leg of the water-perfused suit and no catheters were inserted. Similarly to protocol 1, foil was wrapped around the heated leg and the water circulator controlled the temperature of the water within the water-perfused leg. Participants remained supine whilst a pressure cuff was placed around the finger for the measurement of systemic haemodynamics.

4.2.4. Temperature measurements

Skin thermistors were placed on seven sites: forehead, forearm, hand, abdomen, thigh, calf and foot (Grant Instruments, Cambridge, United Kingdom). Thermistors were securely held in place throughout the protocol by the use of adhesive spray and medical tape. Rectal temperature was measured as previously described. Skin (Squirrel 1000 Series, Grant Instruments, Cambridge, United Kingdom) and rectal (Thermalert, Physitemp, Clifton, New Jersey, USA) temperatures were monitored offline. Weighted mean skin temperature was calculated using methods described previously (Hardy & Dubois, 1937). Mean body temperature was calculated as $[(\text{rectal temperature} * 0.8) + (\text{weighted mean skin temperature} * 0.2)]$ (Hardy & Stolwijk, 1966), while mean leg skin temperature was a composite of the skin temperatures of the thigh and calf.

In 3 additional participants (mean \pm SD age 21 ± 3 years, body mass 67.8 ± 1.3 kg and height 183 ± 11 cm), quadriceps muscle temperature was measured on-line (TC-2000, Sable Systems, Las Vegas, NV, USA) with a T-204A tissue implantable thermocouple microprobe (Physitemp, Clifton, New Jersey, USA). One participant completed the whole body heating protocol while two other participants underwent the isolated leg heating protocol. The muscle thermister was inserted in the left leg of the participant to rest in the vastus lateralis muscle at a depth of approximately 2-3cm. In both whole body and isolated leg heating protocols, muscle temperature was measured together with core and skin temperatures to establish the increases in quadriceps muscle temperature in participants where muscle temperature was not measured. As such, muscle temperature was estimated in 7 participants based upon changes in mean core and skin temperatures.

4.2.5. Systemic haemodynamics and muscle oxygenation

In protocol 1 baseline systemic and leg haemodynamics were measured immediately prior to exercise after a minimum of 10 min supine rest and following the attainment of the desired skin and rectal temperatures. During exercise these measurements were repeated between min 4 and 6. Additionally, arterial and venous blood samples (1 ml for blood gas and electrolyte variables, 2 ml for plasma ATP and plasma haemoglobin and 2 ml for plasma catecholamines) were obtained at rest and after 5 min of exercise. Arterial and venous catheters were also used to measure arterial and venous blood pressure, respectively. In protocol 2, participants remained at rest throughout the heating protocol and temperature and haemodynamic measures were taken every 2 min between 0-10 min and every 10 min thereafter.

In protocol 1, heart rate was obtained from a 3 lead electrocardiogram while arterial and femoral venous pressure waveforms were continuously recorded at the level of the heart via pressure transducers (Pressure Monitoring Kit, Baxter) connected to two amplifiers (BP amp, ADInstruments, Bella Vista, NSW, Australia) and monitored online via a data acquisition system (Powerlab 16/30 ML 880/P, ADInstruments, Bella Vista, NSW, Australia). \dot{Q} was calculated as the product of heart rate multiplied by stroke volume, where stroke volume was estimated using the directly measured arterial pressure waveform via the Modelflow method, as previously described. In protocol 2, blood pressure waveforms were recorded non-

invasively using a finometer (Finapres Medical Systems, Smart Medical, Amsterdam, Netherlands) and heart rate was obtained from a 3 lead electrocardiogram, allowing estimates of systemic haemodynamics as described above. In both studies, systemic oxygen uptake was continuously measured and recorded online (Quark b², Cosmed, Italy).

4.2.6. Leg and skin haemodynamics

In both studies, LBF, leg skin blood flow and muscle oxygenation were measured as previously described.

4.2.7. Statistics

A one-way repeated measures analysis of variance (ANOVA) was performed on all dependent variables to test significance among the control and 3 conditions of heat stress at rest and during exercise. When a significant difference ($P < 0.05$) was found, appropriate *post-hoc* analysis of the data was conducted, using a Bonferroni correction where appropriate ($P < 0.0125$). Where applicable, relationships were determined using Pearson's product moment correlation upon the mean results from all 7 participants ($P < 0.05$).

4.3. Results

4.3.1. Hydration and temperature during whole body heat stress

In protocol 1 body mass and blood electrolytes, osmolality and haematological variables remained unchanged in all experimental conditions (Table 4.0) indicative of a maintained intravascular and extravascular fluid status throughout all heat stress conditions. The individual substances, Na⁺, K⁺ and Cl⁻ will not be discussed individually. Rather they will be indirectly discussed as one in terms of osmolality. With the first stage of whole-body heating, mean skin temperature increased from 32.3 ± 0.3 °C to 36.4 ± 0.2 °C, whereas core temperature was unchanged (37.04 ± 0.08 °C vs. 37.11 ± 0.08 °C; $P > 0.05$). Consequently, mean body temperature increased from 36.1 ± 0.1 to 37 ± 0.1 °C. In the *Skin and Mild Core Hyperthermia* condition, mean skin temperature was maintained (36.9 ± 0.3 °C) while core temperature increased to 38.00 ± 0.05 °C ($P < 0.05$), thus mean body temperature was also elevated (37.7 ± 0.1 °C). This pattern was repeated with further heating to the *Skin and Core Hyperthermia* condition: 37.7 ± 0.2 °C (mean skin), 38.60 ± 0.07 °C (core) and 38.4 ± 0.1 °C (mean body) (all $P < 0.05$). In response to whole-body heating mean leg skin temperature followed the same pattern and magnitude as mean whole-body skin temperature (Fig. 4.4.). In one participant quadriceps muscle temperature increased progressively from 34.9 °C at control rest to 36.6 °C with *Skin Hyperthermia*, 36.9 °C with *Mild Core and Skin Hyperthermia* and finally 38.1 °C with *Skin and Core Hyperthermia*. During exercise, muscle temperature increased progressively from 37.1 °C at control, to 37.5 °C, 38.4 °C, and 39.2 °C, respectively. With the exception of quadriceps muscle temperature, all reported temperatures represent the average of the rest and exercise conditions as skin or core temperatures were not significantly different between rest and exercise ($P > 0.05$) (Fig. 4.4).

Table 4.0. Blood variable responses to whole body heat stress at rest and during exercise.

		Control		Skin Hyperthermia		Skin and Mild Core Hyperthermia		Skin and Core Hyperthermia	
		Rest	Exercise	Rest	Exercise	Rest	Exercise	Rest	Exercise
Hb (g·l ⁻¹)	a	146±4	153±4	145±4	149±4	147±4	150±4	148±4	151±4
	v	145±4	154±4	146±4	150±4	148±4	151±4	148±4	151±5
O ₂ Sat (%)	a	97.5±0.3	97.5±0.4	97.5±0.2	98.0±0.2	97.7±0.5	98.0±0.2	98.7±0.1*†	98.2±0.2
	v	63.4±4.2	30.1±1.5	81.3±1.2*	36.1±1.7	83.2±1.1*	41.6±2.3	84.3±1.5*	47.3±2.0*†
CtO ₂ (ml·l ⁻¹)	a	199±5	209±5	197±5	204±5	200±7	206±5	204±6†	207±6
	v	128±8	65±4	165±3*	75±4	170±5*	87±6*	174±7*	99±5*
Na ⁺ (mmol·l ⁻¹)	a	138±1	139±1	137±1	138±1	136±1	137±1	135±1	135±1*†
	v	138±1	141±1	137±1	140±1	137±1	139±1*	135±1	137±1*†
K ⁺ (mmol·l ⁻¹)	a	4.0±0.1	4.5±0.1	3.9±0.1	4.3±0.1	3.7±0.1	4.0±0.1*†	3.7±0.1*	3.9±0.1*†
	v	4.0±0.1	4.9±0.1	4.0±0.1	4.5±0.1	3.7±0.1*	4.2±0.1*	3.7±0.1*†	4.0±0.1*†
Cl ⁻ (mmol·l ⁻¹)	a	104±1	105±1	104±1	105±1	104±1	104±1	103±1	102±1*#
	v	102±1	102±1	103±1	102±1	103±1	102±1	102±1#	100±1#
Glucose (mmol·l ⁻¹)	a	6.0±0.2	5.8±0.1	8.0±0.4*	7.6±0.4*	9.8±0.3*†	8.5±0.5*	9.3±0.7*	8.1±0.6*
	v	5.6±0.2	5.6±0.1	7.3±0.3*	7.2±0.4*	9.0±0.3*†	8.0±0.4*	8.5±0.6*	7.5±0.6*
Osmolality (mOsm·kg ⁻¹)	a	281±1	284±1	282±1	284±1	282±1	282±1	279±2	279±1
	v	281±1	288±2	282±1	287±1	282±1	285±1	279±1	281±1
ATP (nmol·l ⁻¹)	a	667±91	1032±169	897±101	1238±211	1004±140	1402±270	1242±232	1154±190
	v	685±80	867±114	997±219	851±78	824±115	903±55	874±155	822±145
Noradrenaline (nmol·l ⁻¹)	a	0.7±0.3	1.3±0.4	0.6±0.3	1.1±0.3	1.5±0.6	2.6±0.5	2.0±0.7*	4.6±1.4*
	v	0.8±0.2	2.1±1.3	0.7±0.2	1.3±0.3	1.3±0.6	2.2±1.0	2.0±0.9*	4.9±2.4*

Values are mean±SEM for 7 participants except for noradrenaline (n=6). Notice that a denotes arterial; v, femoral venous; Hb, haemoglobin; O₂ sat, percentage oxygen saturation and CtO₂ oxygen content in blood. ctO₂ and osmolality were corrected for temperature. * Different from control, *P*<0.05. † Different from skin hyperthermia, *P*<0.05. # Different from skin and mild core hyperthermia, *P*<0.05.

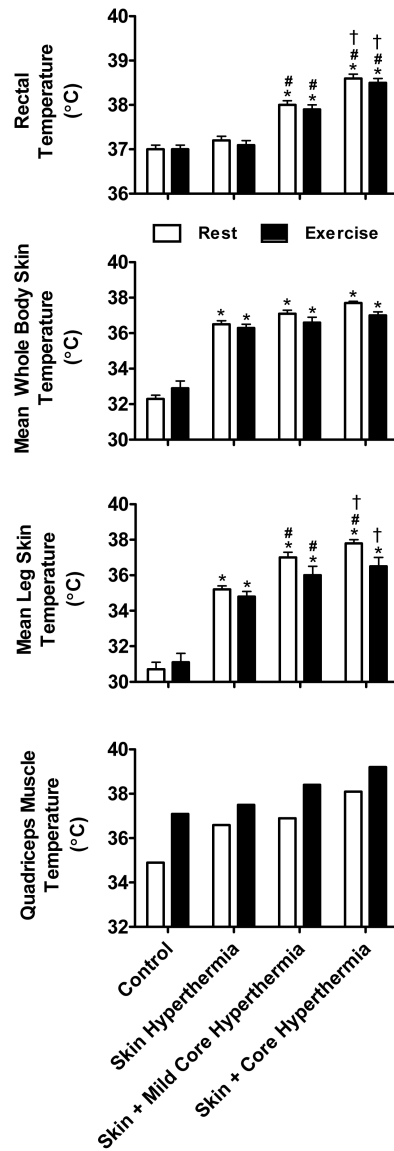


Figure 4.4. Body temperature responses to whole body heat stress

Data are mean \pm S.E.M for 7 participants. With the exception of quadriceps muscle temperature, all reported temperatures represent the average of the rest and exercise conditions as skin or core temperatures were not significantly different between rest and exercise ($P>0.05$) (Fig. 4.4). Quadriceps muscle temperature measured in one participant followed the increase in mean skin temperature at rest and increased rapidly during exercise by 1-2 °C above corresponding resting values. * Different from control, $P<0.05$. † Different from skin hyperthermia, $P<0.05$. # Different from skin and mild core hyperthermia. Significance was accepted at $P<0.05$ and refers to differences in the respective conditions, i.e. either rest or exercise.

4.3.2. Leg and systemic haemodynamics and oxygenation during whole body heat stress

At rest, LBF and SkBF gradually increased with each level of hyperthermia accompanying a decline in leg a-vO₂ difference and a significant but small increase in leg and whole body $\dot{V}O_2$ (peak $\Delta \dot{V}O_2 = 0.02 \pm 0.01$ and 0.15 ± 0.03 l·min⁻¹; respectively, $P < 0.05$, Fig. 4.5).

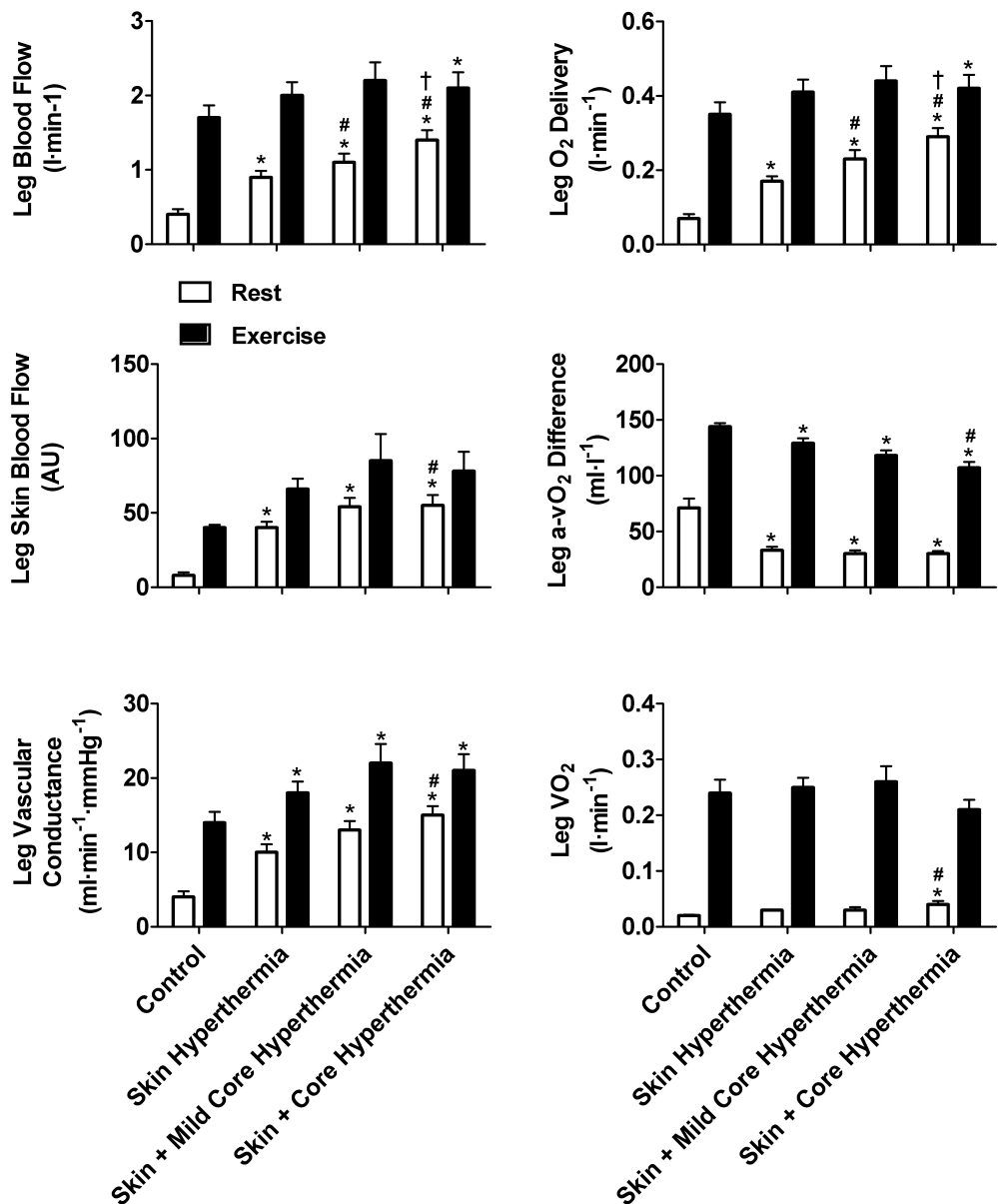


Figure 4.5. Leg haemodynamics and oxygen consumption during whole body heat stress

Data are means \pm S.E.M for 7 participants. * Different from control, $P < 0.05$. † Different from skin hyperthermia, $P < 0.05$. # Different from skin and mild core hyperthermia. Significance was accepted at $P < 0.05$ and refers to differences in the respective conditions, i.e. either rest or exercise.

As measured by NIRS, vastus lateralis muscle oxygenation increased with whole-body heat stress between *Control* and *Skin Hyperthermia* ($76 \pm 2\%$ vs. $89 \pm 2\%$ respectively, $P < 0.05$.) in parallel to an increase in femoral venous oxygenation (63 ± 4 vs. $81 \pm 1\%$, respectively; $P < 0.05$, Table 4.0) but an unchanged arterial oxygenation (97.5 ± 0.3 vs. $97.5 \pm 0.2\%$, respectively). Furthermore, in three participants where a deep femoral venous catheter was placed, blood oxygenation increased with whole body heat stress from control ($60 \pm 11.8\%$) to skin hyperthermia ($79.7 \pm 5.1\%$). Thereafter deep femoral venous oxygenation remained elevated with both skin and mild core hyperthermia and skin and core hyperthermia (75.2 ± 6.4 vs. $76.5 \pm 3.8\%$ respectively). Increases in deep femoral vein oxygenation were reflected by reductions in leg a-vO₂ difference (Table 4.1) indicating a reduced deep femoral O₂ extraction.

Table 4.1: Deep femoral venous oxygenation with whole body heat stress at rest and during exercise

<i>O₂ Sat (%)</i>		<i>Control</i>		<i>Skin Hyperthermia</i>		<i>Skin and Mild Core Hyperthermia</i>		<i>Skin and Core Hyperthermia</i>		
		<i>Rest</i>	<i>Exercise</i>	<i>Rest</i>	<i>Exercise</i>	<i>Rest</i>	<i>Exercise</i>	<i>Rest</i>	<i>Exercise</i>	
N1	a	98.9	99.7	99.3	98.5	99.6	98.4	100.3	99.7	
	vm	76.2	35	73.5	32.8	73.9	34.9	73.1	35	
	vr	32.7	33.7	68.8	35	62.4	31.3	67.9	35.4	
N2	a	97.9	98	98.1	98.4	98.3	97.5	98.1	99	
	vm									
	vr	74.0	40.8	88.5	33.4	87.5	24.8	81.8	48.7	
N3	a	98.8	98.6	98.7	98.6	98.6		97.4	98.3	
	vm	78.2	38.9	81.7	39.9	87.7		83.7	47.7	
	vr	73.3	22.5	81.3	22.2	75.2		79.6	29.3	
<i>CtO₂ (ml·l⁻¹)</i>	N1	a	21.0	22.6	21.0	21.6	21.4	21.8	22.4	22.5
		vm	16.5	8.0	15.7	7.4	15.9	7.9	16.3	7.8
		vr	8.3	7.7	14.8	7.9	13.5	7.1	15.3	8.2
N2	a	18.0	19.0	18.2	19.3	18.5	19.1	19.0	19.3	
	vm									
	vr	14.8	8.1	16.5	6.7	16.9	5.0	16.1	3.1	
N3	a	19.7	20.8	19.9	20.3	20.2		20.0	20.5	
	vm	15.5	8.2	16.4	8.3	17.9		17.0	9.9	
	vr	14.5	4.8	16.3	4.6	15.1		16.3	6.2	
<i>O₂Hb (%)</i>	N1	a	96.8	97.6	97.3	96.2	97.3	96.2	97.7	97.3
		vm	74.8	34.5	72.0	32.3	72.3	34.4	71.3	34.4
		vr	37.1	33.2	67.4	34.5	61.1	30.8	66.4	34.9
N2	a	96.0	96.0	95.9	96.0	96.1	95.4	95.7	96.4	
	vm									
	vr	77.0	40.1	86.5	32.9	85.7	24.5	80	48.2	
N3	a	96.3	96.1	96.1	96.0	96.0		94.9	95.8	
	vm	76.3	38.2	79.5	39.1	85.4		81.5	46.7	
	vr	71.6	22.1	79.3	21.8	73.3		77.6	28.8	
<i>Deep Femoral a-vrO₂ difference (ml·l⁻¹)</i>	N1		149	62	137	79	147	71	143	
	N2		32	109	17	126	16	141	162	
	N3		52	160	36	157	51	37	143	

Values are raw data for each participant. Note that a, denotes arterial; vm, whole mixed femoral venous; vr, retrograde femoral venous; O₂Hb, percentage of oxygen dissolved in haemoglobin; O₂ sat, percentage oxygen saturation and CtO₂ oxygen content in blood. ctO₂ was corrected for body temperature. Blanks indicate where data collection was not possible due to experimental complications.

The increase in LBF with heat stress was associated with a progressive elevation in leg vascular conductance from 4 ± 1 to 15 ± 1 $\text{ml}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$ (Fig. 4.5). Furthermore, blood flow pressure declined owing to a fall in MAP ($P < 0.05$) while femoral venous pressure was stable ($P > 0.05$) (Fig. 4.6). At the level of the systemic circulation, both \dot{Q} and systemic vascular conductance increased progressively with each level of hyperthermia (Fig. 4.6) accompanying gradual increases in heart rate ($P < 0.05$) but a maintained stroke volume.

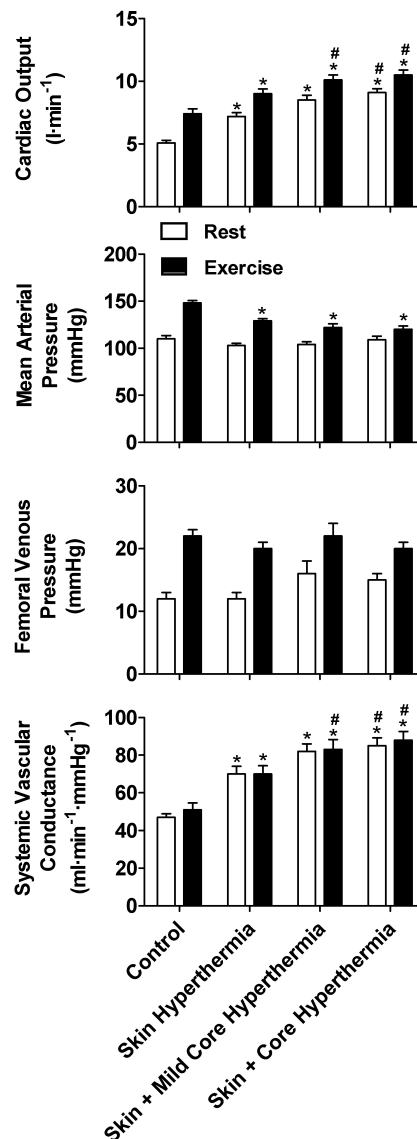


Figure 4.6. Systemic haemodynamics during whole-body heat stress

Data are mean \pm S.E.M for 7 participants. * Different from control, $P < 0.05$. † Different from skin hyperthermia, $P < 0.05$. # Different from skin and mild core hyperthermia. Significance was accepted at $P < 0.05$ and refers to differences in the respective conditions, i.e. either rest or exercise.

During exercise, LBF increased from *control* with whole body heat stress and became significant with *Skin and Core Hyperthermia* ($1.69 \pm 0.17 \text{ l}\cdot\text{min}^{-1}$ vs. $2.05 \pm 0.21 \text{ l}\cdot\text{min}^{-1}$ $P < 0.05$, Fig. 4.5). The increase in LBF was accompanied by proportional decreases in leg a-vO₂ difference ($P < 0.05$). In line with this, leg vascular conductance increased from 14 ± 1 to $21 \pm 2 \text{ ml}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$ from *Control* to *Skin and Core Hyperthermia* ($P < 0.05$). Likewise \dot{Q} , heart rate and systemic vascular conductance progressively increased during exercise with heat stress ($P < 0.05$, Fig. 4.6). With *Skin Hyperthermia*, MAP declined from control but thereafter it remained stable, as was leg and whole body $\dot{V}\text{O}_2$. Additionally while vastus lateralis oxygenation decreased with the onset of exercise both during *Control* and *Skin Hyperthermia* conditions leg skin blood flow increased rapidly ($P < 0.05$; Fig. 4.5).

4.3.3. Effect of whole body heat stress at rest and during exercise

Even though heat stress significantly increased LBF both at rest and during exercise, its rate of increase with elevations in body temperature was significantly attenuated during exercise compared to rest (0.20 ± 0.08 vs. $0.47 \pm 0.07 \text{ l}\cdot\text{min}^{-1} \text{ }^\circ\text{C}^{-1}$; respectively; $P < 0.05$). Similarly increases in \dot{Q} as a function of body temperature were also blunted during exercise compared to rest (1.50 ± 0.12 vs. $1.97 \pm 0.12 \text{ l}\cdot\text{min}^{-1} \text{ }^\circ\text{C}^{-1}$; respectively; $P < 0.05$).

4.3.4. Effect of local and whole body heat stress on resting leg haemodynamics

During isolated leg heat stress, skin hyperthermia induced a significant elevation in LBF and leg vascular conductance while \dot{Q} and systemic vascular conductance were unchanged (Fig. 4.7).

Skin Hyperthermia during both whole body and isolated leg heat stress induced a significant but similar increase in LBF compared to control ($\Delta\text{LBF} = 0.50 \pm 0.07$ vs. $0.49 \pm 0.04 \text{ l}\cdot\text{min}^{-1}$, respectively; $P < 0.05$, Fig. 4.7). However, \dot{Q} only increased during whole body heat stress (i.e., $2.1 \pm 0.3 \text{ l}\cdot\text{min}^{-1}$, $P < 0.05$). With further whole body heat stress, the increase in LBF and \dot{Q} doubled (i.e., 1.05 ± 0.11 and $4.0 \pm$

0.2 $\text{l}\cdot\text{min}^{-1}$, respectively). Thus, the increase in LBF with isolated leg heating accounted for up to $52 \pm 9\%$ of the increase observed with whole-body *Skin and Core Hyperthermia*. The increases in leg blood flow and cardiac output were matched with an elevated leg and systemic vascular conductance (Fig. 4.8).

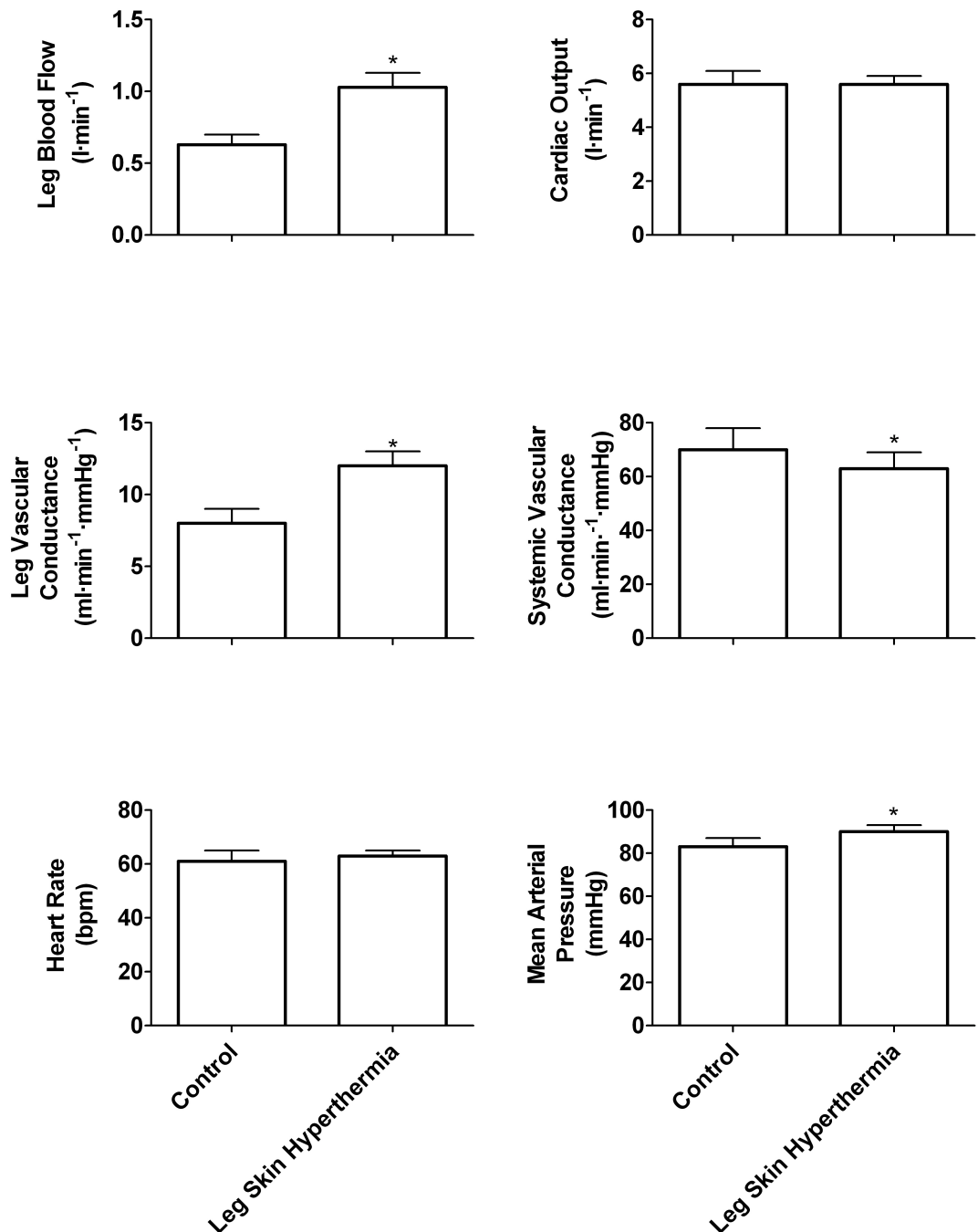


Figure 4.8. Systemic and local responses to isolated leg heat stress from control. Data are mean \pm S.E.M for 7 participants. * Different from control, $P < 0.05$. Significance was accepted at $P < 0.05$.

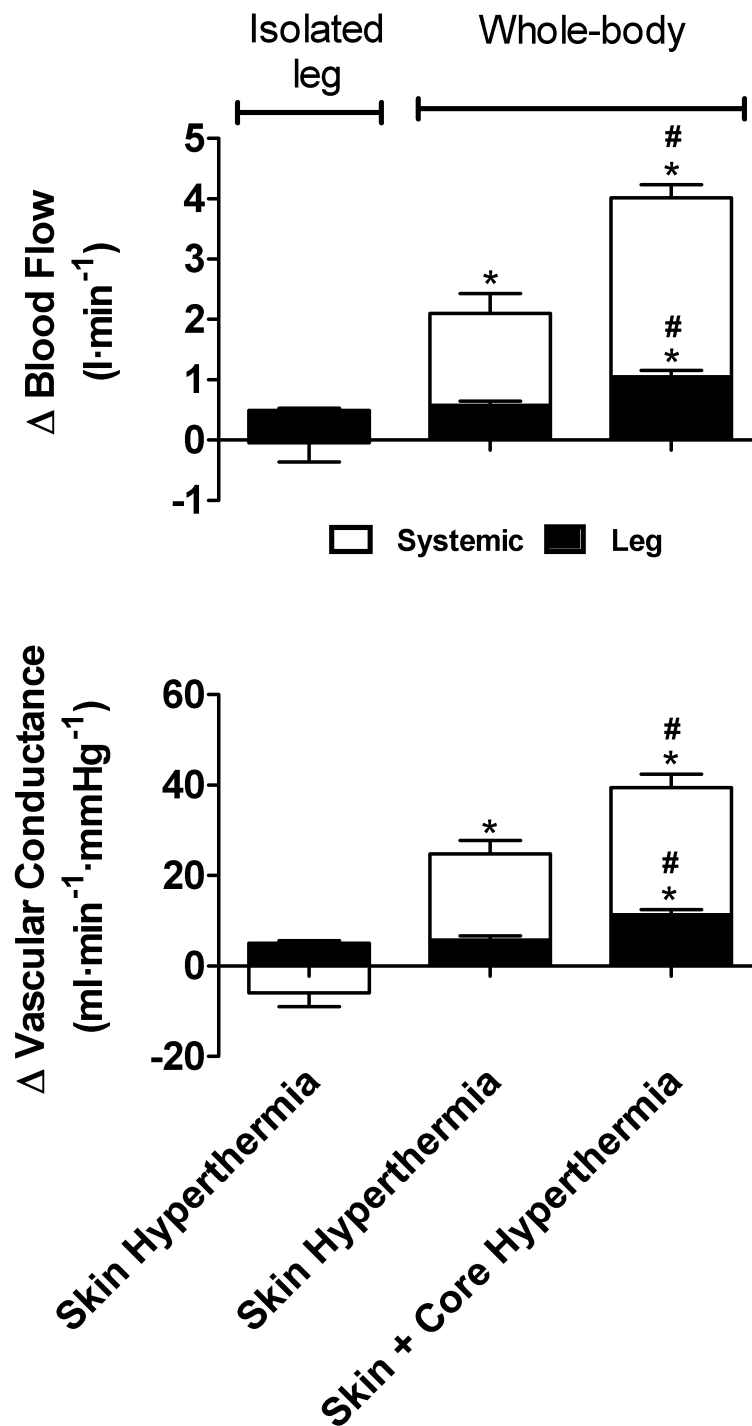


Figure 4.8. Systemic and local responses to whole-body and isolated leg heat stress from control.

Data are mean \pm S.E.M for 7 participants. * Different from control, $P < 0.05$. † Different from skin hyperthermia, $P < 0.05$. # Different from skin and mild core hyperthermia. Significance was accepted at $P < 0.05$ and refers to differences in the respective conditions, i.e. either rest or exercise.

4.3.5. Circulating plasma ATP and catecholamines during whole body heat stress

Plasma noradrenaline increased progressively with whole body heating and became significantly elevated with *Skin and Core Hyperthermia* compared to both rest and exercise control conditions ($P < 0.05$, Table 4.0). At rest, venous plasma ATP remained unchanged throughout ($P > 0.05$, Table 1) while arterial plasma ATP was strongly correlated with increases in leg vascular conductance (Fig. 4.9) and estimated quadriceps muscle temperature ($r^2 = 0.94$; $P < 0.05$ and $r^2 = 0.99$; $P < 0.05$ respectively). During exercise these positive correlations became attenuated ($r^2 = 0.68$; $P = 0.18$ and $r^2 = 0.25$; $P = 0.75$ respectively).

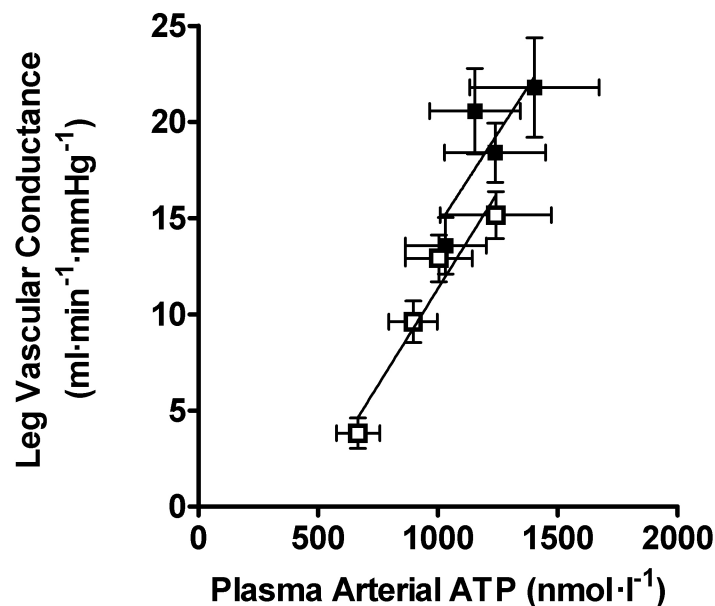


Figure 4.9. Relationship between leg vascular conductance and plasma ATP

With whole body heating at rest LVC shares a strong positive relationship with elevations in plasma arterial ATP ($r^2 = 0.94$, $P = 0.03$) suggesting that plasma ATP may be involved in the observed elevations in leg blood flow.

$$LVC \text{ ml}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1} = [0.02 \cdot \text{plasma arterial ATP (nmol}\cdot\text{l}^{-1})] - 8.739.$$

However, similarly to the attenuation in limb blood flow during exercise with heat stress compared to rest, the relationship between LVC and plasma arterial ATP was also attenuated ($r^2 = 0.71$, $P = 0.15$). Open boxes represent rest while closed boxes represent exercise measurements. Data are mean \pm S.E.M for 7 participants. * Different from previous condition, $P < 0.05$.

4.3.6. Hydration, temperature and haemodynamics during isolated leg heat stress

After 60 min of isolated leg heat stress, mean leg skin temperature increased from $31.8 \pm 0.1^\circ\text{C}$ at control to $37.4 \pm 0.1^\circ\text{C}$ ($P < 0.05$), while quadriceps muscle temperature ($n=2$) also increased from 34 ± 0.4 to $36.8 \pm 0.2^\circ\text{C}$ (Fig. 4.4). However, core temperature ($37.0 \pm 0.1^\circ\text{C}$) and hydration status, as indicated by body mass, remained unchanged. Correspondingly, LBF and muscle oxygenation increased from 0.47 ± 0.08 to $0.96 \pm 0.07 \text{ l}\cdot\text{min}^{-1}$ and $75 \pm 1\%$ to $86 \pm 1\%$ respectively ($P < 0.05$). Furthermore leg SkBF increased from 17 ± 0.5 to 45 ± 2 AU ($P < 0.05$) whereas \dot{Q} , MAP, systemic vascular conductance, heart rate, stroke volume and whole body $\dot{V}\text{O}_2$ remained unchanged.

4.4 Discussion

This study reveals four key findings that provide further insight into the role of local temperature on muscle vasodilation during whole body and isolated leg heat stress and exercise hyperaemia: (1) Leg blood flow, cardiac output and vascular conductance increased progressively with increases in body temperature at rest and during exercise. The increases in blood flow and vascular conductance accompanied a parallel reduction in leg O_2 extraction reflecting elevations in muscle and skin oxygenation and blood flow, while deep femoral vein oxygen saturation also increased. (2) The effects of graded whole body hyperthermia on elevations in leg blood flow and cardiac output were attenuated during mild exercise. (3) Elevations in leg vascular conductance occurred in association with increases in arterial plasma ATP and arterial and femoral venous noradrenaline. Although not strictly implying a cause and effect relationship, this result provides support for the theory that intravascular ATP may be, among other vasodilatory substances, involved in the signalling mechanisms overriding the increases in limb sympathetic vasoconstrictor activity thereby inducing leg vasodilation with hyperthermia. (4) Isolated leg heat stress accounted for approximately one-half of the leg hyperaemia seen during whole body heat stress without increasing cardiac output. Collectively these findings suggest that, locally, heat stress induces leg muscle and skin vasodilation and that skeletal muscle vasodilation contributes to whole body heat stress-mediated hyperaemia. However, the magnitude of increase in resting and exercising limb blood flow with severe whole body heat stress and the blunted hyperaemic response during exercise suggests that local elevations in temperature can only account for a small fraction of exercising limb muscle hyperaemia in humans.

Predictably blood flow and vascular conductance in the whole leg, skin and systemic circulations increased with graded elevations in body temperature both at rest and during mild exercise. At the level of the leg, these responses were accompanied by a parallel reduction in O_2 extraction, which contrast with the unchanged leg blood flow and O_2 extraction reported in a previous knee-extensor exercise study (Savard, et al., 1988). However, the increases in leg blood flow during exercise and heat stress generally agree with a more recent study investigating the effects of isolated leg heating upon leg metabolism during knee-

extensor exercise (Ferguson, et al., 2006). A key question herein was whether whole body heat stress causes vasodilation not only of the skin but also of the muscle vasculature. An important observation from the present study was that quadriceps muscle O₂ saturation measured with near-infrared spectroscopy was elevated with heat stress compared to control conditions both at rest and during exercise, suggesting a corresponding augmentation in quadriceps muscle blood flow.

It has been suggested that measures of skeletal muscle oxygenation using near-infrared spectroscopy (NIRS) during both whole body and isolated limb heat stress are influenced by elevations in skin blood flow (Davis, Fadel, Cui, Thomas & Crandall, 2006). Despite this suggestion, this important study by Davis and colleagues, also illustrated that during the initial exposure to whole body heat stress, muscle oxygenation increased prior to any change in skin blood flow (Fig. 3 in Davis *et al* 2006). The unchanged skin blood flow but increased muscle tissue oxygenation implies that blood flow through skeletal muscle increased during heat stress. This finding is similar to the results reported herein as whole leg blood flow increased while leg skin blood flow reached a plateau during the skin and core hyperthermia condition (Fig. 4.5). Consequently, the results of this study and that of Davis *et al* (2006) suggest that muscle blood flow is elevated together with increases in skin blood flow during heat stress.

There are several additional observations that further support a contribution of muscle vasodilation to heat stress-induced limb hyperaemia. First, deep femoral venous oxygenation increased with whole body heat stress indicating a reduced oxygen extraction reflected in the reduced deep femoral leg a-vO₂ difference (Table 4.1). Secondly, had all of the increase in leg blood flow (up to 1 l·min⁻¹ during whole body heating at rest with skin and core hyperthermia) perfused the cutaneous circulation, quadriceps muscle oxygenation would have remained stable at control levels rather than being augmented as the data presented here and other (Davis, et al., 2006) near-infrared spectroscopy findings indicate.

Finally, during whole body heat stress, increases in saphenous blood flow, the blood vessel of the leg that primarily drains the cutaneous vasculature, has been shown to only account for about ~75% of the increase in whole leg blood flow as

measured through the femoral vein (Abraham, et al., 1994). Therefore a significant proportion of the increase in whole leg blood flow during whole body heat stress remained unaccounted for by elevations in leg skin blood flow. Finally, in this study, skin blood flow demonstrated a plateau with whole body heating during the skin and core hyperthermia condition while whole leg blood flow continued to increase (Fig. 4.5). Taken as a whole, these observations indicate that the increase in whole leg blood flow with whole body and isolated leg heat stress reflects for the most part a shared contribution of skeletal muscle and skin vasodilation.

The results presented here challenge the view that limb blood flow and cardiac output increases with heat stress in sole response to an augmented thermoregulatory demand of the cutaneous circulation (Detry, et al., 1972; Edholm, et al., 1956; Johnson, et al., 1976; Roddie, et al., 1956). The concept of all the elevations in blood flow during heat stress perfusing the circulation of the skin exclusively is principally based upon two observations. Firstly, human forearm studies utilizing the 4-iodoantipyrine-¹²⁵I-clearance method suggest that muscle blood flow stays unchanged in response to heat stress (Detry, et al., 1972; Johnson, et al., 1976). However, it has since been recognized that the isotope clearance technique has several limitations. These limitations include the exchange of isotopes between arterioles and veins and also the solubility of isotopes in different tissues within skeletal muscle, making derived values too low or the methodology insensitive to accurately reflect muscle blood flow (Rowell, 1993).

The measurement of muscle blood flow using isotope clearance methods would be especially problematic in the forearm where the absolute changes in blood flow are small compared to those seen here in the leg. Secondly, it is understood that all increases in \dot{Q} that accompany whole body heat stress are directed to the cutaneous circulation and form the hypothesis that the maximal skin blood flow is $\sim 6-8 \text{ l}\cdot\text{min}^{-1}$. However this concept is based upon indirect measures of changes in \dot{Q} (Detry, et al., 1972; Minson, et al., 1998; Rowell, 1974; Rowell, et al., 1969a) and blood flow redistribution from other territories (Minson, et al., 1998) during whole body heat stress. Furthermore, while it is appreciated that increases in \dot{Q} , as illustrated by changes in whole body skin blood flow and measures of forearm

blood flow share an intimate relationship with one another (Minson, et al., 1998), it need not imply a cause and effect relationship. In this regard, the results from this study suggest that in addition to the increases in skin blood flow that are synonymous with whole body heat stress, blood flow to skeletal muscle also increases. Furthermore, the present findings provide evidence to suggest that skeletal muscle vasodilation also contributes to heat-stress mediated hyperaemia.

The effects of whole body heat stress upon increases in leg blood flow and cardiac output at rest were attenuated during mild exercise. This was evident in the blunted hyperaemic response to exercise and the smaller increase in both leg blood flow and cardiac output with each elevation in body temperature compared to the responses at rest (see Fig. 4.5 and 4.6). Nonetheless, during exercise, leg blood flow was still significantly elevated with heat stress independently of any alterations in leg $\dot{V}O_2$ (Fig. 4.5). Therefore the results suggest that during exercise, heat stress was associated with increases in leg blood flow, which similarly to resting conditions, were associated with thermoregulatory drive as opposed to an elevated metabolic activity.

The enhanced leg blood flow found herein during heat stress and exercise contrasts with the unchanged or reduced limb blood flow reported previously during moderate intensity one-legged knee-extensor exercise and submaximal and maximal cycling during heat stress (González-Alonso & Calbet, 2003; Nadel, et al., 1979; Nielsen, et al., 1990; Savard, et al., 1988). The reduction or blunting of limb blood flow during heat stress found in previous studies could be explained by the influence of reflexes sensing hypohydration and/or the events that underpin cardiovascular limitations to whole body exercise. The design of this study made it possible to isolate the effects of progressive whole body heat stress without the confounding influences of hypohydration or central circulatory limitations. An unchanged blood osmolality, electrolytes and haematological variables demonstrated that hydration status was maintained throughout (Table 4.0). The one-legged knee-extensor exercise model enabled the assessment of the effects of whole body heat stress upon exercise hyperaemia in conditions where the cardiovascular system was functioning far below its maximal capacity (Andersen & Saltin, 1985; González-Alonso, et al., 2008; Mortensen, et al., 2008).

During whole body heat stress, the ~1.6 - 5.4 °C rise in muscle, core and skin temperatures were associated with a ~0.4 l·min⁻¹ increase in exercising leg hyperaemia (Fig. 4.5). Similar elevations in muscle and core temperature are normally associated with an ~8-11 l·min⁻¹ leg hyperaemia during maximal cycling exercise in individuals of comparable characteristics (González-Alonso & Calbet, 2003; Mortensen, et al., 2008; Mortensen, et al., 2005). Thus, although the present findings indicate that locally heat stress induces noticeable muscle vasodilation its overall contribution to maximal exercise hyperaemia is small.

The mechanisms underpinning the heat stress-induced leg muscle and skin vasodilation are likely to involve local vasodilator and vasoconstrictor signals and central neural reflexes (Johnson & Proppe, 1996; Rowell, 1983). At the level of the leg, blood flow increased in the presence of an augmented sympathetic vasoconstrictor activity. This was demonstrated in this study, by the rise in circulating noradrenaline, and in other microneurography studies by the profound increase in muscle and skin sympathetic nerve activity with exposure to heat stress (Bini, et al., 1980; Niimi, et al., 1997; Ray & Gracey, 1997). Together these results indicate that heat stress and/or related factors modulate sympathetic vasoconstrictor activity such that vasodilator activity overrides vasoconstrictor activity.

In this construct the elevations in blood flow found in this study resembled functional sympatholysis, which has been shown to occur in the skeletal muscle vasculature in conditions of an increased sympathetic nervous drive during exercise and hypoxia (Hanada, et al., 2003; Remensnyder, et al., 1962; Rosenmeier, et al., 2004). In support of a role of local temperature towards the functional sympatholysis, it was observed that elevations in leg vascular conductance were intimately related to the rise in the estimated leg muscle temperature at rest ($r^2= 0.99$; $P= 0.0068$). A role of local temperature on the control of the microcirculation is consistent with observations in isolated vessel preparations (Ogura, et al., 1991; Unthank, 1992) and human forearm studies (Barcroft & Edholm, 1943; Barcroft & Edholm, 1946; Johnson, et al., 1976) showing that increases in temperature are associated with arteriolar and forearm vasodilation.

However, the aforementioned increases in leg vascular conductance were also associated with increases in plasma arterial ATP at rest and during exercise (Fig. 4.9). This stimulatory effect of hyperthermia on plasma ATP is in agreement with previous observations during severe heat stress exercise (González-Alonso, et al., 2004). In this context, it is possible that heat stress acted indirectly upon vasodilation and sympatholysis through increases in intravascular ATP. Whilst it is acknowledged that many vasodilator substances and tissue temperature *per se* may be involved in this regulatory process, ATP is an attractive candidate in the light of the recent observations indicating that intravascular ATP can act as a potent vasodilator and sympatholytic molecule in the leg and forearm (González-Alonso, et al., 2008; Kirby, et al., 2008; Rosenmeier, et al., 2004; Rosenmeier, Yegutkin & Gonzalez-Alonso, 2008). Although it is possible that intravascular ATP is involved in the heat stress-mediated limb muscle and skin vasodilation, the significant association between increases in leg vascular conductance and plasma ATP found here, does not necessarily demonstrate a cause and effect relationship.

The comparison of the haemodynamic responses to isolated leg and whole body heat stress provides information on the contribution of elevations in local versus whole body temperatures on leg blood flow and cardiac output. Interestingly, when leg muscle and skin temperatures were similar during both isolated leg and whole body skin hyperthermia conditions, leg blood flow and vascular conductance were equally elevated (Fig. 4.8) as were leg skin blood flow and muscle oxygenation. This would suggest that local temperature elevations accounted for the increased leg hyperaemia with whole body heat stress and that both the muscle and skin vasculature vasodilates in response to increases in local temperature. However, the enhanced leg hyperaemia with isolated leg heat stress accounted for only one-half of the increase in leg blood flow associated during whole body heating with skin and core hyperthermia. In this regard, quadriceps muscle temperature was $\sim 1^{\circ}\text{C}$ lower with isolated leg heating compared to whole body heating in the skin and core hyperthermia condition. Therefore, it remains plausible that elevations in local limb temperature induced by one-legged heat stress can explain all or the majority of the leg hyperaemia that is associated with whole body heat stress.

Unfortunately, it is not possible to increase leg muscle temperature in excess of 38°C while maintaining core temperature at control levels under the present free flow conditions due to the continuous heat fluxes between the leg and the rest of the body. Taken together with previous findings (Johnson, et al., 1976) the results presented here during isolated leg heating indicate that elevations in local temperature account for a large portion of the limb hyperaemia produced by whole body heat stress.

A salient observation was that, in contrast to the similar leg hyperaemia, \dot{Q} only increased during whole body heat stress (Fig. 4.8). This could indicate that when the blood flow demand is relatively small during one-legged heat stress the increase in limb blood flow is met primarily by redistribution of blood flow from other territories, similarly to whole body heat stress (Minson, et al., 1998). Therefore, in line with these earlier observations, the results presented here suggest that when vasodilation occurs throughout the skin and muscle of the whole body, the greater global demand for blood flow invokes an increase in \dot{Q} in addition to blood flow redistribution from other territories.

An experimental limitation of using a water-perfused suit to induce whole body or isolated limb heat stress and help determine the role of temperature upon exercise hyperaemia is that the rise in tissue temperature and limb blood flow is slow in comparison to the rapid hyperaemia that occurs with the onset of muscular contractions. Despite this, analysis of temperature and blood flow kinetics during exercise confirms a rapid increase in muscle temperature and leg blood flow and cardiac output during control and heat stress conditions (D.A. Low, J. Pearson, M. Lotlikar & J. González-Alonso, unpublished observations; González-Alonso *et al* 2000), thus supporting the present conclusions based on the comparison of the steady-state resting and exercise responses.

4.5. Conclusion

In summary, the findings presented here challenge the belief that the augmented leg blood flow and cardiac output with heat stress is due solely to an elevation in skin blood flow. The evidence presented here suggests that elevations in leg blood flow with heat stress are due to vasodilation of both muscle and skin vasculatures. The prevailing limb vasodilation with heat stress, despite an augmented local sympathetic vasoconstrictor activity, is associated with both muscle hyperthermia and the increased arterial plasma ATP. The present observations also indicate that mild exercise attenuates the effects of heat stress upon leg blood flow and cardiac output seen at rest and that local hyperthermia accounts for a small fraction of maximal exercise hyperaemia. While this study provides an insight into the role of elevations in temperature upon leg muscle and skin blood flow and cardiac output, the impact of alterations in blood volume and changes in blood oxygen content and haemoglobin concentrations in resting and mildly exercising humans remains somewhat unclear.

CHAPTER 5

Study 2

5.0. Summary

Hypohydration and hyperthermia reduce active leg blood flow and cardiac output during whole-body exercise in humans. This phenomenon is possibly due to concomitant haemoconcentration and/or a compromised cardiovascular regulation. Whether hypohydration and hyperthermia reduce leg muscle and skin blood flow and cardiac output at rest and during small muscle mass exercise is unknown. To further examine the influence of hypohydration, related haematological alterations and hyperthermia upon cardiovascular function and regulation, leg, skin and systemic haemodynamics, oxygenation and plasma catecholamines and ATP were measured at rest and during 6-min of submaximal one-legged knee-extensor exercise (~23 W) in 7 males in conditions where hydration status and core temperature (T_c) were manipulated: 1) control (0% body mass loss, T_c ~37°C), 2) mild hypohydration (~2%, ~38°C), 3) moderate hypohydration (~3.5%, ~38°C), and 4) rehydration (0%, ~37°C). At rest, leg blood flow (LBF) and cardiac output (\dot{Q}) increased with both mild and moderate hypohydration (LBF: $75 \pm 21\%$ and $112 \pm 35\%$, respectively; $P < 0.05$) and remained elevated after rehydration, while leg skin blood flow (SkBF) and leg a-vO₂ difference were unchanged ($P > 0.05$). During exercise, LBF increased $15 \pm 3\%$ with moderate hypohydration and remained elevated after rehydration ($P < 0.05$) yet leg SkBF, leg a-vO₂ difference and \dot{Q} remained unchanged (all $P > 0.05$). The enhanced LBF at rest and during exercise was accompanied by an increased leg vascular conductance and, during exercise, reductions in blood flow pressure indicating a net leg vasodilation despite increasing plasma noradrenaline and diminishing plasma ATP. The findings presented here suggest that mild and moderate hypohydration and hyperthermia do not compromise leg muscle or skin blood flow or cardiac output in resting and mildly exercising humans. Furthermore, at rest and during mild exercise, where a large cardiovascular reserve is available, rehydration and mild alterations in blood oxygen content, concentration of haemoglobin in blood and blood volume have minimal effects upon leg blood flow and cardiac output in humans.

5.1. Introduction

During prolonged exercise in the heat, the combination of hypohydration and hyperthermia induces significant reductions in cardiac output (\dot{Q}) (Montain & Coyle, 1992b; Sawka, et al., 1979). The reduction in \dot{Q} is suggested to be due to a compromised stroke volume (SV), which in turn is caused by the synergistic effects of hypohydration-induced hypovolaemia (and/or haemoconcentration) and hyperthermia-mediated tachycardia (González-Alonso, et al., 1998; González-Alonso, et al., 1997; González-Alonso, et al., 1999b, 2000; Montain & Coyle, 1992b; Nadel, et al., 1980; Sawka, et al., 1979). The fall in \dot{Q} is accompanied by a small drop in arterial pressure and significant reductions in blood flow to the active skeletal muscle (González-Alonso, et al., 1998) and skin (Fortney, Nadel, Wenger & Bove, 1981; Fortney, Wenger, Bove & Nadel, 1983; Fujii, Honda, Hayashi, Kondo & Nishiyasu, 2008; González-Alonso, et al., 1998; González-Alonso, et al., 1995; Montain & Coyle, 1992b; Nadel, et al., 1980).

The maintenance of hydration status via fluid replacement during exercise prevents the aforementioned negative effects of hypohydration and hyperthermia upon systemic haemodynamics (González-Alonso, et al., 2000; Montain & Coyle, 1992b). Oral fluid replacement helps restore blood haematological and volume changes that occur with exercise-induced hypohydration and hyperthermia, and can sometimes result in haemodilution and plasma volume expansion (Kenefick, et al., 2007). In this regard, reductions in blood flow to the active skeletal muscle during prolonged exercise that causes a significant hypohydration and hyperthermia have been associated with concomitant elevations in blood oxygen content (Calbet, 2000). As such the deleterious effects of hypohydration and hyperthermia upon leg muscle and skin blood flow and cardiac output may, at least in part, be regulatory responses to reductions in blood volume and/or haemoconcentration.

Similar haematological changes to those occurring with hypohydration and rehydration have been implicated in the control of exercising limb blood flow and cardiac output during exercise, possibly via erythrocyte-derived vascular signals such as, but not exclusive to, ATP release (Gonzalez-Alonso, Mortensen, Dawson, Secher & Damsgaard, 2006). However, it remains unknown whether alterations in

the haematological profile of blood occurring with both hypohydration and rehydration independently affect leg muscle and skin blood flow and cardiac output in resting and mildly exercising humans. It was reasoned that if hypohydration-induced haemoconcentration and concomitantly elevated arterial O₂ content were major contributors to the reported reductions in blood flow they should be manifested at rest and during mild exercise. In this construct following rehydration the restoration of blood oxygen content and haemoglobin concentrations to control levels, any reductions in blood flow should be fully restored at rest and during mild exercise.

The effects of hypohydration and hyperthermia and related factors on skeletal muscle and skin blood flow and cardiac output at rest and during mild intensity exercise are unclear. The available literature in resting hypohydrated and hyperthermic humans suggests that \dot{Q} is unchanged whereas limb blood flow is either increased (Fan, et al., 2008; Lynn, et al., 2009) or unaltered (Horstman & Horvath, 1972; Kenney, et al., 1990). However, in these previous studies an assessment as to whether any alterations in blood flow and/or metabolism occurred across the leg was absent and thus an insight into limb muscle and skin blood flow is not available. Furthermore, it is unknown whether leg blood flow and cardiac output decline with hypohydration and hyperthermia during mild intensity small muscle mass exercise where a large cardiovascular reserve is available (Andersen & Saltin 1985; Mortensen et al. 2008).

The purpose of this study was three-fold: 1) to determine whether combined hypohydration and hyperthermia is associated with reductions in leg muscle and skin blood flow and cardiac output in resting and mildly exercising humans, 2) to examine whether rehydration restores any declines in leg muscle and skin blood flow and cardiac output associated with hypohydration and hyperthermia, and 3) to investigate whether the haematological changes occurring with hypohydration and hyperthermia play a role in the reductions in leg blood flow and cardiac output during whole body exercise. To accomplish these aims, leg, skin and systemic haemodynamics, blood oxygenation, plasma catecholamines and ATP were measured at rest and during mild one-legged knee-extensor exercise in healthy male volunteers. Measurements were made during control conditions and both mild and moderate levels of combined hypohydration and hyperthermia and

following acute oral rehydration. It was hypothesised that: 1) leg muscle and skin blood flow and cardiac output will be reduced at rest and during mild exercise, 2) reduced leg blood flow and cardiac output will be closely related to reductions in blood volume, haemoconcentration and concomitant alterations in vasodilator and vasoconstrictor activities reflected, to some extent, by changes in ATP and plasma catecholamines and 3) rehydration will restore leg blood flow and cardiac output to control levels accompanying the restoration of blood volume and haematological variables.

5.2. Methods

5.2.1. Participants

Seven healthy recreationally active males (mean \pm SD age 20 ± 1 years, body mass 74.6 ± 8.8 kg and height 180 ± 3 cm) participated in this study. This study conformed to the code of Ethics of the World Medical Association (Declaration of Helsinki) and was conducted after ethical approval from the Brunel University Research Ethics Committee. Informed written and verbal consent was obtained from all participants before participation.

5.2.2. Design

Leg and systemic haemodynamics and quadriceps muscle oxygenation were examined at rest and during moderate one-legged knee-extensor exercise (mean \pm SEM 23 ± 1 W at ~ 65 rpm for 6 min) in 4 different consecutive conditions whilst supine: 1) *Control*, euhydration, 2) *Mild Hypohydration*, approximately 2% hypohydration as assessed by body mass loss, 3) *Moderate Hypohydration*, approximately 3.5% hypohydration and 4) *Rehydration*, after ingestion of a commercially available carbohydrate-electrolyte beverage equating to participants body mass loss (Fig. 5.0).

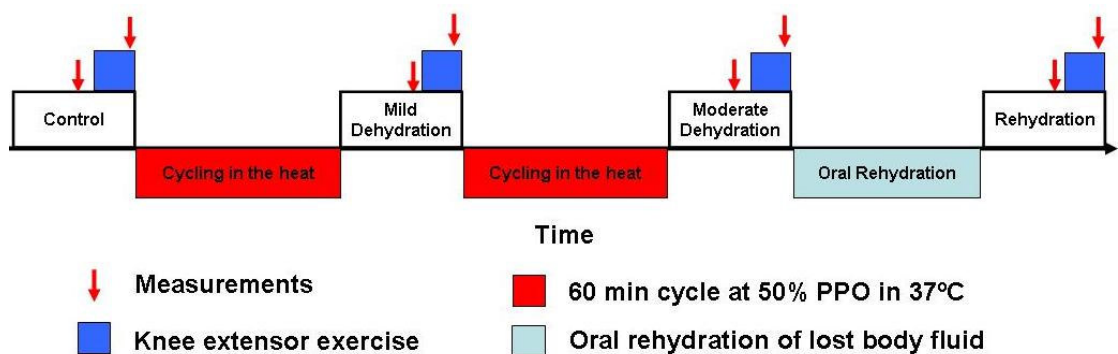


Figure 5.0. The experimental protocol for study 2.

To achieve mild and then moderate hypohydration, participants engaged in 60 min of cycling on an electromagnetically braked cycle ergometer (Excalibur; Lode, Groningen, The Netherlands) in a heat chamber at $\sim 37^\circ\text{C}$ and 60% humidity at

~50% of peak power output to induce hypohydration via water losses due to sweating.

5.2.3. Instrumentation of participants

On the morning of the main experiment participants arrived in the laboratory after eating a light breakfast. After insertion of the rectal thermister and recording of body mass, participants rested in the supine position while catheters were placed into the radial artery and the femoral vein of the exercising leg as previously described in the general methods. The participants then walked to the experimental room and lay supine on the reclining chair that was part of a knee-extensor ergometer (Ergometer LE220, FBJ Engineering, Denmark) and the left foot and ankle were inserted into the boot of the knee extensor ergometer. Participant's lower legs were supported during resting conditions.

5.2.4. Temperature and blood volume measurements

Skin thermisters were placed on six sites: upper back, lower back, chest, abdomen, thigh and foot (Sable Systems, Las Vegas, NV, USA). The thermisters were securely held in place throughout the protocol by the use of adhesive spray and medical tape. Rectal temperature was measured as previously described. Skin and rectal temperatures were monitored continuously online (TC-2000, Sable Systems, Las Vegas, NV, USA). Weighted mean skin temperature was calculated using methods described previously (Taylor, Johnson, Kosiba & Kwan, 1989). At control blood volume was estimated based upon results from previous studies (Sawka, Young, Pandolf, Dennis & Valeri, 1992). Thereafter changes in blood volume were calculated from measurements of haemoglobin as previously described (Dill & Costill, 1974).

5.2.5. Systemic haemodynamics

Baseline systemic haemodynamics were measured immediately prior to exercise after a minimum of 20 min supine rest. During exercise these measurements were repeated between min 4 and 6. As previously described pressure transducers within the arterial and venous catheters were used to measure arterial and venous blood pressure, respectively. Heart rate was obtained from a 3 lead electrocardiogram while arterial and femoral venous pressure waveforms were

continuously recorded at the level of the heart via pressure transducers (Pressure Monitoring Kit, Baxter) connected to two amplifiers (BP amp, ADInstruments, Bella Vista, NSW, Australia) and monitored online via a data acquisition system (Powerlab 16/30 ML 880/P, ADInstruments, Bella Vista, NSW, Australia). \dot{Q} was calculated as the product of heart rate and stroke volume, where stroke volume was estimated using the Modelflow method from direct measures of arterial pressure as described in the general methods. Furthermore, leg and skin haemodynamics and arterial and venous blood samples were obtained at rest and after 5 min of exercise and analysed as previously described.

5.2.6. Statistics

A one-way repeated measures analysis of variance (ANOVA) was performed on all dependent variables to test significance among the control and 3 conditions of altered hydration status and body temperature at rest and during exercise. When a significant difference ($P < 0.05$) was found, appropriate *post-hoc* analysis of the data was conducted using a Bonferroni correction ($P < 0.0125$) where appropriate.

5.3. Results

5.3.1. Hydration and temperature changes with hypohydration and rehydration

Body mass progressively decreased from control to mild and moderate hypohydration (from 74.6 ± 2.8 to 73.3 ± 2.7 and 72.1 ± 2.7 kg; respectively, $P < 0.05$, Fig. 5.1), corresponding to a 1.9 ± 0.1 and 3.4 ± 0.1 % reduction in body mass, respectively.

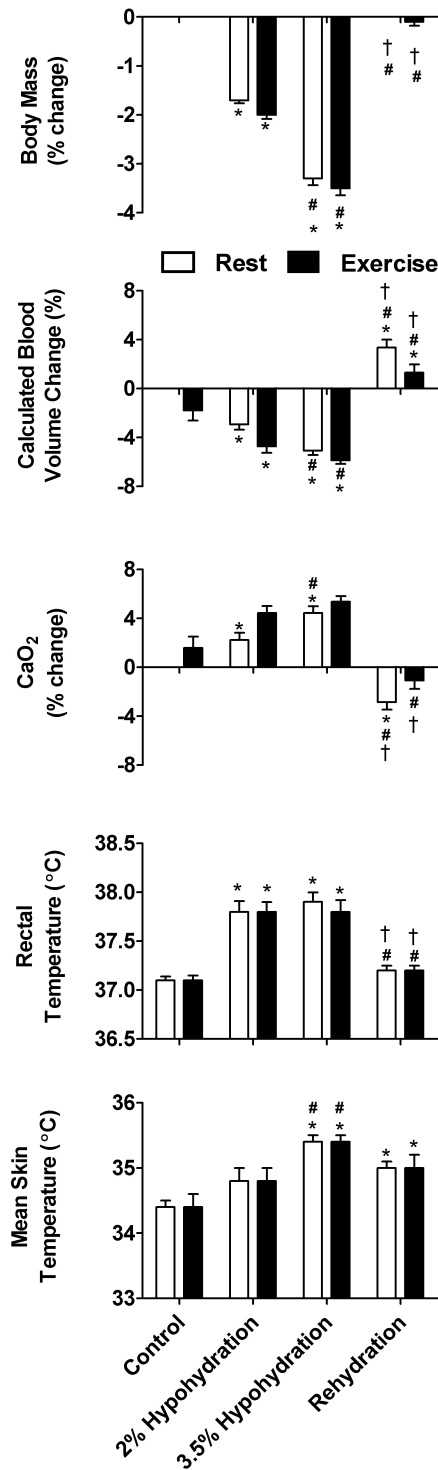


Figure 5.1. Body temperature and markers of hydration status at rest and during exercise.

Data are mean±S.E.M for 7 participants. * Different from control, # different from 2% hypohydration, † different from 3.5% hypohydration. Significance was accepted at $P < 0.05$ and refers to differences in the respective conditions, i.e. either rest or exercise.

Progressive declines in body mass were accompanied by gradual reductions in blood volume (from 5148 ± 229 to 5000 ± 230 and 4887 ± 214 ml; respectively, $P < 0.05$) and plasma volume (from 3208 ± 143 to 3047 ± 144 and 2911 ± 126 ml; P

< 0.05, respectively). Following rehydration, body mass was fully restored (74.6 ± 2.8 kg) while blood and plasma volumes were elevated above control (5322 ± 239 and 3403 ± 165 ml; respectively, both $P < 0.05$). Furthermore, rectal temperature (T_c) was significantly elevated above control with mild and moderate hypohydration (from 37.1 ± 0.1 vs. 37.8 ± 0.1 and $37.9 \pm 0.1^\circ\text{C}$; both $P < 0.05$, respectively) but returned to baseline following rehydration ($37.2 \pm 0.1^\circ\text{C}$). Mean skin temperature (T_{sk}) increased slightly with hypohydration and became significantly elevated with moderate hypohydration compared to control (from 34.4 ± 0.1 vs. $35.4 \pm 0.1^\circ\text{C}$; both $P < 0.05$, respectively). T_{sk} remained elevated following rehydration ($35.0 \pm 0.1^\circ\text{C}$; $P < 0.05$). Hypohydration-induced hypovolaemia was accompanied by increases in blood osmolality, blood electrolytes and arterial oxygen content ($P < 0.05$; Table 5.0). Similarly to study 1, the individual substances, Na^+ , K^+ and Cl^- will not be discussed individually. Rather they will be indirectly discussed as one in terms of osmolality. All reported body masses, blood and plasma volumes and temperatures represent those obtained during resting conditions as no significant change was observed from rest to exercise ($P > 0.05$, Fig. 5.1).

Table 5.0. Blood variable responses to hypohydration and hyperthermia at rest and during exercise.

		Control		2% Hypohydration		3% Hypohydration		Rehydration	
		Rest	Exercise	Rest	Exercise	Rest	Exercise	Rest	Exercise
Hb (g·l ⁻¹)	<i>a</i>	146±1	149±2	151±2*	153±2*	154±1*#	155±2*#	141±2*#†	144±2*#†
	<i>v</i>	147±2	152±2	151±2*	155±2	154±2*#	157±2*#	142±2*#†	145±2*#†
Hct (%)	<i>a</i>	44.9±0.4	45.7±0.5	46.1±0.5*	47.1±0.5	47.3±0.5*#	47.7±0.4*	43.5±0.5*#†	44.4±0.5#†
	<i>v</i>	45.3±0.4	46.8±0.5	46.5±0.5*	47.4±0.6	47.4±0.4*#	48.1±0.4*	43.7±0.4*#†	44.6±0.5*#†
O₂ Sat (%)	<i>a</i>	99.1±0.1	98.7±0.1	98.5±0.2	98.4±0.2	98.0±0.3	98.0±0.2	99.0±0.1	98.0±0.0
	<i>v</i>	66.6±3.8	42.7±2.2	73.0±2.9	38.3±2.0	72.5±2.8	43.2±1.9	72.4±2.5	45.1±2.0#
CtO₂ (ml·l ⁻¹)	<i>a</i>	199±1	202±2	203±2*	208±2	208±2*#	210±2	193±2*#†	197±2#†
	<i>v</i>	134±7	90±5	152±7	82±4*	154±6#	93±4#	141±5	91±4#
Na⁺ (mmol·l ⁻¹)	<i>a</i>	141±1	141±2	143±2*	143±2*	145±2*#	145±2#	137±1*#†	139±2#†
	<i>v</i>	141±1	142±1	143±2*	144±2	145±2*#	147±2*#	138±1*#†	140±2#†
K⁺ (mmol·l ⁻¹)	<i>a</i>	3.7±0.1	3.9±0.1	3.8±0.1	4.1±0.1	4.1±0.1*#	4.3±0.1	3.5±0.05†	3.7±0.05#†
	<i>v</i>	3.7±0.1	4.2±0.05	3.9±0.1	4.3±0.1	4.2±0.1	4.4±0.1	3.5±0.05#†	3.9±0.07#†
Cl⁻ (mmol·l ⁻¹)	<i>a</i>	108±2	108±2	110±2	110±2*	111±2*	111±2*#	105±2*#†	106±2#†
	<i>v</i>	106±2	105±2	108 ±2*	107±2*	110±2*	109±2*#	104±1*#†	104±2#†
Glucose (mmol·l ⁻¹)	<i>a</i>	5.8±0.4	5.3±0.2	5.3±0.1	5.0±0.1	4.5±0.1#	4.4±0.1*#	7.3±0.2#†	6.1±0.3†
	<i>v</i>	5.2±0.2	5.2±0.2	4.9±0.1	4.6±0.1	4.0±0.1*	4.1±0.1*#	6.2±0.3†	5.6±0.3†
Osmolality (mOsm·kg ⁻¹)	<i>a</i>	287±2	288±3	292±3*	291±3	295±3*#	295±3#	281±3*#†	284±3*#
	<i>v</i>	287±2	290±3	291±3	293±3	294±3*#	297±3*#	282±3*#†	285±3#†
ATP (nmol·l ⁻¹)	<i>a</i>	835±133	1087±139	638±72	676±84*	594±44	542±91*	427±68*#†	485±74*#
	<i>v</i>	998±173	1324±173	949±177	894±62*	642±40	591±71*#	611±61	886±144*
Noradrenaline (nmol·l ⁻¹)	<i>a</i>	0.8±0.1	1.0±0.1	1.3±0.2	1.8±0.3	1.3±0.1	1.8±0.1	1.3±0.1	1.4±0.2
	<i>v</i>	1.2±0.4	1.1±0.1	1.4±0.2	1.6±0.1*	1.1±0.1	1.5±0.2*	1.4±0.2	1.9±0.2*
Adrenaline (nmol·l ⁻¹)	<i>a</i>	0.54±0.06	0.53±0.03	0.53±0.05	0.56±0.04	0.55±0.09	0.60±0.10	0.47±0.05	0.54±0.07
	<i>v</i>	0.32±0.03	0.41±0.03	0.36±0.05	0.40±0.02	0.36±0.10	0.48±0.09	0.26±0.03	0.38±0.03

Values are mean±SEM for 7 participants, except for plasma ATP (n=6). Hb denotes haemoglobin; O₂ sat, percentage oxygen saturation; and CtO₂, oxygen content in blood. CtO₂ and osmolality were corrected for core temperature. * Different from control, *P*<0.05. # Different from 2% hypohydration, *P*<0.05. † Different from 3.5% hypohydration *P*<0.05.

5.3.2. Resting haemodynamic responses

At rest, LBF increased from control with both mild and moderate hypohydration (0.38 ± 0.04 vs. 0.64 ± 0.06 and 0.77 ± 0.09 $\text{l}\cdot\text{min}^{-1}$; respectively, $P < 0.05$, Fig. 5.2) but returned towards basal levels following rehydration (0.63 ± 0.05 $\text{l}\cdot\text{min}^{-1}$).

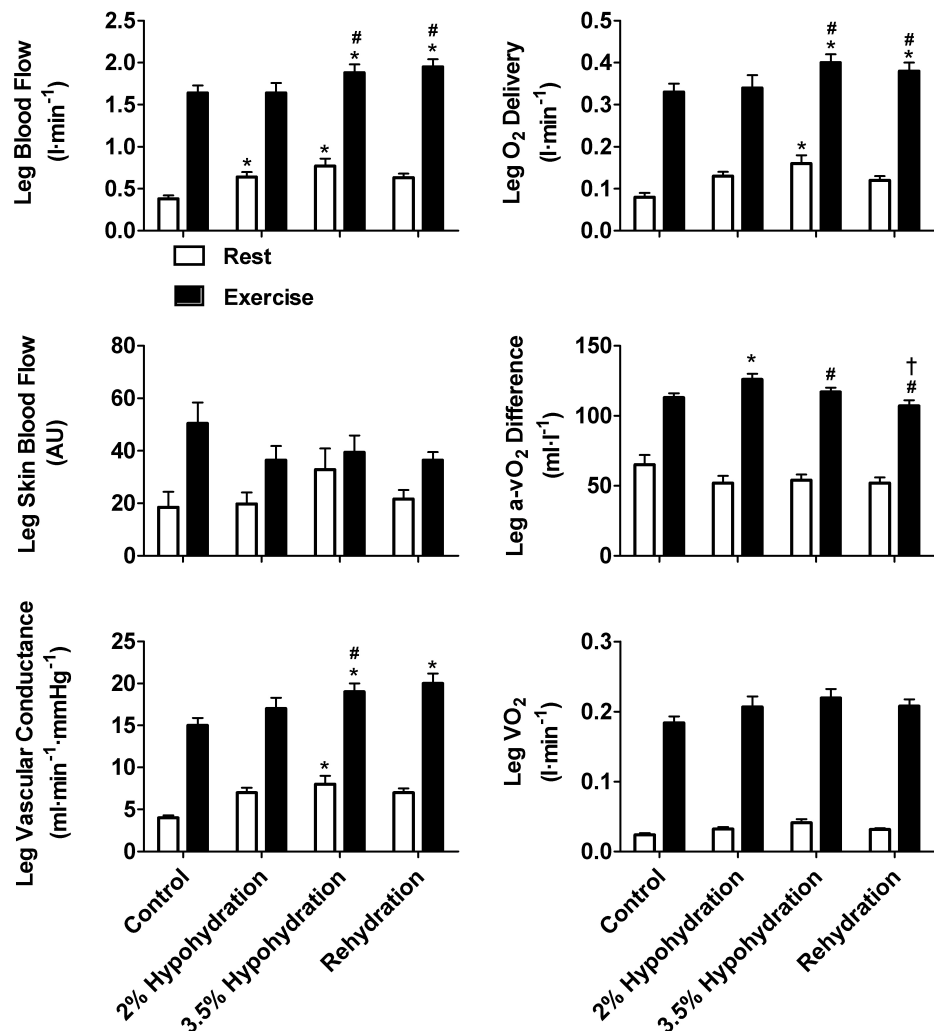


Figure 5.2. Leg haemodynamics and oxygen consumption with hypohydration and hyperthermia

Data are mean \pm S.E.M for 7 participants, apart from leg skin blood flow where $n = 6$. * Different from control, # different from 2% hypohydration, † different from 3.5% hypohydration. Significance was accepted at $P < 0.05$ and refers to differences in the respective conditions, i.e. either rest or exercise.

Elevations in LBF were accompanied by an unchanged leg a-vO₂ difference and leg $\dot{V}O_2$ ($P > 0.05$; Fig. 5.2) while leg SkBF remained unaltered in all conditions ($P > 0.05$). In all resting conditions, leg blood flow pressure was unchanged while elevations in LBF were associated with increases in leg vascular conductance,

which became significant during moderate hypohydration (4 ± 1 vs. 8 ± 1 $\text{ml}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$; $P < 0.05$, Fig. 5.2). Following rehydration leg vascular conductance returned to levels not statistically different from control (Fig. 5.2).

At the level of the systemic circulation, mean arterial pressure remained stable while \dot{Q} and systemic vascular conductance increased with moderate hypohydration (from 5.8 ± 0.3 vs. 6.6 ± 0.3 $\text{l}\cdot\text{min}^{-1}$ and from 58 ± 3 vs. 69 ± 3 $\text{ml}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$, respectively; both $P < 0.05$, Fig. 5.3) and remained elevated after rehydration (6.7 ± 0.2 $\text{l}\cdot\text{min}^{-1}$ and 68 ± 3 $\text{ml}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$; respectively, $P < 0.05$, fig. 5.3). The increased \dot{Q} was due to elevations in heart rate ($P < 0.05$) as stroke volume declined with mild and moderate hypohydration ($P < 0.05$). Following rehydration stroke volume returned to control levels while heart rate remained elevated ($P < 0.05$).

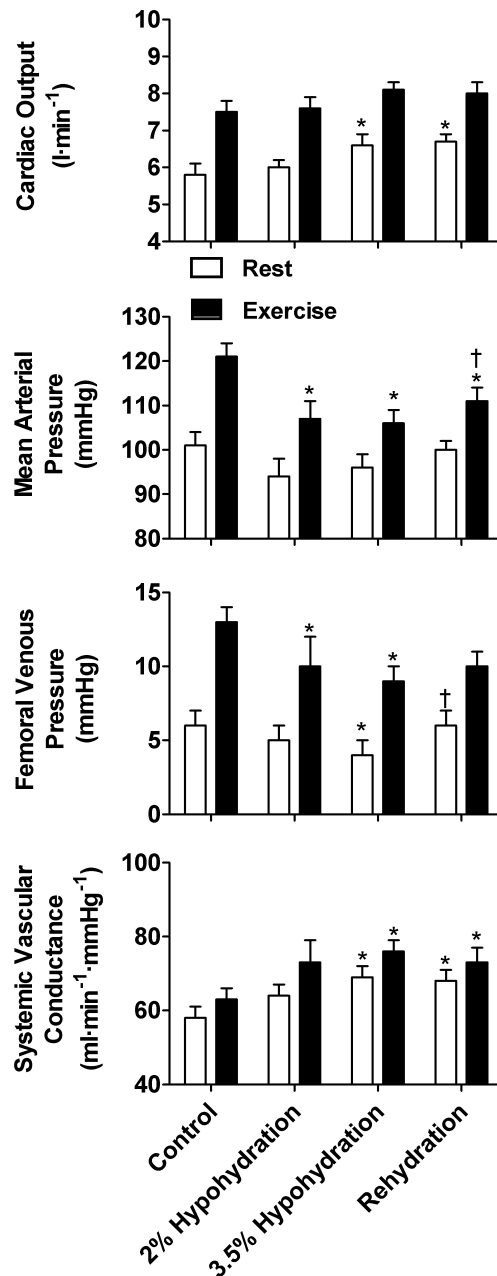


Figure 5.3. Systemic haemodynamics with hypohydration and hyperthermia

Data are mean±S.E.M for 7 participants. * Different from control, # different from 2% hypohydration, † different from 3.5% hypohydration. Significance was accepted at $P < 0.05$ and refers to differences in the respective conditions, i.e. either rest or exercise.

At rest, plasma ATP did not change significantly with mild and moderate hypohydration but declined following rehydration ($P < 0.05$; Fig. 5.4). Plasma arterial and venous adrenaline and noradrenaline were unchanged throughout all resting conditions ($P > 0.05$; Fig. 5.4).

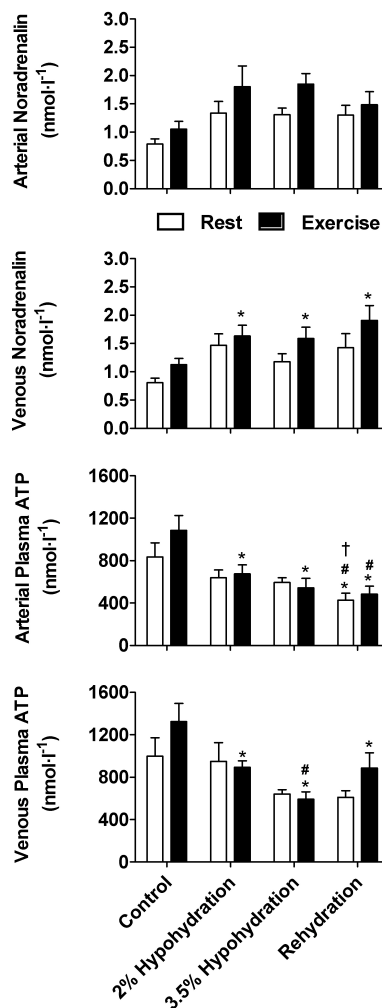


Figure 5.4. Plasma catecholamines and ATP with hypohydration and hyperthermia

Data are mean \pm S.E.M for 7 participants. * Different from control, # different from 2% hypohydration, † different from 3.5% hypohydration. Significance was accepted at $P < 0.05$ and refers to differences in the respective conditions, i.e. either rest or exercise.

5.3.3 Exercising haemodynamic responses

During exercise, LBF was unchanged with mild hypohydration but increased with moderate hypohydration and remained elevated following rehydration (1.64 ± 0.09 vs. 1.88 ± 0.1 and 1.95 ± 0.09 l \cdot min⁻¹, respectively; $P < 0.05$, Fig. 5.2). The increase in LBF was accompanied by reductions in leg blood flow pressure, an enhanced leg vascular conductance (15 ± 1 vs. 19 ± 1 and 20 ± 1 ml \cdot min⁻¹ \cdot mmHg⁻¹; respectively, $P < 0.05$) and an unchanged leg a-vO₂ difference and $\dot{V}O_2$ (Fig. 5.2). Similarly to resting conditions, leg SkBF was unchanged with mild and moderate hypohydration. At the level of the systemic circulation, \dot{Q} remained unchanged with both mild and moderate hypohydration (7.5 ± 0.3 vs. 8.1 ± 0.2 and

$8.0 \pm 0.3 \text{ l}\cdot\text{min}^{-1}$; respectively, $P > 0.05$). However, with moderate hypohydration and following rehydration, systemic vascular conductance increased due to reductions in mean arterial pressure (both $P < 0.05$; Fig. 5.3). Heart rate increased and stroke volume declined with both mild and moderate hypohydration (both $P < 0.05$). Following rehydration, stroke volume returned to control levels while HR remained elevated.

During exercise, arterial and venous plasma ATP declined with both mild and moderate hypohydration and remained below control values following rehydration (all $P < 0.05$; Fig. 5.4). Venous noradrenaline concentration increased with both mild and moderate hypohydration and remained elevated compared to control following rehydration ($P < 0.05$). Plasma arterial and venous adrenaline and arterial noradrenaline were unchanged throughout all conditions during exercise ($P > 0.05$; Fig. 5.4).

5.4. Discussion

There were two novel findings of this study. First, blood flow through the leg and systemic circulations was maintained with hypohydration and hyperthermia at rest and during mild one-legged exercise despite significant core hyperthermia, hypovolaemia and haemoconcentration. While blood flow through the whole leg was stable, both leg a-vO₂ difference and leg skin blood flow were maintained, suggesting that leg muscle and skin blood flow were not reduced with combined hypohydration and hyperthermia at rest or during mild exercise. Secondly, the restoration of body water losses achieved through oral rehydration restored core temperature and was associated with hypervolaemia and haemodilution. However, despite the restoration of blood volume leg blood flow and cardiac output remained unchanged. Taken together, these findings demonstrate that mild and moderate hypohydration and hyperthermia do not compromise leg muscle and skin blood flow or cardiac output in resting and mildly exercising humans. Furthermore, in the present conditions of short-term low cardiovascular strain, rehydration and an altered haematological profile appear to have a negligible effect upon cardiac output and blood flow through the skeletal muscle and skin.

The results presented here suggest that leg muscle and skin blood flow and cardiac output were maintained with hypohydration and hyperthermia in resting and mildly exercising humans. These findings contrast with previous reports at rest (Horstman & Horvath, 1972; Kenney, et al., 1990) and during whole body exercise (González-Alonso, et al., 1998; González-Alonso, et al., 1995, 1997; González-Alonso, et al., 2000; Hamilton, Gonzalez-Alonso, Montain & Coyle, 1991; Montain & Coyle, 1992b; Nadel, et al., 1980; Rowell, 1974; Rowell, et al., 1966).

Previously, reductions in muscle and skin blood flow with combined hypohydration and hyperthermia have been reported to occur due to a compromised cardiac output. Specifically, both a hyperthermia mediated increase in heart rate and a reduction in stroke volume associated with hypovolaemia (González-Alonso, et al., 1998; González-Alonso, et al., 2000). The reduction in stroke volume and elevations in heart rate combine to reduce cardiac output and perfusion pressure, *ergo* exercising leg blood flow declines (González-Alonso, et al., 1998; González-Alonso, et al., 2000). Contrastingly, in the present study \dot{Q} was maintained at rest and during exercise while mean arterial pressure was only slightly reduced during exercise. In line with the maintenance of \dot{Q} , leg a-vO₂ difference and leg $\dot{V}O_2$,

were unchanged throughout. Furthermore, skin blood flow was unchanged with hypohydration and hyperthermia while leg and systemic vascular conductance did not decline. The maintenance of cardiovascular function in this study may be due to a comparatively smaller cardiovascular strain owing to lower levels of hyperthermia, supine body positioning and a small muscle mass exercise model.

The magnitude of hyperthermia upon the response of leg blood flow and cardiac output to combined hypohydration and hyperthermia is important for two reasons. First, during whole body exercise, hyperthermia mediated increases in heart rate have been suggested to reduce cardiac filling time. A reduction in filling time can compromise stroke volume and ultimately contribute to a reduction in cardiac output (González-Alonso, et al., 1999b). In contrast, despite a significant hypohydration, stroke volume is maintained during whole body exercise in a cold environment (González-Alonso, et al., 2000). With a similar level of hypohydration but a lower absolute core body temperature, it was found that reductions in stroke volume were mild and that mean arterial pressure and plasma noradrenaline (Fig. 5.3 and 5.4) were maintained at rest and only slightly altered during exercise. Importantly, the increase in heart rate in the present study fully compensated for reductions in stroke volume, thus cardiac output was maintained.

Secondly, reductions in leg blood flow and cardiac output with hypohydration and hyperthermia are classically associated with an initial elevation in skin blood flow owing to elevations in body temperature and a consequent fall in central blood volume and mean arterial pressure (González-Alonso, et al., 1998; González-Alonso, et al., 1995). In response both high and low pressure baroreceptors are unloaded (Mack, Thompson, Doerr, Nadel & Convertino, 1991; Renlund, Gerstenblith, Fleg, Becker & Lakatta, 1990), which increases vasoconstrictor drive and/or reduces vasodilator signalling and consequently skin blood flow declines (González-Alonso, et al., 1999b). Thus with a lower level of core hyperthermia, the potential impact of tachycardia upon reductions in stroke volume and cardiac output is lower. In this regard, with a lower magnitude of hyperthermia, the drive for skin blood flow would be reduced preventing the large declines in central blood volume which have been suggested to contribute to a reduced systemic cardiovascular functioning. In the present study this was supported by an

unchanged skin blood flow (Fig. 5.2) and plasma noradrenaline concentrations (Fig. 5.4) with combined hypohydration and hyperthermia.

In addition to the effects of a lower absolute core temperature, a supine body position during exercise has been suggested to help maintain central blood volume (González-Alonso, et al., 1999b). The maintenance of central blood volume can ameliorate the reductions in stroke volume, mean arterial pressure and skin blood flow that are normally observed with combined hypohydration and hyperthermia during upright whole body exercise (González-Alonso, et al., 1999b). Despite a significant combined hypohydration and hyperthermia, the protective effect of supine body positioning upon central blood volume may have helped maintain leg blood flow and cardiac output. In addition, the one-legged knee-extensor exercise model is associated with a large cardiovascular reserve and lower vasoconstrictor activity compared to upright whole body exercise (Andersen & Saltin, 1985; González-Alonso, et al., 2008; Mortensen, et al., 2008; Rowell, 2004). A lower evidence of vasoconstrictor activity was also found in this study, as plasma noradrenaline concentrations were unchanged with hypohydration and hyperthermia at rest and during exercise (Fig. 5.4). Consequently local vasoconstrictor activity in muscle and skin vasculature was likely to be lower in comparison to that associated with whole body exercise (Charkoudian et al. 2003; González-Alonso et al. 1995, 1998, 2000). As such, it is possible that the maintenance of leg blood flow and cardiac output at rest and during mild exercise despite a combined hypohydration and hyperthermia was due to: (1) the lower impact of hyperthermia and the supine positioning upon central blood volume and mean arterial pressure and (2) the small muscle mass exercise model which reduced the cardiovascular strain and drive for vasoconstriction.

An unexpected finding was that leg muscle and skin blood flow were unchanged (Fig. 5.2) despite a significant haemoconcentration and hypovolaemia accompanying hypohydration and hyperthermia. In addition leg muscle and skin blood flow remained unaltered following oral rehydration despite a hypervolaemia and haemodilution (Fig. 5.1 and Table. 5.0). The maintenance of leg muscle and skin blood flow was somewhat unexpected for two reasons. First blood oxygenation has been shown to influence limb muscle blood flow regulation during exercise (González-Alonso, et al., 2006). Secondly, hypovolaemia and

haemoconcentration are associated with the reductions in systemic and limb blood flow that accompany whole body exercise with combined hypohydration and hyperthermia (González-Alonso, et al., 2000). Specifically, reductions in blood flow to the exercising limb that occur with combined hypohydration and hyperthermia have been associated with elevations in CaO_2 (Calbet, 2000). In line with these expected responses, plasma ATP declined (Fig. 5.4), and remained below control values following rehydration indicating that in these circumstances plasma ATP may not be active in the maintenance of limb muscle or skin vascular tone.

Leg blood flow and vascular conductance were maintained and in light of increases in blood oxygen concentration owing to hypohydration, oxygen extraction declined accordingly as local metabolic activity was unchanged (Fig. 5.2). Leg vascular tone is the result of the net balance between vasoconstrictor and vasodilator drives and many different vasodilatory substances have been shown to induce vasodilation such as NO (Green, et al., 2005; Stamler, et al., 1997). As such it is possible that other vasodilatory substances including shear stress and/or NO release were active in the maintenance of leg muscle and skin vascular conductance. However, this is speculative as the only vasodilatory substance measured in the present study was ATP. It is also possible that in a construct similar to that discussed in study 1, an increased temperature drive counteracted the opposition to blood flow provided by the significant haemoconcentration. Hence, elevations in temperature may have helped maintain leg blood flow and vascular conductance. In summary it appears that in conditions of low cardiovascular strain, i.e., at rest or during mild exercise akin to habitual activity, reductions in blood volume and haemoconcentration appear to have a negligible effect leg blood flow and cardiac output.

5.5. Conclusion

In summary, the present findings demonstrate that in conditions of low cardiovascular strain combined hypohydration and hyperthermia do not compromise leg muscle and skin blood flows or cardiac output in resting or mildly exercising humans. Rehydration does not seem to alter leg muscle and skin blood flow or cardiac output despite the concomitant hypervolaemia, haemodilution and restored core temperature. These findings suggest that mild alterations in blood volume, oxygen content and haemoglobin concentration that normally accompany hypohydration and hyperthermia and follow rehydration do not play independent roles in the decreases in limb blood flow classically observed during whole body exercise.

CHAPTER 6

General Discussion

6.1. Introduction

The aim of this thesis was to examine the effects of heat stress and combined hypohydration and hyperthermia upon leg muscle, skin and systemic haemodynamics at rest and during mild exercise in humans. In chapter 4, leg muscle, skin and systemic haemodynamic responses to whole body and isolated leg heat stress were examined at rest and in mildly exercising humans. Chapter 5 investigated the effects of combined hypohydration and hyperthermia, and afterwards, rehydration upon leg muscle and skin blood flow and also cardiac output in resting and mildly exercising humans. This chapter summarises the main findings of these studies and discusses the data with relevance to previous literature.

6.2. Summary of main findings

The main findings of this thesis indicate that in resting and mildly exercising humans, skeletal muscle and skin blood flow are increased during whole body and local heat stress. Furthermore, leg muscle and skin blood flow and cardiac output are not compromised by combined hypohydration and hyperthermia and concomitant reductions in blood volume and haemoconcentration. The observed elevations in skeletal muscle blood flow with whole body heat stress suggest that elevations in local tissue and body temperatures are either directly or indirectly involved in the underlying leg muscle and skin vasodilation. However, this heat stress-mediated leg muscle and skin hyperaemia is slightly attenuated during exercise. Therefore it appears that the effect of temperature upon maximal exercise hyperaemia is small. Nevertheless the results presented herein indicate that it is important to consider local temperature as a contributing factor to the regulation of skeletal muscle blood flow in resting and exercising humans. The maintained leg blood flow and cardiac output with combined hypohydration and hyperthermia suggests that significant haemoconcentration and hypovolaemia do not reduce leg muscle or skin blood flow at rest or during mild exercise. The influence of hydration status upon limb blood flow and cardiovascular functioning during mild intensity exercise akin to habitual activity appears to be negligible as indicated by the unchanged leg muscle and skin blood flow and cardiac output following rehydration and consequent haemodilution and hypervolaemia. Together the studies comprising this thesis indicate that skeletal muscle blood flow is increased with elevations in leg tissue temperature, while the effect of changes in

blood volume and haematological profile are apparently small in the present experimental conditions of rest and mild small muscle mass exercise. Furthermore, the impact of elevations in body temperature upon increases in leg blood flow became slightly reduced during mild exercise, while no reduction in blood flow was evident with hypohydration.

6.3. Leg muscle and skin blood flow with heat stress and combined hypohydration and hyperthermia.

The aim of this thesis was to examine the effects of heat stress and combined hypohydration and hyperthermia upon leg skeletal muscle, skin and systemic haemodynamics at rest and during mild exercise in humans. The specific aims were to: 1) examine the effect of heat stress upon leg muscle, skin and systemic haemodynamics at rest and during mild exercise, 2) examine the effect of increases in isolated leg skin and muscle temperature and elevations in whole body skin temperature upon the limb muscle and skin haemodynamic responses to whole body skin and core hyperthermia, 3) examine the effect of graded hypohydration and hyperthermia upon leg muscle, skin and systemic haemodynamics at rest and during mild exercise, and 4) to determine whether rehydration which restored blood volume and haematological changes to control restored any alterations in leg muscle and skin blood flow and cardiac output associated with combined hypohydration and hyperthermia. The following section discusses each of these aims.

6.3.1 Influence of heat stress and hypohydration upon skeletal muscle, skin and systemic haemodynamics in resting and mildly exercising humans

As expected, leg blood flow, cardiac output and vascular conductance increased progressively with heat stress at rest and during mild exercise. At the level of the leg, elevations in blood flow were accompanied by reductions in oxygen extraction as indicated by a fall in leg a-vO₂ difference. Although leg $\dot{V}O_2$ was slightly elevated during the final stage of whole body heat stress at rest (Fig. 4. 5, 0.02 l·min⁻¹), this augmentation was too small to account for all of the increase in leg blood flow with heat stress. Leg blood flow and leg $\dot{V}O_2$ have been shown to share a close linear relationship during incremental one-legged knee-extensor exercise to exhaustion where an increase in leg blood flow of 1 l·min⁻¹ corresponded with an increase in leg $\dot{V}O_2$ of 0.16 l·min⁻¹. (Mortensen, et al., 2008). However, in study 1, leg blood flow increased ~1 l·min⁻¹ at rest with whole body heating from control to skin and core hyperthermia with only a 0.02 l·min⁻¹ increase in leg $\dot{V}O_2$. In addition to these responses at rest, the further ~0.4 l·min⁻¹ increase

in leg blood flow during combined heat stress and exercise compared to control exercise were observed in absence of any alterations in leg $\dot{V}O_2$ (Fig, 4.5). Thus in conjunction with the significant reductions in leg a-v O_2 difference, elevations in leg blood flow during heat stress were, for the most part, independent of increases in leg tissue metabolism.

Along with increases in leg blood flow, oxygenation of the vastus lateralis muscle, as measured by NIRS, was elevated during both whole body and isolated leg heat stress. The elevated leg muscle tissue oxygenation increased in line with whole leg blood flow. Thus, given that leg $\dot{V}O_2$ was largely unchanged and that the small increase in leg metabolism with heat stress at rest is unable to explain increases in leg blood flow, these findings suggest that muscle blood flow was elevated with heat stress. This finding is not unique in its support for an increased muscle blood flow during heat stress.

In three additional participants, deep femoral vein blood oxygenation was measured during whole body heat stress as described for study 1. Given that the length of the catheter used to take deep femoral venous blood samples was 12 cm in length, the tip of the catheter would have been distal to the saphenofemoral junction. Therefore, the blood sampled from the deep femoral catheter was not representative of blood flowing through the saphenous vein which itself drains the cutaneous vessels of the leg. The deep femoral venous blood samples, for the most part, reflected changes in oxygen extraction within the muscle tissue itself. This difference was represented in control conditions where blood oxygenation in the deep femoral vein was lower than that in mixed femoral venous blood (Table 4.1). This was the case for two participants where this comparison could be made. The comparison was not possible in one participant due to problems associated with the placement of the mixed femoral venous catheter

The difference in oxygen saturation and therefore oxygen extraction between mixed and deep femoral venous blood reflected differences in tissue metabolism between the location of the deep femoral catheter and the mixed venous catheter. Given the low metabolic requirement of skin tissues (Tayefeh, et al., 1997) and contrastingly large O_2 extraction of muscles tissue this was an expected finding. In this regard, venous blood oxygenation was lower when sampled from the deep

femoral venous catheter as opposed to the mixed femoral venous catheter (Table 4.1).

During heat stress deep femoral blood oxygenation increased while arterial-deep femoral venous O₂ difference declined (Table 4.1). Consistent with results from the initial whole-body heating protocol, muscle tissue oxygen delivery increased due to an elevated blood flow. Due to the increased oxygen supply, oxygen extraction relative to blood flow, reduced in order to maintain leg tissue metabolism. Although only measured in 3 participants, this increase in oxygenation of deep femoral venous blood suggests that the blood flow of leg muscle tissue is increased with heat stress. Consequently the results from these three additional participants provide direct evidence, supporting indirect measures of muscle blood flow obtained using NIRS. As such these results support previous conclusions based upon the initial whole body heat stress protocol (study 1) suggesting that muscle blood flow increases with heat stress.

In further support of this conclusion, during whole body heat stress the increase in skin blood flow displayed a plateau from the skin and mild core hyperthermia to skin and core hyperthermia conditions. Despite this plateau in leg skin blood flow, whole leg blood flow continued to increase. As whole leg blood flow increased but skin blood flow remained unchanged during these stages of heat stress, it is possible that the increased flow through the leg perfused skeletal muscle.

A commonality between study 1 and 2 was that skin blood flow increased in the active limb (leg) during exercise, not only in control conditions, but also during all levels of heat stress (Fig. 4.4). The elevation in leg skin blood flow was prominent in study 1 during whole body heat stress. This is a notable finding given that previous studies have reported a decline in forearm skin blood flow and increases in forearm vascular resistance during prolonged exercise attributed to an enhanced vasoconstrictor activity (Bevegard & Shepherd, 1966; Hirata, et al., 1983; Johnson, 1992; Kenney & Johnson, 1992). The reduction in skin blood flow during exercise is also reported to be exacerbated during heat stress (González-Alonso, et al., 2008; Johnson, 1986; Johnson & Park, 1982). However, the findings from study 1 do not agree with these earlier reports.

In study 1, leg skin blood flow increased with exercise at each level of heat stress from corresponding values at rest. The increase in leg skin blood flow occurred despite significant elevations in plasma noradrenaline and a significant hyperthermia (~38.6 °C). Previous research has suggested that the increase in skin blood flow during exercise in a hot environment plateaus after core temperature exceeds approximately 38°C (González-Alonso, et al., 2008). It is notable that previous reports of reductions in skin blood flow during isolated limb exercise have been obtained from the forearm, while here it was observed that leg skin blood flow increased during one-legged knee-extensor exercise. However, previous studies reporting a reduction in skin blood flow during exercise have utilised a supine cycling exercise model (Bevegard & Shepherd, 1966; Hirata, et al., 1983; Johnson & Park, 1982). Sympathetic vasoconstrictor activity increases with both whole body compared to an isolated limb exercise model and relative to the active muscle mass (Savard, et al., 1989). As such, it is possible that the increased sympathetic nervous activity accompanying whole body exercise as evidenced in previous research, could be responsible for reductions in skin blood flow during whole body exercise and heat stress compared to mild one-legged knee extensor exercise as utilised here.

An explanation of the increases in leg skin blood flow during exercise in studies 1 and 2 is that elevations in tissue temperature due to local muscle contraction are somehow involved. This could occur via a similar mechanism attributed to elevations in limb muscle vascular conductance with heat stress. That is where elevations in tissue temperature of the limb occur, such as during one-legged knee-extensor exercise, skin blood flow increases to aid the dissipation of heat produced in the working muscles. However, more evidence is needed before drawing a firm conclusion.

The investigation into the partition of heat stress mediated increases in blood flow between skeletal muscle and skin vasculatures dates back to the 1950's. The results from study 1 agree with some of the early research but contrast the findings of more recent studies where muscle blood flow, was unchanged during heat stress (Detry, et al., 1972; Edholm, et al., 1956; Johnson, et al., 1976; Roddie, et al., 1956). The results from those more recent studies reported an unchanged muscle blood flow during heat stress and gave rise to the classical

dogma suggesting that all elevations in cardiac output during heat stress are accounted for by increases in skin blood flow.

The more recent studies investigating changes in muscle blood flow during heat stress have used a number of techniques including, isotope clearance and iontophoresis techniques (Detry, et al., 1972; Johnson, et al., 1976). These measurements of muscle blood flow during heat stress, although novel at the time of investigation, are now regarded as unreliable (Heymann, et al., 1977; Rowell, 1993). In addition, the contention that all increases in \dot{Q} during heat stress perfuse the cutaneous circulation was based on indirect measurements of regional blood flow distribution (Minson, et al., 1998) and maximal cardiac output during heat stress (Detry, et al., 1972; Rowell, 1974; Rowell, et al., 1969a). While acknowledging that skin blood flow is elevated in line with cardiac output during heat stress (Minson, et al., 1998), arguably this relationship is not cause and effect. As such, it does not demonstrate that muscle blood flow is unaltered or reduced during heat stress.

The measurement of skeletal muscle blood flow is problematic. Although newer techniques have been developed to those mentioned previously, their reliability to detect muscle blood flow during heat stress has been questioned. One such technique is near-infrared spectroscopy (NIRS). The validity of the NIRS technique to measure skeletal muscle oxygenation and serve as an index of muscle blood flow in times of elevated skin blood flow has been questioned (Davis, et al., 2006). Specifically, Davis and colleagues reported that measures of skin blood flow increased in line with NIRS measurements of muscle tissue oxygenation during heat stress. As such it was concluded that measures of muscle tissue oxygenation obtained through NIRS were influenced by elevations in skin blood flow and not representative of increases in muscle blood flow.

However, simply because skin blood flow and muscle tissue oxygenation share a similar pattern of increase during heat stress does not indicate that measures of muscle tissue oxygenation using NIRS are invalid. In closer examination of the relationship between skin blood flow and tissue oxygenation (Fig. 3, Davis *et al* 2008) it can be seen that tissue oxygenation and skin blood flow do not respond in a manner equally consistent with one another. This is evident at the onset of whole

body heat stress where muscle oxygenation increased but skin blood flow was unchanged. Thus to imply a cause and effect relationship between these two parameters could be inaccurate in the absence of more appropriate evidence. While agreeing that skin blood flow increases with heat stress, the results of study 1 suggest that muscle blood flow is also elevated. Furthermore, skin and muscle blood flow may demonstrate a similar pattern of increase in response to heat stress.

The evidence presented here suggesting that muscle blood flow increases with heat stress has important implications for thermoregulation. Previous estimates of maximal skin blood flow ($6-8 \text{ l}\cdot\text{min}^{-1}$) were based upon changes in cardiac output during heat stress and the redistribution of blood flow from other regions (Detry, et al., 1972; Minson, et al., 1998; Rowell, 1974; Rowell, et al., 1969b). In light of the evidence presented here it appears that previous values for maximal skin blood flow during whole body heat stress are possibly overestimates. It is important to track changes in skin blood flow in times of elevated body temperature as it allows assessment of the contribution of convection to heat dissipation. Thus a lower skin blood flow during heat stress is representative of a reduction in cooling capacity through the skin. However, elevations in muscle temperature do participate in the thermoregulatory response to heat stress although the percentage contribution is unclear. In this regard, it could be of particular interest to examine the thermoregulatory responses and skeletal muscle blood flow in skin graft patients where cutaneous vasodilation in grafted areas is either reduced or absent during heat stress (Davis, et al., 2007). This could provide a valuable insight into the role of skeletal muscle in the thermoregulatory response to heat stress in humans.

In study 1 elevations in leg vascular conductance were strongly associated with elevations in estimated muscle temperature at rest, indicating the role of local temperature in the control of the leg microcirculation. Leg blood flow, vascular conductance, skin blood flow and muscle oxygenation were equally elevated between isolated leg and whole body heat stresses when leg muscle and skin temperatures were similar. This suggests that local elevations in deep leg tissue temperature contributed to the overall hyperaemic response. A comparable phenomenon has previously been observed during *in situ* and *in vitro* experiments where arteriolar diameter increased in response to elevations in temperature

(Ogura, et al., 1991; Unthank, 1992). Further, whole limb blood flow has also been shown to increase with isolated limb heat stress. The isolated limb heat stress induced comparable elevations in temperature to those mentioned above *in situ* and *in vitro* experiments (Barcroft & Edholm, 1943; Barcroft & Edholm, 1946; Johnson, et al., 1976).

The possibility that local elevations in skin and muscle tissue temperature account for all of the increase in leg blood flow during heat stress contrasts with the small amount of literature available (Johnson, et al., 1976). Johnson and colleagues described that elevations in limb blood flow during isolated forearm heat stress were approximately one half of that seen during whole body heat stress. However, forearm muscle temperatures during whole body heat stress were not reported. So directly comparing blood flow between isolated limb and whole body heat stress studies is problematic. In study 1 while the increases in leg skin temperatures were consistent between severe whole body and isolated leg heat stress, leg muscle temperatures were elevated further ($\sim 1^\circ\text{C}$), during whole body heat stress.

A larger increase in muscle tissue temperature during isolated leg heating could have been achieved with prolonged heat stress. However, under normal 'free blood flow' conditions leg tissue temperatures cannot be elevated to a similar magnitude to whole body heat stress without elevating both core and tissue temperatures in non-experimental leg areas. Thus to assess the contribution of elevations in isolated leg skin and muscle tissue temperatures to leg and systemic haemodynamic responses to heat stress independently of core temperature, the magnitude of increase was slightly lower. Despite this limitation, it is clear from the present data that elevations in local skin and muscle tissue temperature account for at least one-half of the whole body heat stress-induced leg hyperaemia.

In further examination, elevations in skeletal muscle blood flow may have been mediated directly by increases in muscle tissue temperature. However, plasma ATP was also associated with increases in leg vascular conductance during whole body heat stress. Plasma ATP approximately doubled at rest with whole body heat stress in study 1, and so elevations in plasma ATP may have been involved in the elevations in skeletal muscle blood flow. This is an attractive possibility given the powerful vasodilator and sympatholytic properties of ATP (González-Alonso, et al.,

2008; Kirby, et al., 2008; Rosenmeier, et al., 2004; Rosenmeier, et al., 2008). Although it is not directly possible to positively identify this role for plasma ATP here, whole leg blood flow did increase in spite of an elevated sympathetic vasoconstrictor activity, as indicated by the increase in norepinephrine concentrations. However, this mechanism remains speculative and to demonstrate a direct involvement of intravascular ATP in heat stress-mediated muscle vasodilation further investigation is required.

Despite the strong association between plasma ATP and increases in muscle tissue temperature reported in study 1, elevations in core body temperature were not accompanied by increases in plasma ATP in study 2 (Fig 5.4.). In study 2 increases in core temperature were likely to have been accompanied by elevations in muscle tissue temperature. Increases in muscle tissue temperature would have been pronounced given that in order to reach the required level of hypohydration participants cycled in a hot environment. Thus muscle tissue temperature would increase due to metabolic heat production owing to the intensity of exercise, and the hot environmental temperature. Previously it has been shown that muscle tissue temperature remained elevated for ~45 min following a bout of mild knee-extensor exercise (Kenny, et al., 2003). In this regard, it is highly likely that muscle tissue temperatures exceeded ~38°C at each stage of combined hypohydration and hyperthermia. Despite this the relationship between muscle tissue temperature elevations and plasma ATP observed in study 1 was uncoupled in study 2.

Leg blood flow and cardiac output increased with hypohydration and hyperthermia at rest and during exercise in study 2 despite the uncoupling between body temperatures and plasma ATP. As such it is important to consider the many different vasodilatory substances and mechanisms that are associated with the regulation of blood flow in addition to alterations in plasma ATP, e.g., shear stress (Green, et al., 2004). Shear stress can be increased in line with blood viscosity, which itself would increase in line with progressive hypohydration (study 2). Shear stress has been linked to the release of endothelial derived nitric oxide, and consequently vasodilation and increased blood flow (Green, et al., 2004). Although it was beyond the scope of this thesis to measure nitric oxide, its involvement in the maintenance and slight increase in blood flow observed with hypohydration is

an area for future investigation. Further research into the specific mechanisms of the limb muscle vasodilation suggested here to occur with heat stress is important.

The findings of study 2 indicated that leg muscle blood flow and cardiac output were unaltered with mild and moderate hypohydration and hyperthermia at rest and during exercise. Previously, it has been reported that combined and hyperthermia result in a decline in exercising limb blood flow (González-Alonso, et al., 1998). The previously reported decline in leg blood flow occurred while leg vascular conductance was unaltered in the face of an elevated sympathetic vasoconstrictor activity (González-Alonso, et al., 1998). While this particular study of González-Alonso and colleagues is unique in its presentation of such findings during prolonged cycling in the heat, it also indicated that leg blood flow could be maintained during exercise and hypohydration so long as cardiac output was not compromised. Accordingly, the unchanged cardiac output and leg blood flow in combination with an unchanged leg a-vO₂ difference and leg skin blood flow, suggest that leg muscle and skin blood flow were maintained with hypohydration and hyperthermia at rest and during mild knee-extensor exercise. This maintenance of skin and muscle blood flow occurred despite a significant hypovolaemia and haemoconcentration (Fig. 5.2).

In study 2 hypohydration, plasma volume expansion and haemodilution due to oral rehydration, had no impact upon skeletal muscle or skin blood flow at rest or during exercise. The results presented here are in partial agreement with recent research where blood volume and oxygen content were manipulated (González-Alonso, et al., 2006). Specifically, the combination of anaemia and hypovolaemia, and polycythaemia and hypervolaemia in humans did not alter either resting or exercising limb blood flow or plasma ATP (González-Alonso, et al., 2006). However, the combination of anaemia with plasma volume expansion resulted in a large haemodilution and a marked increase in leg blood flow and a corresponding increase in \dot{Q} (González-Alonso, et al., 2006). In this light, alterations in haemoglobin oxygenation have been shown to influence limb muscle blood flow regulation during exercise (González-Alonso, et al., 2006).

Reductions in leg blood flow during whole body exercise with hypohydration and hyperthermia were closely associated with reductions in blood volume and

haemoconcentration (González-Alonso, et al., 2000). In further analysis, it was suggested that the reductions in blood flow could be due to a link between alterations in blood oxygenation with hypohydration and blood flow regulation (Calbet, 2000; González-Alonso, et al., 2000). In this study the level of hypohydration and subsequent rehydration were mild compared to the larger changes that were induced previously (González-Alonso, et al., 2006). Reductions in limb blood flow that have been previously reported with hypovolaemia and haemoconcentration were reported during a higher whole body exercise intensity and greater degree of hyperthermia (González-Alonso, et al., 2000). In contrast, the findings from study 2 suggest that during combined hypohydration and hyperthermia, leg muscle blood flow and cardiac output were not reduced in resting and mildly exercising humans.

An important observation from the isolated leg heat stress protocol of study 1 was that cardiac output remained unchanged when leg blood flow increased by $0.5 \text{ l}\cdot\text{min}^{-1}$. This increase in leg but not systemic blood flow suggests that, under these conditions, increases in leg blood flow occur primarily via redistribution of blood flow from other territories when leg tissue temperature increases. This is plausible in so much as the contribution of blood flow redistribution to elevations in blood flow during heat stress is $\sim 1 \text{ l}\cdot\text{min}^{-1}$ (Minson, et al., 1998). This magnitude of blood flow redistribution with heat stress could encompass the increase in leg blood flow during one-legged heat stress. This idea is plausible given that with single limb heating blood flow in the contralateral limb and upper body territories other than visceral organs is likely to remain unchanged (Johnson, et al., 1976). Although this is a possibility, more research is required to confirm or reject this hypothesis.

In study 1 no measurements of blood flow in other regions of the body were made during isolated leg heat stress. Further, with comparable elevations in temperature renal vascular conductance is unaltered during isolated limb heat stress (Kuipers, Sauder, Kearney & Ray, 2009). If the maintenance of renal vascular conductance is common to the splanchnic region, it could indicate that no blood flow redistribution away from central organs occurs during isolated limb heat stress. While skin and core temperatures are important to the understanding of blood flow during heat stress, study 1 indicates that the cardiac output response is dependent upon the magnitude and/or distribution of elevations in tissue temperature.

In study 1, leg blood flow and cardiac output were elevated with heat stress during exercise while leg $\dot{V}O_2$ was unchanged and contrasts previous research (González-Alonso & Calbet, 2003; Nadel, et al., 1979; Nielsen, et al., 1990; Savard, et al., 1988). However, the effect of increases in temperature upon elevations in leg blood flow and cardiac output were attenuated during exercise compared to rest by approximately 65%. The attenuation is indicated by the ~ 1 $\text{l}\cdot\text{min}^{-1}$ increase in leg blood flow with whole body heat stress at rest compared to the ~ 0.35 $\text{l}\cdot\text{min}^{-1}$ increase during exercise. The significantly increased plasma norepinephrine ($P < 0.05$) with heat stress during exercise compared to rest suggests that vasoconstrictor activity was elevated. Plasma arterial noradrenaline concentrations increased markedly during exercise, especially in conditions where core temperature was elevated (Table 4.0). Hence the augmented vasoconstrictor drive may have been responsible for the attenuation in blood flow during exercise.

With the exception of Savard and colleagues (1988), previous investigation into the effects of heat stress and combined hypohydration and hyperthermia upon exercise hyperaemia has been conducted using whole body exercise protocols. Whole body exercise gives rise to significant limitations to central and local blood flow (Rowell, 2004). In contrast isolated limb exercise is associated with a large cardiovascular reserve (Andersen & Saltin, 1985). Although Savard and colleagues (1988) utilised a one-legged exercise model, they did not describe whether or not hydration status was maintained. Consequently, hypohydration may have influenced the hyperaemic response to heat stress and exercise. Contrastingly, in study 1, hydration status was carefully controlled as demonstrated by an unchanged blood osmolality and body mass, and only a small increase in arterial blood oxygenation from control with whole body heating ($\sim 1\%$). In this regard, the results from study 1 indicate that when hydration status is maintained blood flow to the exercising limb increases with whole body heat stress.

The results from study 2 suggest that, during mild one-legged exercise hypohydration and hyperthermia do not compromise leg muscle and skin blood flow or cardiac output (Fig. 5.2 and 5.3). In study 1 using an isolated limb exercise model, we were able to determine the effects of heat stress upon exercise

hyperaemia where hydration status was controlled for and limitations to perfusion were minimal. Exercise hyperaemia increased with heat stress although the magnitude of increase represented only a small fraction (~5%) of the hyperaemic response to maximal exercise and also heat stress exercise, which are reported to be ~8.5-11.0 l·min⁻¹ (Mortensen, et al., 2005). Thus in comparison, the large increases in leg blood flow that are associated with maximal exercise, the small increase associated here with heat stress is small. As such while heat stress increases exercise hyperaemia its impact during maximal intensity whole body exercise appears to be small.

The maintained systemic and limb blood flow with hypohydration and hyperthermia during exercise contradicts previous research conducted using a whole body exercise model (González-Alonso, et al., 1998; González-Alonso, et al., 1997; González-Alonso, et al., 1999b, 2000; Montain & Coyle, 1992b; Nadel, et al., 1980; Sawka, et al., 1979). Reductions in cardiac output and active muscle blood flow during prolonged exercise with hypohydration and hyperthermia have been attributed, at least in part, to an impaired stroke volume (González-Alonso, et al., 1998). The reduction in stroke volume has, in turn, been associated with hypohydration-induced reductions in blood volume and a hyperthermia-induced tachycardia (González-Alonso, et al., 2000).

The different findings between the present and previous studies may have been due to the lower cardiovascular strain associated with an isolated limb exercise model compared to whole body exercise. In this regard, the haemodynamic effects of 4% hypohydration are blunted during exercise in a cold environment where \dot{Q} is not reduced and the small reductions in stroke volume are fully restored by plasma volume restoration (González-Alonso, et al., 2000). Similarly, in study 2 the influence of exercise intensity upon heart rate was reduced with one-legged knee-extensor compared to whole body cycling exercise. Given that exercise intensity was lower than in the aforementioned studies the metabolic demand for blood flow was smaller. As such the increase in cardiac output required to match metabolic demand necessitated a smaller elevation in heart rate. Consequently, the potentially deleterious influence of tachycardia upon stroke volume via reductions in cardiac filling time and cardiac output were reduced. Similarly, where cardiac output has been shown to decline during exercise with hypohydration and

hyperthermia (González-Alonso, et al., 1998; González-Alonso, et al., 1997; González-Alonso, et al., 1999b, 2000; Montain & Coyle, 1992b; Nadel, et al., 1980; Sawka, et al., 1979), the core temperature was larger (~38.6-39°C) than reported here (~38°C). Accordingly a greater magnitude of core temperature increase would induce a greater drive for skin blood flow. An increase in skin blood flow could further reduce central blood volume thereby compromising systemic haemodynamics and ultimately lead to a decline in cardiac output and leg blood flow.

Cutaneous blood pooling has been suggested to be unrelated to the decline in cardiac output (González-Alonso, et al., 2000). However, a reduced central blood volume could initiate a vasoconstrictor response due to the unloading of high and low pressure baroreceptors (Mack, et al., 1991; Renlund, et al., 1990). While this response could raise central blood volume and reduce skin blood flow (González-Alonso, et al., 2000), heat dissipation would become impaired, further increasing core temperature and heart rate. This mechanism becomes noteworthy given that hypohydration provokes a more sensitive baroreflex defence of blood pressure (Charkoudian, et al., 2005). This further highlights the impact of hyperthermia upon the systemic response to hypohydration and hyperthermia. Thus the lower magnitude of hyperthermia and the low intensity, small muscle mass exercise model, could help explain the maintenance of systemic and leg skin blood flow during exercise despite combined hypohydration and hyperthermia.

The practical implications of the findings from study 2 suggest that mild changes in blood volume and oxygen content do not adversely affect leg muscle blood flow or cardiac output during low intensity exercise. As such these findings may have implications for the impact of hypohydration and hyperthermia upon habitual activity. Furthermore, limb and systemic haemodynamics were largely unchanged following rehydration after ~2 and 3.5% hypohydration. Thus the results question the importance of rehydration in order to maintain leg blood flow and cardiac output in a subsequent bout of mild intensity exercise.

Reductions in \dot{Q} during whole body exercise can be fully restored during a subsequent exercise bout following the adequate oral replenishment of lost fluids (Mitchell, Phillips, Mercer, Baylies & Pizza, 2000). However, in study 2, both at rest

and during mild one-legged knee-extensor exercise, \dot{Q} was maintained following both ~2% and ~3.5% hypohydration as indicated by body mass loss. Thus with mild and moderate levels of hypohydration and hyperthermia, leg blood flow and cardiac output were not reduced. Consequently with regard to short-term acute cardiovascular function these results question the need to remain hydrated during repeated bouts of mild intensity exercise.

The conclusions drawn from study 2 indicate that cardiovascular functioning is largely unaffected by combined hypohydration and hyperthermia in resting humans. Even with much larger elevations in skin temperature i.e., in the classic model of passive heat stress, cardiac output increases concomitant with thermoregulatory demand (Rowell, et al., 1969a). A similar increase in cardiac output has been shown during heat stress also despite hypohydration as indicated by elevations in haematocrit and a small reduction in mean arterial pressure (Crandall, et al., 2008). In contrast, mean arterial pressure was stable at rest both in study 1, during passive whole body heat stress, and during study 2, with combined hypohydration and hyperthermia. Accordingly, the separate effects of whole body heat stress and combined hypohydration and hyperthermia at rest could be exacerbated when present in combination. The combination of heat stress and hypohydration could result in a reduction in mean arterial pressure, as indicated by the work of others (Crandall, et al., 2008). Consequently, this cardiovascular strain could lead to a slight compromise in cardiac output and leg blood flow. Despite this, it is unclear whether elevations in cardiac output and leg blood flow with passive heat stress would be greater if hypohydration were prevented. Furthermore, in many of the aforementioned studies participants have been measured whilst supine. As a result it is likely that exposure to high skin temperatures in combination to core hyperthermia and hypohydration whilst in an upright position could exacerbate the cardiovascular strain as evident in reductions in blood pressure found in supine participants (González-Alonso, et al., 1999b). When exercise is conducted in an upright position, the combined elevations in skin and core temperatures and hypohydration could have implications for health.

6.3.2 Limitations

It was not possible to increase leg tissue temperature to the same levels reached during severe whole body heat stress at rest or during exercise. Currently, a

method to increase leg tissue temperature to such levels while maintaining core temperature at ~37 °C under the present free flow conditions is unavailable. As a result it was not possible to investigate the contribution of local muscle and skin temperatures of the magnitude seen during whole body intense exercise upon cardiac output and leg blood flow. Nevertheless, given these experimental limitations, evidence has been discussed suggesting that at least 50% of the hyperaemia associated with whole body heat stress is due to isolated limb heat stress. Consequently elevations in local muscle and tissue temperatures appear to be important factors governing increases in blood flow and vascular conductance.

An experimental limitation in using a water-perfused suit or leg to determine the role of temperature on exercise hyperaemia is that the elevations in tissue temperature and limb blood flow is relatively slow compared to the rapid hyperaemia that occurs upon onset of exercise. However, analysis of the temperature and flow kinetics during exercise demonstrates a rapid increase in muscle temperature, leg blood flow and cardiac output during control and heat stress conditions (D.A. Low, J. Pearson, M. Lotlikar & J. González-Alonso, unpublished observations; González-Alonso *et al* 2000). This was deemed sufficient to allow a comparison to be made, thus supporting the present conclusions based upon the steady-state resting and exercise responses.

A limitation of study 2 is that due to the complexity of the intervention and protocol, the experiment lasted approximately 10 hours. As such time may have influenced the systemic and haemodynamic responses to hypohydration and hyperthermia. A control trial to account for the effect of time was not included in study 2 due to the invasive nature of the experiment. A time-control study would involve the same measurements being taken over the same duration but without the effects of hypohydration and hyperthermia. However, such a control trial would require catheterisations on two different occasions in order to have a control, euhydration, trial and measure blood oxygenation. It is therefore possible that the observed elevations in leg blood flow and cardiac output during moderate hypohydration and hyperthermia compared to the initial control conditions are typical to diurnal variation and/or responses akin to post-exercise *per se*. To gain insight into this possibility, participants ingested a large volume of fluid to restore; body fluids, body temperatures and haematological variables to basal levels following

hypohydration. Thereafter the haemodynamic responses were examined at rest and during exercise to determine whether rehydration affected leg muscle and skin blood flow and cardiac output compared to control and moderate hypohydration and hyperthermia. However, despite the effectiveness of the rehydration procedure in expanding blood volume, producing haemodilution and restoring body mass and internal body temperature, no noticeable circulatory alterations were observed. The results thereby suggested that the haemodynamic responses to moderate hypohydration and hyperthermia were not necessarily due to an effect of exercise *per se*.

The coefficient of variation in measurement of leg blood flow at rest and during exercise was 8.1% and 4.9% respectively. While this error in leg blood flow measurement may appear large, any alteration in leg blood flow reported during Studies 1 and 2 exceeded this variance. This is justified, in so much as, given a resting leg blood flow of 0.4 l min^{-1} and an exercising leg blood flow of 1.7 l min^{-1} the potential error in measurements are $\sim 0.06 \text{ l min}^{-1}$ and 0.17 l min^{-1} respectively. The magnitude of the changes in leg blood flow exceeded these potential errors. Additionally, this level of error, 8.1% at rest and 4.9% during exercise, are both well within the acceptable range reported elsewhere (Rådegran, 1999; Shoemaker, et al., 1996). Although the ultrasound used to measure common femoral artery diameter in this thesis is very sophisticated, even more complex systems have been reported elsewhere in the measurement of brachial artery diameter (Green, et al., 2005). The use of a system capable of both an unbiased and greater accuracy of blood vessel diameter measurement may reduce the current coefficient of variation reported here for vessel diameter thereby leading to an even more precise measurement of leg blood flow. However, this system was not available for use in the present thesis.

It is acknowledged that the sample size, i.e., $n=7$ in study 1 and 2 is small. As such some variables such as plasma ATP in study 1 demonstrated a large percentage increase in mean values yet did not reach statistical significance. This may have been due to the small sample size. As such a greater number of participants in each study may have helped with the clarity of findings. However, invasive studies such as study 1, protocol 1 and study 2 are complex. In this regard it was scheduled that 9 subjects would participate in both studies in order to avoid this

limitation. Yet due to illness and pre-experimental complications the tests could only go ahead on 7 participants in each protocol.

In study 1, muscle temperature was measured directly in one participant along with core and skin temperatures. Consequently changes in skin and core temperatures of this participant were used to estimate changes in muscle temperature in other participants. This is a limitation of study 1 and the direct measurement of muscle blood flow in all participants would lead to an enhanced accuracy of results. Thus a direction for future work would involve continuous measures of muscle temperature in all participants in order to obtain more reliable data.

Finally, the ecological validity of some aspects of studies 1 and 2 was low. In study 1, subjects effectively maintained their hydration status by drinking a warm carbohydrate-electrolyte solution. The temperature of the solution at the time of ingestion was $\sim 35^{\circ}\text{C}$. While this temperature was essential in order to keep participants hydrated without reducing core body temperature, it compromised the ecological validity of the study. Future studies could examine the impact of heat stress upon leg blood flow and cardiac output while ingesting cooler fluids, at temperatures that fluids are ingested at during athletic scenarios. By ingesting cooler fluids the duration of exposure to heat stress would have been prolonged. As such hyperthermia would have been prolonged and the findings would be more applicable to athletic competition.

6.4. Future directions

During study 1, a salient observation was that, in contrast to the similar leg hyperaemia during one-legged heating, \dot{Q} only increased during whole body heat stress (Fig. 4.8). This indicates that when the blood flow demand is relatively small during one-legged heat stress the increase in limb blood flow is met primarily by redistribution of blood flow from other territories. The magnitude of blood flow redistribution can amount to 1 l min^{-1} during whole body heat stress (Minson, et al., 1998). In line with these earlier observations, when vasodilation occurs throughout the skin and muscle of the whole body, the greater global demand for blood flow invokes an increase in \dot{Q} in addition to blood flow redistribution from other territories. However, this interpretation is open to criticism given the lack of direct measures of blood flow in other territories. Therefore, a future direction would be to measure blood flow in visceral regions whilst also measuring leg blood flow during isolated leg heat stress. This could be conducted using gamma camera technology that has recently been utilised in a heat stress experiment focusing upon blood flow distribution during heat stress (Crandall, et al., 2008).

Heat stress mediated increases in leg vascular conductance with rest and exercise were associated with elevations in intravascular plasma ATP. ATP has been strongly linked to vasodilation and sympatholysis (González-Alonso, et al., 2008; Kirby, et al., 2008; Rosenmeier, et al., 2004; Rosenmeier, et al., 2008).

However, the possibility that intravascular ATP is involved in the heat stress-mediated limb muscle and skin vasodilation warrants further investigation as the significant association between the increases in leg vascular conductance and plasma ATP found here does not necessarily demonstrate a causal link. Furthermore, many other substances and mechanisms have been suggested to be involved in the regulation of blood flow. However in the present thesis only one such marker was measured, plasma ATP. Future studies could be conducted in a similar manner to study 1 and study 2 measuring many more of these vasodilatory candidates and thereby providing a deeper insight into the mechanisms of an increased or maintained vascular tone. This is most notable in study 2, where combined hypohydration and hyperthermia and rehydration were associated with a reduced plasma ATP but a maintained leg blood flow. Given that vascular tone is the balance between many different vasodilatory substances and vasoconstrictor

drive the measurement of all vasodilatory candidates would yield a deeper insight into the regulation of blood flow in such conditions.

The one-legged knee-extensor exercise model enables the assessment of the effects of whole body heat stress upon exercise hyperaemia in conditions where the cardiovascular system functions far below its maximal capacity (Andersen & Saltin, 1985; González-Alonso, et al., 2008; Mortensen, et al., 2008). In study 1, the $\sim 1.6 - 5.4$ °C rise in muscle, core and skin temperatures was associated with a ~ 0.4 l min⁻¹ increase in leg blood flow during exercise (Fig. 4.4). Similar elevations in muscle and core temperature are normally associated with an $\sim 8 - 11$ l min⁻¹ leg hyperaemia during maximal cycling exercise in individuals of comparable characteristics (González-Alonso & Calbet, 2003; Mortensen, et al., 2008; Mortensen, et al., 2005). Thus, although the present findings indicate that locally heat stress induces noticeable muscle vasodilation its overall contribution to maximal exercise hyperaemia is small. However the effect of progressive whole body heat stress, in the manner induced in study 1, upon incremental one-legged knee-extensor exercise to exhaustion is yet to be investigated and could help elucidate the effects of temperature upon maximal skeletal muscle hyperaemia.

An incremental one-legged knee-extensor exercise protocol could also be applied during combined hypohydration and hyperthermia to investigate maximal exercise hyperaemia without a severely compromising systemic haemodynamics. In study 2, cardiac output was maintained with combined hypohydration and hyperthermia during one-legged knee-extensor exercise thereby sharply contrasting previous results found during whole body exercise. It has been reported that the intensity of whole body exercise determines the magnitude of decline in \dot{Q} combined hypohydration and hyperthermia (Montain, Sawka, Latzka & Valeri, 1998). An incremental one-legged knee-extensor exercise test to exhaustion during combined hypohydration and hyperthermia could indicate the specific point at which \dot{Q} becomes compromised. Furthermore, given the concomitant haemoconcentration a study of this nature could provide a further insight into the influence of local versus systemic factors upon exercise hyperaemia.

Within this thesis, the investigation of combined leg blood flow and cardiac output during isolated limb heat stress is novel as previous studies did not report \dot{Q}

during isolated forearm heat stress (Johnson, et al., 1976). However, the present finding of an unchanged cardiac output with isolated leg heat stress indicates that increasing the temperature of a single limb is not sufficient to increase \dot{Q} . This finding might have some clinical implications. During surgery cardiac output might decline in association with hypothermia as well as cerebral blood flow and oxygen delivery, which can result in post-operative health complications (Shaw, et al., 1987). Often hypothermia is induced during surgery in order to slow down the activity of the heart, notably during cardiac surgery (Shaw, et al., 1987) which could restrict cerebral oxygenation (Nollert, Mohnle, Tassani-Prell & Reichart, 1995). However, the results from study 1 could suggest that in heating a large tissue mass such as the leg, local blood flow increases owing to elevations in local tissue temperature without increasing cardiac output. This is noteworthy, given that heart rate and stroke volume were also unaltered and that the change in blood flow through the leg ($\sim 0.5 \text{ l min}^{-1}$) was over half of the estimated values of cerebral blood flow at rest in control conditions (González-Alonso, et al., 2004). Thus an area for future investigation could concern the elevation of cerebral temperature and its effects upon cerebral blood flow and oxygenation and cardiac output.

6.4.1 Hypotheses

Study 1

1. *Research hypothesis:* local hyperthermia will induce vasodilation in resting and exercising human limb muscle, thereby contributing to heat stress and exercise hyperaemia. **ACCEPT**
2. *Research Hypothesis:* the whole leg and systemic hyperaemic response to heat stress will be attenuated during exercise. **ACCEPT**
3. *Research Hypothesis:* local hyperthermia will account for a large portion of leg hyperaemia during whole body heat stress. **ACCEPT**

Study 2

1. *Research Hypothesis:* hypohydration and hyperthermia will cause a reduction in leg muscle, skin and systemic haemodynamics at rest and during exercise. **REJECT**
2. *Research Hypothesis:* rehydration will restore leg muscle, skin and systemic haemodynamics to control levels at rest and during exercise. **REJECT**

6.5. Summary

This thesis has provided evidence to suggest that heat stress is associated with elevations in both skeletal muscle and skin blood flow. The present findings suggest that elevations in muscle temperature contribute to increases in blood flow at rest and also exercise hyperaemia, although its contribution to maximal exercise hyperaemia appears to be small. The findings of this thesis also contradict previous results during whole body exercise with hypohydration and hyperthermia. In this regard, mild and moderate hypohydration and hyperthermia do not compromise leg muscle and skin blood flow and cardiac output in resting and mildly exercising humans. The results from study 2 indicated that hypohydration and hyperthermia do not significantly affect the cardiovascular system during mild intensity, short duration exercise. Similarly during exercise and following hypohydration, the haemodynamic effects of rehydration under the present conditions of low cardiovascular strain are negligible. Taken in context with previous research the results suggest that the requirements for fluid replacement are different depending on the intensity and type of exercise.

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Appendix

I

Informed Consent Sheet

Informed Consent Form

The participant should complete the whole of this sheet himself

**Please tick the
appropriate box**
YES NO

Have you read the Research Participant Information Sheet?

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 understand that you are free to withdraw from the study:

- at any time

- without having to give a reason for withdrawing?

- *(where relevant)* without affecting your future employment as a member of staff of the University or your progression or assessment as a student of the University.

Do you agree to take part in this study?

Signature of Research Participant:

Date:

Name in capitals:

Witness statement

I am satisfied that the above-named has given informed consent.

Witnessed by:

Date:

Name in capitals:

Appendix

II

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 medial 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:.....
Doctors Surgery
Address:.....
Emergency Contact Name:.....

Please answer the following questions:

		YES	NO
1.	Has your doctor ever diagnosed a heart condition or recommend only medically supervised exercise?	<input type="checkbox"/>	<input type="checkbox"/>
2.	Do you suffer from chest pains, heart palpitations or tightness of the chest?	<input type="checkbox"/>	<input type="checkbox"/>
3.	Do you have known high blood pressure? If yes, please give details (i.e. medication)	<input type="checkbox"/>	<input type="checkbox"/>
4.	Do you have low blood pressure or often feel faint or have dizzy spells?	<input type="checkbox"/>	<input type="checkbox"/>
5.	Do you have known hypercholesteremia?	<input type="checkbox"/>	<input type="checkbox"/>
6.	Have you ever had any bone or joint problems, which could be aggravated by physical activity?	<input type="checkbox"/>	<input type="checkbox"/>
7.	Do you suffer from diabetes? If yes, are you insulin dependent?	<input type="checkbox"/>	<input type="checkbox"/>
8.	Do you suffer from any lung/chest problem, i.e. Asthma, bronchitis, emphysema?	<input type="checkbox"/>	<input type="checkbox"/>
9.	Do you suffer from epilepsy? If yes, when was the last incident?	<input type="checkbox"/>	<input type="checkbox"/>
10.	Are you taking any medication?	<input type="checkbox"/>	<input type="checkbox"/>
11.	Have you had any injuries in the past? E.g. back problems or muscle, tendon or ligament strains, etc...	<input type="checkbox"/>	<input type="checkbox"/>
12.	Are you currently enrolled in any other studies?	<input type="checkbox"/>	<input type="checkbox"/>
13.	I have already participated in a blood donation program	<input type="checkbox"/>	<input type="checkbox"/>
14.	Are you a smoker?	<input type="checkbox"/>	<input type="checkbox"/>
15.	Do you exercise on a regular basis (at least 60 min a week)?	<input type="checkbox"/>	<input type="checkbox"/>
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.

Participant's name & signature: _____ Date: _____

Investigator's name & signature: _____ Date: _____

Appendix

III

Letter of Ethical Approval – Study 1

University Research Ethics Committee

31 January 2008

Proposers: Mr James Pearson and Mr Eric Stöhr (submitted by Prof. Jose González-Alonso)

Title: The effects of hyperthermia on the regulation of human skeletal muscle blood flow and cardiac function

Dear Professor González-Alonso,

The Research Ethics Committee has approved your application for research ethical approval for the above-named project, which is to be undertaken in February 2008.

Any changes to the protocol contained in your application, and any unforeseen ethical issues which arise during the project, must be notified to the Committee.
Kind regards,

David Anderson-Ford

Chair, Research Ethics Committee

Brunel University

Appendix

IV

Letter of Ethical Approval – Study 2

University Research Ethics Committee

11 September 2008

Proposer: Mr. James Pearson
Mr. Eric Stöhr
Centre for Sports Medicine & Human Performance
Heinz Wolff

Title: **The effects of graded dehydration and hyperthermia on the regulation of human skeletal muscle blood flow and cardiac function**

Dear Mr. Pearson and Mr. Stöhr,

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 unforeseen ethical issues which arise during the project, must be notified to the Committee.
Kind regards,

David Anderson-Ford
Chair, Research Ethics Committee
Brunel University

Appendix

V

Letter of Ethical Approval – Study 1 - Muscle Temperature
Measurements

Research Ethics Committee

16 February 2009

Proposer:	Prof. José González-Alonso
	School of Sport & Education

Title: The effects of hyperthermia on the regulation of human skeletal muscle blood flow and cardiac function

Dear Professor González-Alonso,

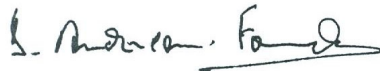
The Research Ethics Committee has approved your application for research ethical approval for the above-mentioned project, which is to be undertaken in 2009.

Any changes to the protocol contained in your application, and any unforeseen ethical issues which arise during the project, must be notified to the Committee.

Please make note of the following:

- Reference to anaesthetists being present during the procedure should be added to the application form (question 15), and returned to the Secretary of the University Research Ethics 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,



David Anderson-Ford
Chair, Research Ethics Committee
Brunel University

Appendix

VI

Conference abstracts and publications from the present thesis

Heat stress increases leg muscle and skin blood flow in resting and exercising humans

James Pearson¹, David Low¹, Eric Stöhr¹, Leena Ali², Horace Barker², José González-Alonso¹

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Introduction: Heat stress increases cardiac output (Q) at rest and during exercise in humans largely to meet the augmented thermoregulatory demands of the skin circulation (González-Alonso *et al.* 2008). Whether heat stress causes muscle vasodilatation and thereby increases muscle perfusion remains uncertain. This study tested the hypothesis that local leg and systemic hyperthermia increases leg muscle, leg skin and systemic perfusion at rest and during exercise. **Methods:** Leg and systemic hemodynamics, O₂ transport and VO₂ were measured at rest and during 6-min of one-legged knee-extensor exercise (25±3 W) in 7 active males (21±2 yr) in 4 conditions, in which participants' hydration status was maintained: 1) control (T_{core} ~37°C, T_{skin} ~33°C), 2) skin hyperthermia (T_c ~37°C, T_{sk} ~36°C), 3) skin and mild core hyperthermia (T_c ~38°C, T_{sk} ~37°C), and 4) high skin and core hyperthermia (T_c ~39°C, T_{sk} ~37°C). Femoral artery blood flow (LBF; Doppler ultrasound), vastus lateralis skin blood flow (SkBF; laser Doppler flowmetry) and blood gas and haematological variables (ABL 825, Radiometer) were measured in each condition. Data were analysed using a one-way ANOVA with repeated measures and Tukey's post hoc analysis with significance accepted at $P < 0.05$. Data represent mean±SEM. **Results:** At rest and during exercise, LBF and Q increased with each elevation in heat stress compared to control (peak delta LBF= 1.1±0.1 and 0.9±0.2 L/min from 0.5±0.1 and 2.4±0.2 L/min, respectively; peak delta Q= 4.0±0.2 and 3.1±0.3 L/min from 5.1±0.2 and 7.4±0.4 L/min, respectively). However, the increase in LBF and Q due to exercise (exercise hyperemia) was the same (~1.6 L/min) in all heat stress conditions. Correspondingly, SkBF initially increased with skin hyperthermia and skin and mild core hyperthermia (8.5±1.4-fold) but showed no additional elevation with high skin and core hyperthermia. The increased muscle perfusion accounted for the further increase in LBF. In addition, the increase in SkBF due to exercise was not different among conditions. Mean arterial and perfusion pressure declined, yet leg vascular conductance increased with heat stress, indicating that the increased leg perfusion was due to local vasodilatation. The elevation in leg muscle and skin temperature alone accounted for by >50% of the increase in LBF and SkBF with high skin and core hyperthermia. The elevated leg perfusion with each level of heat stress was accompanied by a parallel reduction in leg O₂ extraction such as that leg VO₂ remained unaltered either at rest or during exercise. **Conclusion:** These findings demonstrate that heat stress increases leg muscle and skin blood flow in resting and exercising humans. Further, the results suggest that increases in muscle tissue temperature *per se* might contribute to local muscle blood flow regulation and exercise hyperaemia.

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Increases in leg and systemic perfusion with dehydration and hyperthermia in resting and mildly exercising humans

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Introduction: Dehydration and hyperthermia reduce muscle, skin and systemic perfusion during intense whole body exercise in humans, possibly as a result of adjustments to concomitant hemoconcentration and/or compromised cardiovascular regulation. Whether dehydration and hyperthermia also reduces leg muscle perfusion, skin blood flow (SkBF) and cardiac output (Q) at rest and during small muscle mass exercise remains unknown. **Methods:** To further examine the influence of dehydration and hyperthermia upon cardiovascular function and regulation we measured leg, skin and systemic hemodynamics and leg muscle oxygenation at rest and during 6-min of one-legged knee-extensor exercise (~18 W) in 7 active young males in 4 conditions where hydration status (DE; body weight loss) and core temperature (T_c) were manipulated through exercise in an environmental chamber: 1) control (DE 0%, T_c ~37°C), 2) mild dehydration (DE ~2%, T_c ~38°C), 3) moderate dehydration (DE ~3.5%, T_c ~38°C), and 4) rehydration (DE 0%, T_c ~37°C). **Results:** At rest, leg blood flow (LBF) and Q increased with both mild and moderate dehydration (peak Δ = 0.4±0.1 and 0.8±0.4 l/min from 0.4±0.04 and 5.8±0.3 l/min, respectively; both P<0.05) accompanying slight reductions in leg a-vO₂ difference but unchanged muscle oxygenation, SkBF and leg VO₂ (all P>0.05). During exercise, LBF increased with moderate dehydration (Δ LBF = 0.25±0.05 l/min from 1.64±0.09 l/min; P<0.05) yet Q, leg a-vO₂ difference, muscle oxygenation, SkBF and VO₂ remained unchanged (all P>0.05). Elevations in LBF and Q at rest and during exercise were associated with increases in leg and systemic vascular conductance (P<0.05) while perfusion pressure declined indicating a net vasodilation despite the concurrent augmented plasma noradrenaline and diminished plasma ATP. After rehydration, muscle oxygenation increased (P<0.05) and leg vascular conductance remained elevated, while leg a-vO₂ difference declined further (P<0.05) and SkBF and leg VO₂ remained unchanged. **Conclusion:** Our findings demonstrate that dehydration and hyperthermia do not compromise cardiovascular function and regulation in resting and mildly exercising healthy humans and suggest that the impact of these stresses upon cardiovascular function is dependent upon the intensity of exercise. Further, they support that the need for fluid replacement is less during habitual physical activity than during intense exercise.

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