Limb Tissue Haemodynamic Responses and Regulation in the Heat-Stressed Human: Role of Local vs. Central Thermosensitive Mechanisms at Rest and During Small Muscle Mass Exercise

A thesis submitted for the degree of Doctor of Philosophy

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ABSTRACT

Limb haemodynamic responses during heat-stress and the importance of local *vs.* central temperature-sensitive mechanisms towards their regulation remain poorly understood, both at a whole-limb level and within individual tissues (i.e. skeletal muscle and skin). The aims of this thesis were to 1) investigate the haemodynamic responses at rest to direct thermal challenges both at a local level and during progressive elevations in systemic heat stress, 2) to ascertain the contribution of local vs. systemic mechanisms towards this regulation, and 3) to investigate the same responses during single-legged small-muscle mass exercise to near maximal levels. Results from Chapters 4 and 5 characterised the haemodynamic responses during isolated cooling and heating of the arm and leg, and provided evidence of alterations in both skin and skeletal muscle blood flow controlled solely through local temperature-sensitive mechanisms. While local cooling led to modest decreases in limb blood flow due to decreases in mean blood velocity alone, increases during heating occurred as a result of an increased antegrade flow, a diminished retrograde flow, and a reduction in the potentially pro-atherogenic oscillatory shear index. In Chapter 6, whole-body heating with isolated single leg cooling displayed the continued control of limb blood flow via local thermosensitive mechanisms alone, as cooled leg blood flow remained unchanged despite significant elevations in core temperature, cardiac output, and opposing heated leg blood flow. Furthermore, elevations in heated leg $\dot{V}O_2$ suggested a possible metabolic contribution to the observed skeletal muscle hyperaemic response. During incremental single-legged knee-extensor exercise to near maximal levels, blood flow was determined by a combination of metabolic workload and local tissue temperatures, regardless of whether systemic heat stress was present. Chapter 7 revealed that whilst skin and muscle blood flow in the leg continued to increase in line with local temperatures to levels of severe heat stress, rapid cooling of the leg when hyperthermic resulted in a similar reverse response in muscle tissues only, as skin blood flow remained elevated despite the abolition of high skin and subcutaneous temperatures. In addition, evidence was provided that moderate levels of whole-body heat stress provided little additional benefit to anti-atherogenic shear profiles than that experienced during isolated limb heating alone. Taken together, these findings suggest that local thermosensitive mechanisms dominate limb blood flow control during direct rapid

heating in humans both at rest and during small muscle mass exercise, but that underlying central mechanisms may act to maintain flow when local temperatures are reduced in the face of high core temperatures.

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LIST OF ABBREVIATIONS

$a\text{-}vO_2$ diff – Difference between arterial and venous oxygen concentrations ($ml \cdot l^{-1}$)
BA – Brachial artery
CFA – Common femoral artery
ctO_2 – Oxygen content of blood ($ml \cdot l^{-1}$)
DBP – Diastolic blood pressure (mmHg)
eNOS – Endothelial nitric oxide synthase
FVP – Femoral venous pressure (mmHg)
FMD – Flow-mediated dilation
GSV – Great saphenous vein
[Hb] – Haemoglobin concentration ($g \cdot l^{-1}$)
HR – Heart rate ($beats \cdot min^{-1}$)
LBF – Leg blood flow ($l \cdot min^{-1}$)
LBNP – Lower body negative pressure
LVC – Leg vascular conductance ($ml \cdot min^{-1} \cdot mmHg^{-1}$)
MAP – Mean arterial pressure (mmHg)
MBF – Muscle blood flow (LDU)
OSI – Oscillatory shear index
PFA – Profunda femoral artery
PO_2 – Partial pressure of oxygen (mmHg)
PCO_2 – Partial pressure of carbon dioxide (mmHg)
$\dot{Q}$ – Cardiac output ($l \cdot min^{-1}$)
SBP – Systolic blood pressure (mmHg)
SFA – Superficial femoral artery
SkBF – Skin blood flow (LDU)
SR – Shear rate (s^{-1})
SR_{mean} – Mean shear rate (s^{-1})
SR_{ant} – Antegrade shear rate (s^{-1})
SR_{ret} – Retrograde shear rate (s^{-1})
SV – Stroke volume (ml)
SVC – Systemic vascular conductance ($ml \cdot min^{-1} \cdot mmHg^{-1}$)
TAMV – Time-averaged mean velocity measured by Doppler ultrasound ($cm \cdot s^{-1}$)
T_b – Blood temperature (°C)
T_c – Core body temperature (°C)
T_{sk} – Skin temperature (°C)
T_m – Muscle temperature (°C)
V_{mean} – Mean blood velocity ($cm \cdot s^{-1}$)
V_{ant} – Antegrade blood velocity ($cm \cdot s^{-1}$)
V_{ret} – Retrograde blood velocity ($cm \cdot s^{-1}$)
$\dot{V}O_2$ – Oxygen uptake ($ml \cdot min^{-1}$)

CHAPTER 1

Introduction

1.1 – Background

During exposure to thermal challenges (i.e. cold or heat stress), a variety of physiological responses are triggered within the human body in an attempt to maintain body temperature within the narrow limits required for optimal physiological function. With a change in core temperature of as little as 3-4 °C potentially proving fatal (Crandall and González-Alonso; 2010), the presence of lowered or raised body tissue temperatures results in profound changes in vascular tone and a redistribution of blood volume to or from peripheral tissues in an attempt to maximise (heat stress) or minimise (cold stress) heat loss from the body (Tipton and American College of Sports Medicine; 2006). The magnitude of increase in blood flow can be substantial, with reported increases in systemic, leg, and arm flows during heat stress of up to 6 $l \cdot min^{-1}$ (Rowell *et al*, 1969), 1.4 $l \cdot min^{-1}$ (Pearson *et al*, 2011), and 0.6 $l \cdot min^{-1}$ (Naylor *et al*, 2011), respectively. These increases are second only to that observed during exercise and result in significant cardiovascular strain, as increased tissue perfusion and adrenal and sympathetic nerve activity are accompanied by reductions in arterial and central venous pressure (Rowell *et al*, 1969; Niimi *et al*, 1997; Minson *et al*, 1998; Crandall *et al*, 1999). This challenge to the cardiovascular system can be further exacerbated when exercise is performed under conditions of heat stress, with the heart required to supply blood flow to the vast vascular beds of both skin and skeletal muscle for combined thermoregulatory and metabolic purposes (Rowell, 1974; Kenney *et al*, 2014).

The control of these haemodynamic responses is complex and multi-factorial, consisting of both local (Holzer, 1998; Kellogg *et al*, 1999; Bailey *et al*, 2004; Bailey *et al*, 2005; Yamazaki *et al*, 2006; Yamazaki, 2010; Pearson *et al*, 2011; Heinonen *et al*, 2011) and central (Wyss *et al*, 1974; Wyss *et al*, 1975; Wenger *et al*, 1975; Proppe *et al*, 1976; Wenger *et al*, 1985) feedback mechanisms, as well as non-thermoregulatory inputs (Stephenson *et al*, 1984; Taylor *et al*, 1988; Crandall *et al*, 1998; Jackson and Kenny; 2003). Although there exists a wealth of previous research on haemodynamic responses to thermal stress; the multitude of techniques used to manipulate tissue temperatures, the different durations and intensities of these

thermal challenges, and the different exercise modalities employed have made it difficult to separate and identify the importance of the role of local temperature-sensitive mechanisms in the regulation of limb blood flow, both at a whole-limb level and within individual tissues (i.e. skeletal muscle and skin). Characterising this blood flow distribution and regulation is important for a number of reasons. Thermal stress can be both detrimental and beneficial to human health and performance depending on the manner in which it is experienced and its magnitude. The presence of an increased cardiovascular strain at rest and a decrease in exercise capacity during numerous forms of exercise are well documented in the literature (MacDougall *et al*, 1974; González-Alonso *et al*, 1999; Tatterson *et al*, 2000; Ely *et al*, 2007). In contrast, however, a potential use for cold and heat stress for therapeutic benefit can also exist when appropriately applied (Imamura *et al*, 2001; Kihara *et al*, 2002; Hoedemaekers *et al*, 2007). Both localised and whole-body cold stress are commonly applied for the treatment of muscle fatigue, tissue injury, and as a protective mechanism during major medical trauma (MacAuley, 2001; Polderman, 2004; Bleakley *et al*, 2004; Leeder *et al*, 2012), whilst the presence of haemodynamic responses during heat stress similar to that experienced during mild to moderate exercise has been postulated as a potential method for improving vascular health in certain clinical populations (Imamura *et al*, 2001; Kihara *et al*, 2002; Simmons *et al*, 2011; Carter *et al*, 2014). With data on limb blood flow responses and regulation in both conditions unclear – and in the case of therapeutic interventions often contradictory – a clearer picture of the magnitude of change, distribution to specific tissues in the limbs, and mechanisms regulating this control is required.

1.2 – Thesis aims

The primary aims of this thesis therefore were to 1) investigate the haemodynamic responses at rest to direct thermal challenges both at a local level and during progressive elevations in systemic heat stress, 2) to ascertain the contribution of local vs. systemic mechanisms towards this regulation, and 3) to investigate the same responses during single-legged small-muscle mass exercise to near maximal levels.

Four invasive experimental studies were carried out in order to achieve these aims. In Chapter 4, one hour of local cooling or heating of the human arm *in vivo* was used to characterise haemodynamic responses to localised changes in tissue temperature. This protocol was repeated in the leg in Chapter 5 due to the possibility of heterogeneity between the limbs. In Chapter 6, the distribution and regulation of blood flow in the leg was investigated during moderate whole-body heat stress both at rest and during incremental single-legged exercise, before Chapter 7 expanded further upon these findings by extending whole-body heat stress to severe levels approaching the participants' level of thermal tolerance.

The following chapter contains a review providing a synopsis of the existing literature most relevant to the studies described above. The review will start with a description of the methods by which body temperature is manipulated in a laboratory setting as this will impact upon the magnitude of response observed. Focus will then switch to peripheral haemodynamic adjustments to changes in temperature and the underpinning local regulatory mechanisms, before moving on to address the systemic haemodynamic alterations and concomitant central regulatory reflexes that accompany diverse isolated limb and whole-body homeostatic thermal challenges. The combined effects of exercise and heat stress will then be covered, before the aims and hypotheses of each of the studies are presented.

CHAPTER 2

Literature Review

2.1 – Manipulation of body temperature and the definition of thermal stress

Previous investigations studying limb blood flow responses to thermal challenges have employed a wide-ranging spectrum of methods, durations, and techniques in order to both characterise responses and identify the underlying control mechanisms responsible for these alterations. With the control of limb blood flow potentially operating under the influence of both local and central regulatory factors, the use of numerous direct and indirect temperature manipulations reported previously in the literature has made it difficult to ascertain the relative contribution of local vs. central mechanisms in its regulation. Further complications arise in the vastly differing levels of thermal stress often reported in the literature, with the severity of thermal challenge experienced likely to affect both the magnitude and regulation of flow within the limbs. The opening section of this literature review will outline the different methods used to manipulate body temperatures and the different levels of thermal stress commonly achieved in the literature.

2.1.1 – Methods of manipulating body and tissue temperatures

Methods of manipulating body or tissue temperatures at rest can be broadly separated into three categories:

1) Local cooling/heating – A peripheral tissue mass is altered in temperature without any corresponding decrease/increase in core or mean body skin temperature, allowing the local effects of temperature alone to be investigated without the confounding influence from systemic or reflex feedback mechanisms. The volume of tissue exposed to this type of challenge can vary substantially, ranging from an area of skin < 3 cm wide (Black *et al*, 2008) to an entire lower limb (Pearson *et al*, 2011). Commonly employed methodologies involve the manipulation of skin and underlying tissue

temperatures using ice and frozen gel packs (cold stress only) (Baker and Bell; 1991; Taber *et al*, 1992; Karunakara *et al*, 1999; Selkow *et al*, 2011), airflow (Wenger *et al*, 1986), water immersion (Barcroft and Edholm; 1943; Barcroft and Edholm; 1946; Naylor *et al*, 2011; Padilla *et al*, 2011), or water perfused cuffs (Keller *et al*, 2010; Heinonen *et al*, 2011; Pearson *et al*, 2011); with the major mechanism of heat transfer occurring via the conductance of heat through the tissues either to or from the cooled or heated skin. Local skin temperatures can differ significantly using these procedures, with temperatures reported from 5 °C during cold stress (Merrick *et al*, 1993) to 42 °C during heat stress (Barcroft and Edholm; 1943), although the majority of studies fall somewhere between these limits.

2) Whole-body indirect cooling/heating – Exposure of peripheral tissues to a cold or hot medium alters core temperature through the convective heat transfer of returning venous blood. The alteration of core temperatures in this technique without simultaneously controlling variations in skin temperature can significantly affect the magnitude of haemodynamic responses observed (Rowell, 1974). Common methods for this technique involve the immersion of the hands or forearms in cold water to decrease core temperature in previously heat-stressed humans (DeGroot *et al*, 2013) or the immersion of the lower legs in hot water to raise core temperature (Barcroft and Edholm; 1946; Barcroft *et al*, 1947; Roddie *et al*, 1956; Carter *et al*, 2014). In clinical settings, the insertion of an invasive heat-exchange catheter into the inferior vena cava via the femoral vein can allow the manipulation of internal blood temperature and permit wide-ranging core temperature manipulations (Hoedemaekers *et al*, 2007).

3) Whole-body direct cooling/heating – The manipulation of core temperature through whole-body exposure to a significant cold or heat stress. Although achievable through exposure to low/high environmental temperatures or cold/warm water immersion, the development and use of water-perfused suits in the last 50 years (Rowell *et al*, 1969; Detry *et al*, 1972; Wyss *et al*, 1974; Wyss *et al*, 1975; Minson *et al*, 1998; Ooue *et al*, 2007; Pearson *et al*, 2011; Heinonen *et al*, 2011) has allowed the investigation of thermoregulatory-

controlled haemodynamic responses without the confounding skin temperature variations experienced in outdoor environments/climatic chambers or the hydrostatic effect of whole-body water immersions.

With local and central mechanisms both shown to have a powerful regulatory effect on peripheral blood flow (described in more detail in later sections), the choice of technique used to alter tissue temperature can potentially alter both the magnitude of the blood flow response observed in the limb and its mechanisms of control. In order to systematically alter and compare these responses and mechanisms, the same method of temperature manipulation must be employed, and for this reason, all studies carried out in this thesis employed direct cooling or heating protocols, both locally and at a whole-body level.

2.1.2 – Defining thermal stress

Although commonly reported in the literature as two distinct conditions (i.e. cold stress during lowered temperatures and heat stress during raised temperatures), thermal stress is a continuous spectrum that ranges from extreme lows to extreme highs. For simplicity, this thesis will also refer to the lowering of temperatures below normal ambient conditions as cold stress and the elevation above as heat stress. However, it should be noted that the mechanisms controlling blood flow during each of these conditions should not be viewed as distinct responses but rather as an overlapping continuum as thermal stress progresses from cold to hot. The term thermal stress itself refers to the conditions responsible for changes in body temperature; such as low/high external environmental temperatures, cold-water immersion, high humidity, excess/warm clothing, and metabolic heat production through exercise; while thermal strain (e.g. lowered/elevated core temperature) occurs as a physiological response to this stress (Sawka *et al*, 2011). Although indices categorising the severity of thermal stress do exist (e.g. wet bulb globe temperature), laboratory studies tend to classify the levels of a thermal challenge by measuring changes in parameters of the resultant thermal strain, with core or mean body temperatures being that most commonly reported. Whole-body cold stress

results in lowered core temperatures and the onset of hypothermia, which can be classed as mild (< 35 °C), moderate (< 32 °C) or severe (< 28 °C) (Stocks *et al*, 2004). Conversely, heat stress results in elevations in core temperature and subsequent hyperthermia, with increases of as little as 3 °C proving potentially life-threatening even in healthy populations (Crandall and González-Alonso, 2010). Results reported in Table 2.1 show the large variations in thermal strain reported during previous localised and whole-body heat stress studies, with core temperatures ranging from 0 – 2.3 °C (average ~ 1 °C).

Table 2.1 – Previous studies investigating limb blood flow during heat stress

Author	Year	Measurement Site	Method of Heating	Duration	Method of Blood Flow Measurement	Δ T_c (°C)	Δ T_{sk} (°C)	Δ Blood Flow (ml·dl^{-1}·min^{-1})
Caldwell et al	2014	Hand	WB WWI with altered local temperature	-	Displacement Plethysmography	1.5	10	16
Abramson et al	1939	Forearm	Local WWI	-	VOP	-	12	10
Barcroft and Edholm	1943	Forearm	Local WWI	60	VOP	-	12	12
Barcroft and Edholm	1946	Forearm	Local WWI	-	VOP	-	7	4
Barcroft and Edholm	1946	Forearm	Indirect feet immersion	30	VOP	-	5	7
Barcroft et al	1947	Forearm	Indirect feet immersion	50	VOP	-	-	11
Bonde-Petersen	1992	Forearm	WB WWI	15	Xe133	0.8	1	1
Carter et al	2014	Forearm	Indirect feet immersion	25	Doppler ultrasound	0.7	-	11.5
Detry et al	1972	Forearm	WPS	30	VOP	1.1	6	23
Edholm et al	1956	Forearm	Indirect leg immersion and WPS	30	VOP	1	-	9
Fujii et al	2013	Forearm	Indirect leg immersion and WPS	30	VOP	2.3	4	16
Minson et al	1998	Forearm	WPS	60	VOP	1.9	6	30
Ooue et al	2007	Forearm	WPS	-	Doppler ultrasound	0.6	-	11.5
Ooue et al	2008	Forearm	LBE	30	Doppler ultrasound	1	-	15
Naylor et al	2010	Forearm	Local WWI	25	Doppler ultrasound	-	-	20
Padilla et al	2011	Forearm	Local WWI	60	Doppler ultrasound	-	-	17
Prinzmetal and Wilson	1936	Forearm	Local WWI	-	VOP	-	12	4
Roddie et al	1956	Forearm	Indirect feet immersion	50	VOP	-	12	9
Simmons et al	2010	Forearm	LBE	30	Doppler ultrasound	0.4	3	9.5
Smolander et al	1985	Forearm	Sauna	15	VOP	0.5	6	11
Smolander et al	1992	Forearm	Sauna and opposing limb exercise	80	VOP	0.9	8	11
Wenger et al	1975	Forearm	LBE with constant local temperature	30	VOP	1	0	11
Wenger et al	1985	Forearm	LBE with local arm temperature altered	15	VOP	-	6	5
Wenger et al	1986	Forearm	Warm local airflow	160	VOP	-	12	12
Wyss et al	1975	Forearm	WPS	60	VOP	2	6	9
Wyss et al	1976	Forearm	WPS	30	VOP	0.8	5	21
Caldwell et al	2014	Foot	WB WWI with altered local temperature	-	Displacement Plethysmography	1.5	10	9
Heinonen et al	2011	Calf	WPC	-	PET	0	8	1
Heinonen et al	2011	Calf	WPS	-	PET	1	6	1
Keller et al	2010	Calf	WPC	-	Doppler ultrasound	0.3	7	4.2
Pearson et al	2010	Leg	WPC	-	Doppler ultrasound	0	7	4.7
Pearson et al	2010	Leg	WPS	-	Doppler ultrasound	1.8	6	11.5

Missing values represent data not reported in results. Some values estimated from figure representations. Measurements not made by venous occlusion plethysmography normalised to ml·dl^{-1}·min^{-1} for comparison using limb volumes from Wang *et al* 1999. WB, whole-body; WWI, warm-water immersion; WPS, water-perfused suit; LBE, lower-body exercise; WPC, water-perfused cuff; VOP, venous-occlusion plethysmography; Xe133, Xe^{133} isotope washout; PET, positron-emission tomography.

Whilst these differences in thermal strain alone can affect limb blood flow due to changes in central haemodynamics and thermoregulatory reflexes (discussed in more detail in following sections), the profound direct impact of local temperatures on skin and potentially skeletal muscle blood flow can also cause additional dramatic alterations in peripheral haemodynamics through independent pathways, as illustrated in Table 2.2.

Table 2.2 – Estimated whole-body skin flow requirements during severe running exercise at different T_c and T_{sk}

T_c (°C)	T_{sk} (°C)	Gradient (°C)	SkBF ($l \cdot min^{-1}$)
38	30	8	1.1
38	34	4	2.2
38	36	2	4.4
39	36	3	3.0

Reproduced from Sawka *et al*, 2011. T_c, core temperature; T_{sk}, skin temperature; SkBF, whole-body skin blood flow.

The wide-ranging variety of temperature manipulations (local, indirect, direct), durations (15 to 160 min), locations (arm, leg), core temperatures (0 to + 2.3 °C), and skin temperatures (0 to + 12 °C) reported in Table 2.1 make it difficult to systematically analyse and elucidate the effect of local vs. central mechanisms on the regulation of limb haemodynamic responses.

2.1.3 – Summary

Both the methodology used to manipulate body temperature and the severity of the thermal stress achieved can potentially alter the magnitude of change in limb blood flow, the distribution to different tissues within the limb, and the mechanisms responsible for its control. In order to systematically investigate each of these responses, care must be taken to control for these potential confounding factors in order to compare responses between studies.

For the purpose of this thesis, the following core temperatures will be used to classify the intensity of the heat stress experienced in order that haemodynamic responses and regulatory mechanisms can be systematically investigated: < 0.5 °C – mild heat stress, 0.5 < 1.0 °C – moderate heat stress, 1.0 < 1.5 °C – intense heat stress and 1.5 < 2 °C – severe heat stress. In addition, the use of a water-perfused suit will allow changes in core temperature whilst simultaneously clamping skin temperature at a chosen level, thereby controlling for additional haemodynamic responses resulting from changes in local skin temperature.

2.2 – Peripheral haemodynamic adjustments to passive local and whole-body thermal challenges

The overall acute circulatory adjustments to cold and heat stress result from co-ordinated autonomic responses designed to minimise or maximise heat loss to the surrounding environment. The primary mechanism in humans to achieve this goal is through alterations in vascular tone and a redistribution of blood volume either from (cold stress) or to (heat stress) the periphery. These alterations may differ between different vascular beds within the limb (e.g. skin/muscle) and can be driven through local mechanisms, central mechanisms, or a combination of the two. The following section will provide an in-depth look at these responses and the known mechanisms controlling them during localised cold stress, localised heat stress, and whole-body heat stress. Attention will be paid to individual skin and skeletal muscle haemodynamic changes as well as whole-limb responses, as the magnitude of change and mechanisms controlling these vascular beds are most likely heterogeneous in nature. Whole-body cold stress is not addressed in this thesis, and as such will only be briefly discussed for the sake of completeness.

2.2.1 – Localised cold stress

Localised cold stress is defined in this thesis as the lowering of peripheral tissue temperatures alone without an associated drop in core or mean body skin temperatures. This technique is commonly used in athletic and clinical settings both to aid muscle recovery and manage acute soft-tissue injuries (MacAuley, 2001; Bleakley *et al*, 2004). The potential therapeutic benefits of localised cold stress are proposed to result from a myriad of interrelated factors; such as reductions in cell metabolism, muscle spasm, pain sensation, and secondary cell apoptosis in the days following injury (Bleakley *et al*, 2004). Another benefit reported almost universally in the literature is a reduction in tissue blood flow, with a subsequent attenuation of both oedema formation and inflammatory responses (Baker and Bell; 1991; Karunakara *et al*, 1999; Bleakley *et al*, 2004; Nadler *et al*, 2004; Kanlayanaphotporn and Janwantanakul; 2005; Long *et al*, 2005; Tomchuk *et al*, 2010; Selkow *et al*, 2011). Whilst this reduction in blood flow is well supported in isolated cutaneous tissues, studies investigating whole-limb responses *in vivo* are far from conclusive, while those specifically investigating skeletal muscle responses to cooling are surprisingly lacking given its widespread use.

Whole-limb blood flow

Data regarding whole-limb blood flow responses to localised cooling remain unclear. Whilst numerous studies have reported the expected decreases in limb blood flow (Thorsson *et al*, 1985; Taber *et al*, 1992; Karunakara *et al*, 1999; Yanagisawa *et al*, 2007; Gregson *et al*, 2011), only one has quantified absolute blood flow changes, with Gregson and colleagues (2011) reporting a decrease in superficial femoral arterial flow from ~ 90 to 60 ml·min^{-1} following lower-body cold water immersion. This 30% decrease broadly agrees with other relative changes reported in the literature using techniques such as impedance (Taber *et al*, 1992; Karunakara *et al*, 1999), NIRS (Yanagisawa *et al*, 2007), or Xe^{133} washout (Thorsson *et al*, 1985). In contrast to the aforementioned studies, however, other groups have reported no change in flow (Baker and Bell; 1991; Fiscus *et al*, 2005; Selkow *et al*, 2011), or

even a paradoxical increase through cold-induced vasodilation (Clarke *et al*, 1958; Ducharme and Radomski; 1990).

Discrepancies in these studies may once again come down to methodological differences or the extent and duration of cooling. Firstly, the cooling of non-exercised limbs in previous studies may have minimal influence on blood flow responses due to the relatively high level of vasoconstriction already present in the limbs in resting thermoneutral conditions. Studies into the localised cooling of previously exercised tissues are limited, but appear to suggest a significant vasoconstriction when baseline blood flows are elevated (Vaile *et al*, 2011; Ihsan *et al*, 2013). Alternatively, water immersion and compression can both decrease blood flow through non-thermoregulatory mechanisms (Merrick *et al*, 1993; Tomchuk *et al*, 2010), whilst the presence of significant thermal gradients in large muscle groups may overestimate the extent of cooling achieved when measuring skin temperatures alone (Merrick *et al*, 1993; Myrer *et al*, 1998; Jutte *et al*, 2001; Myrer *et al*, 2001; Enwemeka *et al*, 2002; Merrick *et al*, 2003; Kanlayanaphotporn and Janwantanakul; 2005). Differing responses within individual limb tissues (i.e. skin and muscle) may also impact upon whole-limb blood flow responses.

Skin blood flow

Studies on skin blood flow are more clear-cut than that of the whole-limb, with decreasing local skin temperatures resulting in a pronounced vasoconstriction of cutaneous vessels which is maintained throughout the duration of the cold stress (Kellogg, 2006; Johnson and Kellogg; 2010; Minson, 2010), albeit with the presence of repeated transient vasodilation known as a hunting response evident in some tissues (Flouris *et al*, 2008). A limitation of all skin blood flow studies is the inability to measure absolute changes in flow, with the commonly employed method of laser Doppler flowmetry capable of picking up relative changes in blood volume and velocity alone. Venous occlusion plethysmography has been used previously to represent changes in skin blood flow (Rowell, 1974), but this technique works on the assumption of an unchanged underlying skeletal muscle blood flow, which – as

discussed in following sections – is incorrect. Although still not fully understood, cutaneous vasoconstrictor responses are thought to be exerted through a variety of temperature-sensitive mechanisms, as direct cooling of cutaneous vessels *in vitro* appears to have no effect on vessel tone (Vanhoutte and Shepherd; 1970). Upon initial exposure, post-synaptic translocation of α_2-adrenergic receptors has been shown to result in an increased number of receptors for binding and augmented vasoconstriction (Bailey *et al*, 2004). This translocation has been suggested to be mediated mainly through the Rho-Rho kinase system (Bailey *et al*, 2004), which is itself activated through the generation of reactive oxygen species within the smooth muscle cells of the vascular vessels (Bailey *et al*, 2005; Yamazaki, 2010) (Fig. 2.1). With continued cooling, low skin temperatures have been shown to exert a similar effect on vascular tone as application of the NO blocker L-NAME, suggesting an additional inhibition of either the NOS system or its effects downstream (Yamazaki *et al*, 2006).

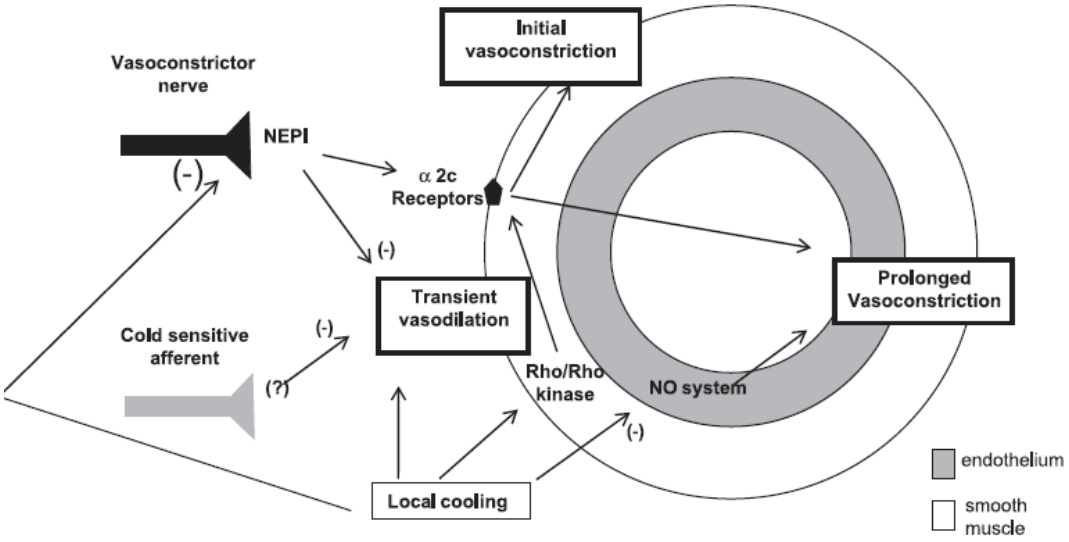

Fig. 2.1: Proposed mechanisms controlling skin blood flow during localised cold stress. Although still not fully elucidated, these effects include an upregulation of α_2-adrenergic receptors through activation of the Rho/Rho kinase system and an attenuation of NO-mediated vasodilation through either the inhibition of NOS or its downstream mechanisms. (Adapted from Johnson & Kellogg 2010).

Skeletal muscle blood flow

Skeletal muscle vasoconstriction in response to localised cold stress has previously been documented in rats (Curl *et al*, 1997; Schaser *et al*, 2007) and hamsters (Thorlacius *et al*, 1998). Despite the widespread use of localised cooling in the treatment of muscle fatigue and soft tissue injuries following exercise, results in five studies specifically assessing changes in muscle blood flow in humans are mixed, with one reporting vasodilation (Ducharme and Radomski; 1990), one no change (Selkow *et al*, 2011) and the others vasoconstriction (Thorsson *et al*, 1985; Yanagisawa *et al*, 2007; Gregson *et al*, 2011). The prolonged cooling in the former study suggests that an initial vasoconstrictor response to localised cooling may in fact be reversed if maintained over longer durations, possibly to maintain blood flow for metabolic purposes, as is also evident in cutaneous tissues at certain sites (Flouris *et al*, 2008). The mechanisms responsible for these potential decreases in flow have yet to be fully elucidated, but could be due in part to increased muscle sympathetic nerve activity (Mizushima *et al*, 1998) which could potentially lead to a decreased metabolic rate (Merrick *et al*, 1999).

2.2.2 – Whole-body cold stress

Whole-body cold stress results in extensive vasoconstriction throughout peripheral sites of the body. This response is mediated in the skin both by the mechanisms mentioned previously and via an additional reflex vasoconstriction pathway most likely controlled through the co-transmission of noradrenaline and neuropeptide Y (Stephens *et al*, 2004). The relative contribution of local vs. central thermal and non-thermal mechanisms to this vasoconstrictor response was investigated in a number of studies from the lab of Johnson and colleagues, who reported a suppression of the whole-body reflex response to cooling when local cooling was carried out simultaneously (Alvarez *et al*, 2006). A later study attributed this to an effect mediated at least in part by initial baseline vascular conductance values, as the use of sodium nitroprusside and isoproterenol to re-establish these baseline values following localised cooling led to the re-establishment of the majority of the reflex response (Hodges *et al*, 2007). Taken together, these results indicate that with

uniform whole-body cooling, reflex responses appear to dominate the overall change in skin blood flow. However, if whole-body cold stress is combined with more aggressive local cooling, the local mechanisms will dominate responses at the affected peripheral site. In contrast to responses in skin, no studies have assessed the local vs. central contribution to underlying skeletal muscle blood flow, although this could be challenging due to the presence of significant confounding factors such as shivering responses.

2.2.3 – Localised heat stress

Localised heat stress has been consistently shown to elevate limb blood flow in both the arm (Barcroft and Edholm; 1943; Barcroft and Edholm; 1946; Black *et al*, 2008; Naylor *et al*, 2011; Padilla *et al*, 2011) and leg (Keller *et al*, 2010; Pearson *et al*, 2011; Heinonen *et al*, 2011). Whilst the presence of increased limb perfusion is universally accepted, the magnitude of the response, the distribution to the tissues within the limb and their respective control mechanisms still remain unclear. With local heat application commonly used for therapeutic purposes and recent data emerging that cardiovascular adaptations to localised heat stress may provide improvements in endothelial function (Naylor *et al.* 2010; Carter *et al.* 2014), further insight into the haemodynamic responses and control mechanisms responsible for these is required.

Whole-limb blood flow

The vast majority of localised heating studies have focused on responses in forearm models, with measurements using venous occlusion plethysmography and Doppler ultrasound reporting increases in flow ranging from 70 – 400 ml·min^{-1} (Prinzmetal and Wilson; 1936; Abramson *et al*, 1939; Barcroft and Edholm; 1943; Barcroft and Edholm; 1946; Wenger *et al*, 1985; Wenger *et al*, 1986; Padilla *et al*, 2011; Naylor *et al*, 2011). These increases are mediated through vasodilation in both the conduit (brachial) artery (Simmons *et al*, 2011; Padilla *et al*, 2011) and downstream resistance vessels (Padilla *et al*, 2011; Naylor *et al*, 2011; Carter *et al*, 2014), leading

to concurrent alterations in the velocity and shear rate profiles of blood entering the arm. In normothermic conditions, the blood flow profile travelling through peripheral conduit arteries during a single cardiac cycle at rest tends to be triphasic in nature; consisting of a large antegrade flow component during systole, a reversal of blood flow during early diastole, and a return to forward flow during late diastole (Blackshear Jr *et al*, 1979). Elevations in temperature have been shown to not only increase mean flow and shear rates, but also to attenuate or even abolish the oscillatory nature of the flow profile within the artery (Simmons *et al*, 2011; Padilla *et al*, 2011). The presence of oscillatory flow has been shown to be detrimental to endothelial function both *in vitro* (Chiu *et al*, 1998; Ziegler *et al*, 1998; Conway *et al*, 2010) and *in vivo* (Thijssen *et al*, 2009; Tinken *et al*, 2009; Carter *et al*, 2013; Schreuder *et al*, 2014), and has been implicated in the formation of atherosclerotic plaques in areas of turbulent flow and low shear rate profiles (Caro *et al*, 1969; Ku *et al*, 1985). The anti-atherogenic flow profiles resulting from tissue temperature elevations have been postulated to offer the potential for improvements in endothelial function similar to that experienced during mild exercise, and limited recent studies appear to confirm this in forearm models of both healthy and chronic heart failure populations (Imamura *et al*, 2001; Kihara *et al*, 2002; Carter *et al*, 2014). Despite the higher prevalence of atherosclerotic disease in the leg (Kroger *et al*, 1999), however, no study to date has assessed the impact of local heat stress on flow and shear rate profiles in this limb.

While leg blood flow exhibits a greater absolute increase in flow during prolonged heating (increases up to 1.1 $l \cdot min^{-1}$; Pearson *et al*, 2011), flow (and therefore potentially shear rate) per unit volume is substantially less than that in the arm (~ 10 *vs.* 18 $ml \cdot dl \cdot min^{-1}$; Keller *et al*, 2010; Pearson *et al* 2011). A reason for this could involve the presence of vascular heterogeneity between the upper and lower limbs, with vessels in the leg shown to have an augmented vasoconstrictor (Pawelczyk and Levine; 2002) and attenuated vasodilator responsiveness (Newcomer *et al*, 2004) when compared to the arm. Alternatively, the greater skin-to-muscle tissue ratio in the arm compared to the leg could also play a part if the vasodilatory response is predominantly confined to the cutaneous tissues alone. The relative contribution of

each tissue to the heat stress response remains contentious, and will be discussed in more detail below.

Skin blood flow

Much like localised cold stress, the cutaneous response to localised heat stress has received much attention and is well characterised; consisting of an early transient vasodilation, followed by a partial recovery of tone and a final slower prolonged vasodilation that is capable of maximally dilating cutaneous vessels even in the absence of systemic hyperthermia (Fig. 2.2).

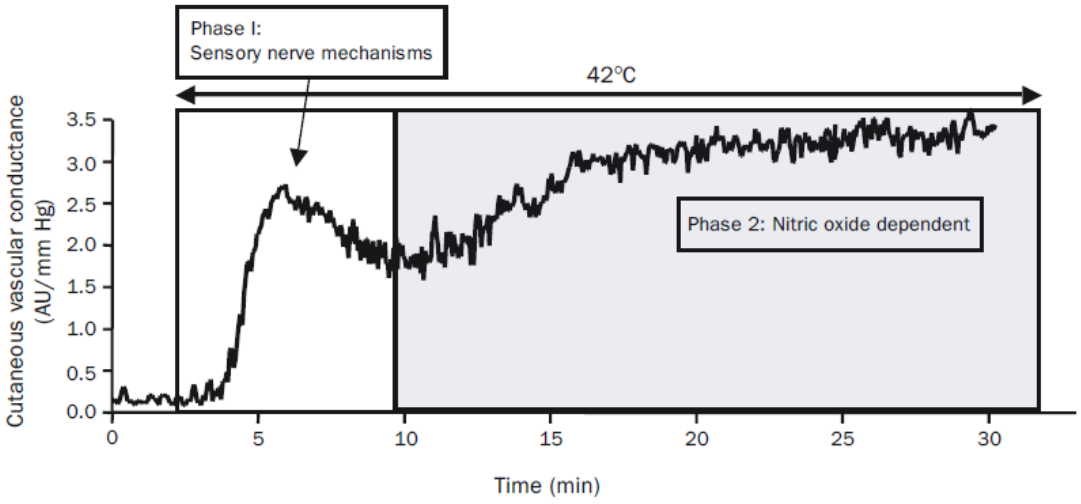

Fig. 2.2: Cutaneous vascular response following rapid skin warming to 42 °C. Phase I consists of rapid vasodilation mediated predominantly through an afferent axon reflex. This is followed by a transient vasoconstriction before a prolonged plateau is established in Phase II through NO-dependent pathways (Adapted from Charkoudian 2003).

The initial transient vasodilation observed during local heating of the skin occurs independently to central feedback and is mediated predominantly through a local axon reflex, as shown by its inhibition by topical anaesthetic (Minson *et al*, 2001) but not proximal neural blockade (Wenger *et al*, 1986). This neural modulation appears to be activated through the stimulation of heat-sensitive vanilloid receptors located within the skin and controlled through the release of substances such as calcitonin

gene-related peptide (CGRP), neurokinin A, and substance P (Holzer, 1998), potentially aided by sympathetic nerve contribution (Houghton *et al*, 2006). As local heating continues, important contributions from the NO system help to establish and maintain the prolonged vasodilator response (Kellogg *et al*, 1999), most likely through augmented activation of eNOS (Kellogg *et al*, 2008) or alterations downstream of nitric oxide generation (Johnson and Kellogg; 2010) (Fig. 2.3). The failure of NO antagonists such as L-NAME to completely abolish the response, however, displays the significant contribution of other, possibly unidentified, mechanisms (Minson *et al*, 2001; Black *et al*, 2008).

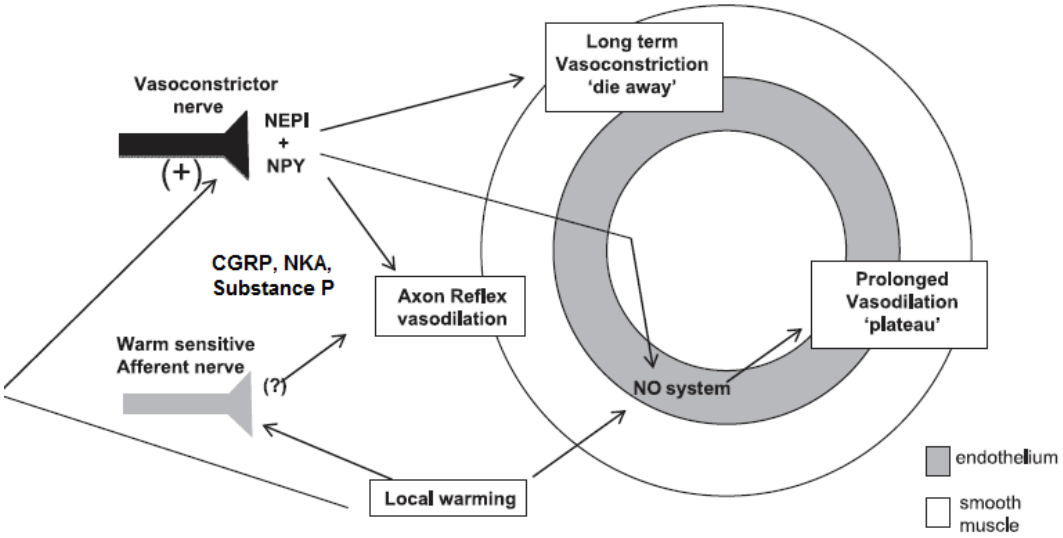

Fig. 2.3: Proposed mechanisms controlling skin blood flow during localised heat stress. The activation of warm sensitive afferent nerves result in an initial axon reflex vasodilation mediated through calcitinon gene related peptide (CGRP), neurokinan A (NKA), and Substance P, potentially aided by sympathetic nerve contributions. Important contributions from the NO system help to establish and maintain a prolonged plateau as heating continues (Adapted from Johnson and Kellogg 2010).

Skeletal muscle blood flow

The question of whether these increases in skin blood flow account for the full hyperaemic response observed in the limbs has remained highly controversial for many years, due to the difficulty in separating skin and skeletal muscle tissue perfusion *in vivo* and the variety of experimental techniques employed in previous

studies. Early work in forearm models using techniques such as superficial and deep venous blood oxygen content comparisons (Detry *et al*, 1972) and antipyrine^{125}-I washout (Detry *et al*, 1972; Johnson *et al*, 1976) concluded the presence of a local vasodilation confined to the skin alone, as did animal studies in conscious baboons (Hales *et al*, 1979). Together, these findings shaped the commonly held view that skeletal muscle tissue was as a whole unresponsive to local changes in temperature, with increases in limb blood flow presumably occurring in the skin alone. However, later studies in cancer patients using 20 min of intense local deep muscle heating via microwave diathermy – where tissue temperatures are rapidly elevated to 45 °C – have shown elevations in thigh skeletal muscle perfusion to levels approaching 32 $ml \cdot dl^{-1} \cdot min^{-1}$, displaying a clear ability for profound muscle vasodilation in response to heat stress (Wyper and McNiven; 1976; Lehmann *et al*, 1978; Sekins *et al*, 1984; Song, 1984; Giombini *et al*, 2007). Later studies involving techniques such as Contrast-Enhanced Ultrasound (Akyürekli *et al*, 1997), Near Infra-Red Spectroscopy (Okada *et al*, 2005), Xe^{133} clearance (Keller *et al*, 2010), Positron-Emission Tomography (Heinonen *et al*, 2011), duplex Doppler ultrasound (Pearson *et al*, 2011), and laser Doppler flowmetry (Binzoni *et al*, 2012) have confirmed this response using more traditional methods of heating. Heinonen and colleagues (2011) expanded upon these findings further by demonstrating that this increase in flow occurred in response to direct tissue heating alone, suggesting that earlier results may have been influenced by the indirect body heating protocol employed (Edholm *et al*, 1956; Roddie *et al*, 1956). Although the mechanisms controlling this increased flow are still unresolved, they do not appear to be through a decreased sensitivity to vasoconstrictor drive, as α-adrenoreceptor responsiveness is preserved (Keller *et al*, 2010). This finding points to the local release of competing vasodilator metabolites as the most promising candidates for increasing perfusion. Recent *in vivo* work by Pearson *et al*, (2011) reported increases in arterial ATP in line with progressive increases in core temperature and muscle blood flow during heat stress, potentially implicating intravascular ATP release as a potential modifier of vascular tone during a thermal challenge. Follow-up work from the same laboratory identified erythrocytes as the sole contributor to this response (Kalsi and González-Alonso; 2012), operating through a separate pathway to the oxygen-sensitive mechanism previously reported during exercise (González-Alonso *et al*, 2002; Rosenmeier *et al*, 2004; Mortensen *et al*, 2011). Other factors responsible could involve known

sympatholytic metabolites such as NO (Thomas and Victor; 1998), or an increased metabolic drive for blood flow through a Q_{10} effect of temperature and increased $\dot{V}O_2$, although whether this is altered during passive heat stress is still contentious (Rowell *et al*, 1970; Savard *et al*, 1988; Nielsen *et al*, 1990; Febbraio *et al*, 1994; Koga *et al*, 1997; Ferguson *et al*, 2002; Ferguson *et al*, 2006; Pearson *et al*, 2011; Koga *et al*, 2013).

2.2.4 – Whole-body heat stress

Whole-limb blood flow

Whole-body heat stress results in extensive vasodilation throughout peripheral sites and can be mediated through a combination of local, central, and non-thermoregulatory mechanisms. Direct whole-body heating can result in core and skin temperatures of up to ~ 40 °C (Rowell *et al*, 1969; Minson *et al*, 1998; Pearson *et al*, 2011; Stöhr *et al*, 2011). These extreme levels of heat stress can lead to substantial increases in peripheral blood flow, with limb blood flow alone being shown to reach levels of 35 $ml \cdot dl^{-1} \cdot min^{-1}$ in the arm (Minson *et al*, 1998) and 11.5 $ml \cdot dl^{-1} \cdot min^{-1}$ in the leg (Pearson *et al*, 2011) during direct whole-body heating (Table.2.1). However, how much of this increase can be attributed to the local temperature-sensitive responses described previously remains unclear. Early research addressing this question has indicated a predominantly reflex control of the limb hyperaemic response, with studies using proximal nerve blockade (Grant and Holling; 1938; Edholm *et al*, 1957; Blair *et al*, 1960), atropine blockage of muscarinic receptors (Roddie *et al*, 1957), and sympathetic denervation (Doupe *et al*, 1943) all significantly attenuating increases in limb blood flow. However, although the indirect heating methods employed throughout each of these studies would have resulted in an elevated core temperature; skin and local tissue temperatures at the site of measurement in the forearm would most likely have been significantly lower. With evidence of significant vasodilation in both skin and skeletal muscle tissues during exposure to local increases in tissue temperature, the mechanisms responsible for flow increases in these previous studies may be different to those experienced during direct whole-body heating. In line with this, data from Pearson *et al*, (2011)

indicated a substantial local contribution to leg blood flow during progressive levels of heat stress, further suggesting that limb blood flow during direct and indirect heating may operate under different mechanistic control.

Skin Blood Flow

The control of skin blood flow during whole-body heat stress is a complex multi-factorial process involving both central and peripheral thermoregulatory networks, and can be modified by numerous non-thermoregulatory feedback mechanisms such as exercise (Taylor *et al*, 1988), circadian rhythms (Stephenson *et al*, 1984), metaboreceptors (Crandall *et al*, 1998), and baroreceptors (Jackson and Kenny; 2003). In short, the presence of an elevated blood temperature perfusing the pre-optic anterior hypothalamus within the brain during increases in core temperature is integrated with afferent feedback from deep tissue and skin thermosensors to provide an indication of the overall thermal load experienced by the body (Sawka *et al*, 2011) (Fig. 2.4).

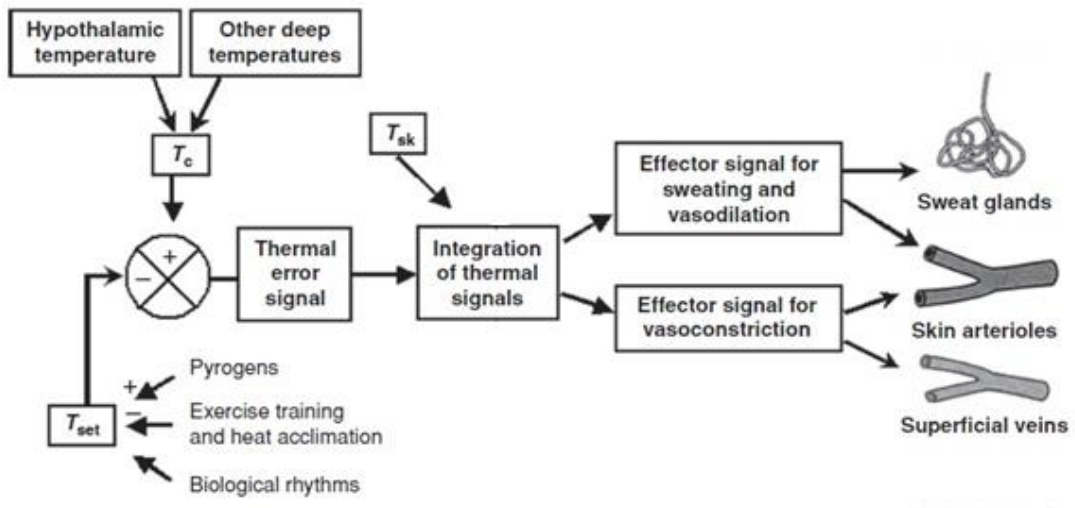

Fig. 2.4: Schematic diagram of the factors controlling skin blood flow during whole-body thermal challenges in humans (Reproduced from Sawka et al 2011).

According to this model, any deviation from the body core's preferred temperature 'set-point' (typically ~ 37 °C) triggers efferent signals to instruct cutaneous resistance vessels to vasodilate, thereby redistributing blood to the periphery for proportionate heat loss. In glabrous skin (e.g. palms of hands and soles of feet), this vasodilation is achieved exclusively through the withdrawal of tonic sympathetically-mediated vasoconstrictor tone (Roddie *et al*, 1957). In non-glabrous (hairy) skin, however, increases in cutaneous flow are achieved through both sympathetic vasoconstrictor withdrawal and the additional activation of a sympathetically-driven active vasodilatory pathway unique to humans. This latter pathway can account for up to 90% of the increase in skin blood flow during indirect heating (Roddie *et al*, 1957) and is proposed to work through as-yet-unidentified cholinergic co-transmitters binding to muscarinic receptors (Kellogg *et al*, 1995), with contributions from VIP (Bennett *et al*, 2003), substance P (Wong and Minson; 2006), neuronal NOS (Kellogg *et al*, 2008), and histamine (Wong *et al*, 2004) all implicated in its control. This increased reflex drive during whole-body heat stress occurs in tandem with the locally-acting mechanisms described in the previous section, which can act to augment or attenuate the overall blood flow response occurring at the peripheral site measured. The relative inputs of central vs. local control is generally reported to be ~ 10:1 (i.e. a 1 °C increase in core temperature will result in a skin blood flow response 9x that of the same increase in skin temperature) (Wyss *et al*, 1974; Wyss *et al*, 1975; Wenger *et al*, 1975; Proppe *et al*, 1976; Wenger *et al*, 1985). Whilst this initially suggests a minimal contribution of local mechanisms to skin (and therefore limb) blood flow control, the wide-ranging capacity for altering skin temperature – from under < 10 °C (Merrick *et al*, 1993) to over 40 °C (Black *et al*, 2008) – coupled with modest changes in core temperature (usually < 3 °C), results in a much greater influence of local temperature than is often appreciated. The major effect of local temperature changes is to modify the threshold core temperature at which skin blood flow starts to increase (Johnson and Proppe; 2011), although changes in the sensitivity of the response have also been observed (Wenger *et al*, 1985). As displayed in Fig 2.5. despite the same core temperature and therefore identical central reflex control, an earlier rise in skin blood flow due to the direct effect of high local temperatures will result in significantly higher leg blood flow at Site A (40 °C) compared to the cooler Site B (30 °C), where increases in blood flow will occur solely due to central reflex drive alone (Barcroft and Edholm; 1943).

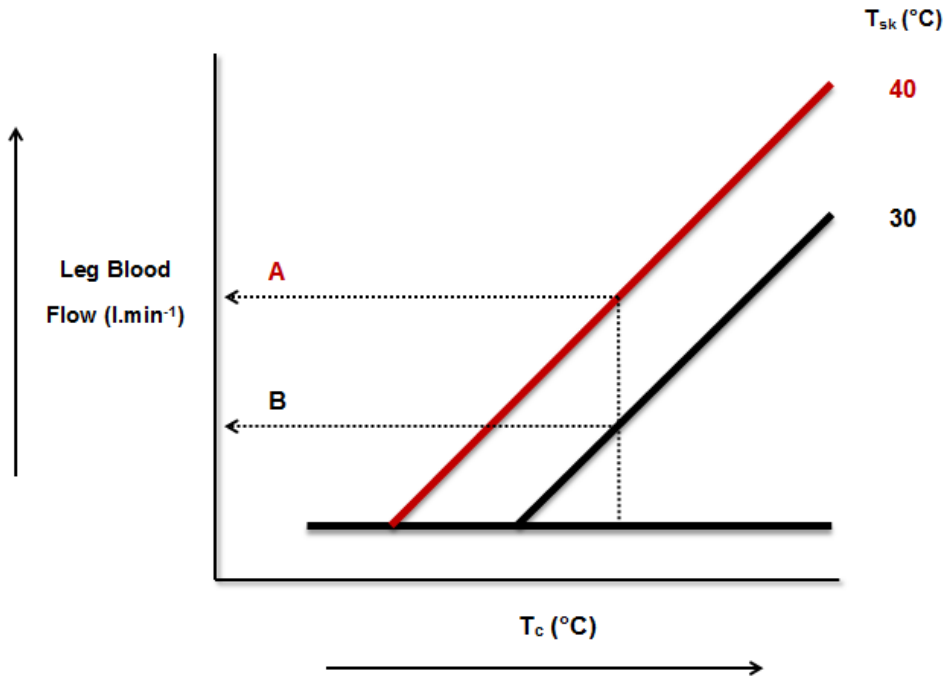

Fig. 2.5: Inter-relationship of leg blood flow control by core (T_c) and skin (T_{sk}) temperatures. The earlier threshold for vasodilation afforded by high local skin temperatures results in a significantly higher leg blood flow per °C increase in T_c when local T_{sk} is 40 °C (A) compared to 30 °C (B) (Adapted from Johnson and Proppe 1996).

Skeletal muscle blood flow

Changes in underlying deep tissue perfusion also have the potential to affect whole-limb responses to heat stress . Early estimates of maximal whole-body skin blood flow of 7-8 l·min^{-1} relied on the assumption of an unchanged or even decreased skeletal muscle blood flow (Rowell, 1974). As mentioned previously, this assumption has recently been challenged by results showing clear increases in muscle blood flow when exposed to direct local heat stress (Okada *et al*, 2005; Keller *et al*, 2010; Pearson *et al*, 2011; Heinonen *et al*, 2011; Binzoni *et al*, 2012), making this estimate of maximal skin blood flow invalid when participants are directly heated. In contrast to local heating, whole-body heating leads to a hyperadrenergic state, characterised by increases in circulating catecholamines (Kim *et al*, 1979) and increased muscle and skin sympathetic nervous activity (Niimi *et al*, 1997; Crandall *et al*, 1999). Although the presence of active sympathetic vasodilator innervation in the skin has been shown to attenuate this competing vasoconstrictor drive (Wilson *et al*, 2001), the lack of similar known innervation in the skeletal muscle, along with a

preserved α-adrenoreceptor responsiveness (Keller *et al*, 2010), has led some authors to postulate that increasing resistance within skeletal muscle tissue may aid blood pressure regulation during severe levels of heat stress (Niimi *et al*, 1997; Kenney *et al*, 2014). Addressing this theory, Heinonen and colleagues (2011) observed increases in skeletal muscle, adipose tissue and bone blood flow during both local and moderate whole-body heating, although with a slightly attenuated response in the whole-body protocol. For skeletal muscle blood flow to be elevated at all (or even simply maintained) under this increased adrenergic and sympathetic drive would require opposing sympatholytic mechanisms, as discussed in the previous section. No study to date has assessed muscle blood flow responses to more severe levels of heat stress, however, with further increases in adrenergic drive potentially restricting perfusion when blood pressure regulation is exposed to yet greater challenges.

2.2.5 – Summary

The control of peripheral haemodynamics during exposure to both cold and heat stress is a complex combination of local and central mechanisms that may act both in tandem and in competition. Although the ratio of central to local control is often reported as 10:1, the wider range of skin temperatures that can be experienced relative to core temperature changes may result in a substantial contribution from local mechanisms during direct cooling and heating challenges, and as such the relative contribution of these local and central mechanisms during direct heat stress remains unclear. In addition, many forms of mild intensity exercise (e.g. slow walking) can result in significant elevations in local blood flows and temperatures without a corresponding change in whole-body core temperature. The changes observed in whole-limb blood flow occur as the sum of perfusion changes in all tissues of the limb, which are most likely heterogeneous in both their response and mechanistic control.

2.3 – Systemic haemodynamic adjustments to passive whole-body thermal challenges

The often substantial changes in peripheral blood flow during thermal challenges must be supported by equivalent changes at a systemic level in order to maintain perfusion within the tissues. Tissue blood flow at any site within the body occurs as an integrated result of the tissue perfusion pressure and the conductance of its vascular tree (Laughlin *et al*, 2011). Whilst localised cooling and heating have little to no effect on total peripheral resistance, the profound changes in vascular conductance during whole-body cold or heat stress require proportional central adjustments in order to maintain systemic blood flow and perfusion pressure throughout the body. Whilst numerous studies in exercising dogs and humans have indicated that these central adjustments during exercise are predominantly regulated by peripheral metabolic demand (Guyton, 1968; González-Alonso *et al*, 2008; Bada *et al*, 2012; Munch *et al*, 2014), the question of whether the same applies for thermoregulatory function remains to be elucidated.

2.3.1 – Whole-body cold stress

As mentioned previously, whole-body cold stress is not covered in this thesis and will therefore only be briefly described for the sake of completeness. The redistribution of blood from peripheral to central vessels during exposure to cold stress results in increases in mean arterial pressure, stroke volume, cardiac output, and decreases in heart rate (Stocks *et al*, 2004). As cold exposure is prolonged, increased metabolic heat production via brown adipose tissue stores (van Marken Lichtenbelt, Wouter D *et al*, 2009) and shivering thermogenesis (Iampietro *et al*, 1960) results in further increases in cardiac output, mediated predominantly through further increases in stroke volume (Peter *et al*, 1970).

2.3.2 – Whole-body heat stress

In contrast to whole-body cold stress, the pronounced increases in peripheral blood flow and the reductions in arterial and central venous pressures during whole-body heating at rest places a significant cardiovascular strain on the human body second only to that experienced during exercise. In order to prevent excessive decreases in mean arterial pressure in the face of these alterations, the body must maintain peripheral perfusion through a combination of increased cardiac output and the redistribution of blood from other compliant vascular beds. Modulation of the baroreflex set point acts to maintain baroreceptor-mediated regulation of blood pressure in the face of these significant cardiovascular adjustments (Crandall, 2008). Cardiac output increases in line with whole-body core temperature at a rate of ~ 3 $l \cdot min^{-1} \cdot °C^{-1}$, as shown in Fig. 2.6 (Koroxenidis *et al*, 1961; Rowell *et al*, 1969; Bonde-Petersen *et al*, 1992; Niimi *et al*, 1997; Minson *et al*, 1998; Peters *et al*, 2000; Pearson *et al*, 2011; Nelson *et al*, 2011; Ganio *et al*, 2012; Ogoh *et al*, 2013).

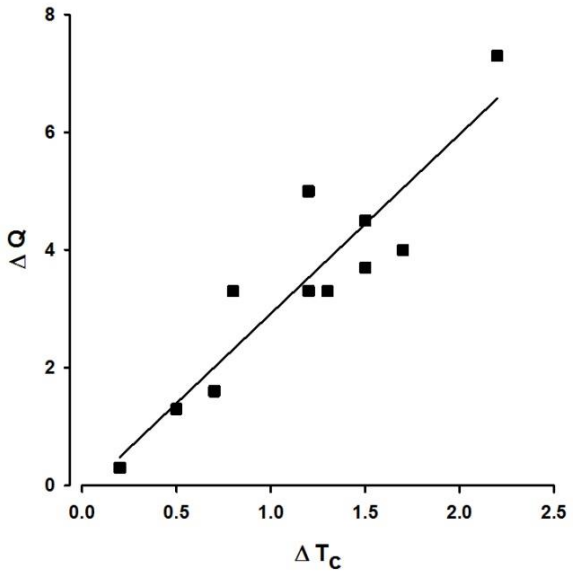

Figure 2.6: Analysis of changes in cardiac output vs. changes in core temperature in 11 independent research articles. Each data point represents mean change in one study. Regression equation y = 3.05x – 0.13; R^2 = 0.84; P < 0.05.

These progressive increases in cardiac output are predominantly achieved through increases in heart rate of ~ 35 beats·min^{-1}·°C^{-1} (Rowell *et al*, 1969; Wyss *et al*, 1975; Bonde-Petersen *et al*, 1992; Niimi *et al*, 1997; Minson *et al*, 1998; Ganio *et al*, 2012; Ogoh *et al*, 2013), as stroke volume has been shown to either remain essentially unchanged (Peters *et al*, 2000; Nelson *et al*, 2011; Stöhr *et al*, 2011; Ganio *et al*, 2012; Ogoh *et al*, 2013) or even slightly increase (Rowell *et al*, 1969; Bonde-Petersen *et al*, 1992). The mechanisms responsible for heart rate increases are multi-factorial, but appear to consist of a combination of a direct effect of blood temperature on intrinsic heart rate (Badeer, 1951; Jose *et al*, 1970; Gorman and Proppe; 1984), baroreceptor-mediated feedback caused by reduced arterial and central venous pressures (Wilson and Crandall; 2011), and the presence of increased circulating catecholamines (Rowell, 1990). The ability of the heart to maintain or even slightly increase stroke volume is remarkable given that passive heat stress results in reductions in central blood volume (Crandall *et al*, 2008) alongside a lack of muscular contractions and their associated peripheral muscle pump action. This movement of blood volume away from the core results in reductions in central venous and right atrial mean pressures (Rowell *et al*, 1969; Minson *et al*, 1998; Wilson *et al*, 2007; Keller *et al*, 2009), left ventricular filling pressure (Wilson *et al*, 2007; Wilson *et al*, 2009) and end diastolic volume (Stöhr *et al*, 2011); all of which should theoretically compromise the heart's ability to maintain stroke volume. However, both the detrimental effects of a decreased preload and associated leftward shift of the Frank-Starling curve (Wilson *et al*, 2009) appear to be effectively counteracted in young healthy humans by a number of compensatory adjustments in cardiac function, which act in unison to enhance left ventricular ejection fraction and increase cardiac output to meet the blood flow requirements of the extensive vasodilation present in the periphery. These adjustments include a preservation of diastolic function in the face of decreased filling pressures (Brothers *et al*, 2009), an increased left atrial and ventricular systolic function (Brothers *et al*, 2009), an increased twisting velocity of the ventricles (Stöhr *et al*, 2011), and an increased left ventricular ejection fraction (Crandall *et al*, 2008). Taken together, these results provide strong evidence of an inotropic effect of heat stress on cardiac contractility, acting to maintain stroke volume in the face of decreased left ventricular filling pressure and permitting the large increases in cardiac output.

2.3.3 – Summary

Whole-body thermal challenges lead to profound peripheral and central haemodynamic adjustments. Of these challenges, whole-body heat stress offers by far the larger cardiovascular strain, as a substantial increase in blood supply to peripheral tissues compromises cardiac filling and in some individuals the maintenance of stroke volume. As a result, increases in heart rate and cardiac function are experienced in order to elevate cardiac output to the levels that minimise the drop in perfusion pressure in the tissues.

2.4 – The cardiovascular challenge of combined exercise and heat stress

The combination of exercise and heat stress can lead to one of the greatest cardiovascular challenges experienced by healthy humans, as the vast vascular beds of both skeletal muscle and skin dilate in response to joint metabolic and thermoregulatory demands. Whether this regulation manifests as a competition (Rowell, 1993) or commensalism (Kenney *et al*, 2014) between the two circulations remains open to debate, although whichever it may be, the end result remains an elevated cardiac strain (Williams *et al*, 1962; Rowell *et al*, 1966; Rowell *et al*, 1969; Savard *et al*, 1988; Nielsen *et al*, 1990; González-Alonso and Calbet; 2003; Trinity *et al*, 2011; Pearson *et al*, 2011; Schlader *et al*, 2013) and impaired performance capacity (Galloway and Maughan; 1997; González-Alonso and Calbet; 2003; Arngrímsson *et al*, 2004; Ely *et al*, 2010; Periard *et al*, 2011). Much like passive heat stress, the haemodynamic responses to combined exercise and heat stress are complex and can be affected by a myriad of factors such as the volume of muscle-mass engaged, the intensity and duration of the exercise, and the severity of the heat stress experienced. The next section of this literature review will focus on factors controlling and ultimately limiting limb blood flow during exercise in thermoneutral ambient conditions, before expanding upon these responses with the added challenge

of heat stress. Finally, the potential relative influences of central vs. local mechanisms to this control of blood flow within the limb will be discussed.

2.4.1 – Haemodynamic responses to exercise in thermoneutral conditions

The transition from rest to exercise results in profound changes in the cardiovascular system at both the peripheral and systemic level. For prolonged dynamic exercise to occur, sufficient oxygen delivery to the exercising tissues is required in order to maintain aerobic metabolism and prevent the rapid onset of muscular fatigue. In order to facilitate this increased oxygen demand, working muscle blood flow increases in line with metabolic work rate, thereby matching oxygen delivery and uptake ($\dot{V}O_2$) to tissue metabolic demand. This substantial increase in skeletal muscle perfusion is achieved through the combination of an intensity-dependent increase in cardiac output (Åstrand *et al*, 1964; Grimby *et al*, 1966; Mortensen *et al*, 2005) alongside a much smaller contribution from the redistribution of blood flow from other compliant beds such as the splanchnic (Rowell *et al*, 1964; Rowell *et al*, 1965) and renal (White and Rolf; 1948; Chapman *et al*, 1948; Grimby, 1965) circulations. It has long been recognised that there is a maximum oxygen uptake ($\dot{V}O_2max$) that can be achieved in humans during intense levels of exercise (Hill *et al*, 1924). With the intake and delivery of oxygen to the tissues dependent on the respiratory, cardiovascular and metabolic systems, there are numerous sites at which increases in $\dot{V}O_2$ could potentially be compromised. With the exception of a small population of highly-trained individuals (Dempsey and Wagner; 1999), oxygen diffusion from the lungs to the blood stream does not appear to be a major limiting factor to this phenomenon as arterial oxygen content is remarkably well maintained even at maximal exercise intensities (Mortensen *et al*, 2005; Mortensen *et al*, 2008). This finding implicates a cardiovascular limitation in the attainment of $\dot{V}O_2max$, and the precise physiological mechanisms contributing to this have been studied for almost a century.

With oxygen uptake determined by a combination of blood flow delivery to the tissues and the extraction of oxygen upon arrival, the question as to whether central (i.e. the pumping capacity of the heart) or peripheral (i.e. maximal vasodilatory or oxygen extraction capacity within skeletal muscle) factors are responsible for the upper limit of oxygen uptake has long been studied. Early work measuring exercising skeletal muscle blood flow using Xe^{133} washout techniques reported maximum values of 40-50 $ml \cdot 100g^{-1} \cdot min^{-1}$ (Grimby *et al*, 1967; Pirnay *et al*, 1972) during levels approaching $\dot{V}O_2max$. With an estimated exercising muscle mass of ~ 15 kg during whole-body dynamic exercise, these figures would equate to a maximal skeletal muscle blood flow of 7.5 $l \cdot min^{-1}$; well within the pumping capacity of the heart and suggestive of a peripheral limitation to exercise capacity. This theory was refuted, however, by a seminal paper published by Andersen and Saltin (1985), in which the development of a single-legged exercise technique combined with accurate measurements of leg blood flow using thermodilution reported skeletal muscle blood flows up to 300 $ml \cdot 100g^{-1} \cdot min^{-1}$ when exercise was carried out using the thigh extensors alone. These findings have been confirmed in numerous additional studies (Rowell *et al*, 1986; Richardson *et al*, 1995; Mortensen *et al*, 2005; Mortensen *et al*, 2008; Munch *et al*, 2014), and if extrapolated to whole-body maximal exercise would equate to skeletal blood flow requirements approaching 45 $l \cdot min^{-1}$; a value far outstripping cardiac pumping capacity in even the fittest populations. This central limitation was neatly illustrated by Mortensen *et al* (2008) through the measurement of leg blood flow, cardiac output, and leg $\dot{V}O_2$ during incremental exercise to exhaustion using both small and large muscle-masses. Findings from this study indicated that in contrast to the linear increase in all three variables during small muscle-mass exercise to exhaustion; leg blood flow, cardiac output and leg $\dot{V}O_2$ were all attenuated during whole-body exercise when approaching the limits of exercise capacity, indicating a restriction in peripheral conductance in line with a plateau in cardiac output (Fig. 2.7).

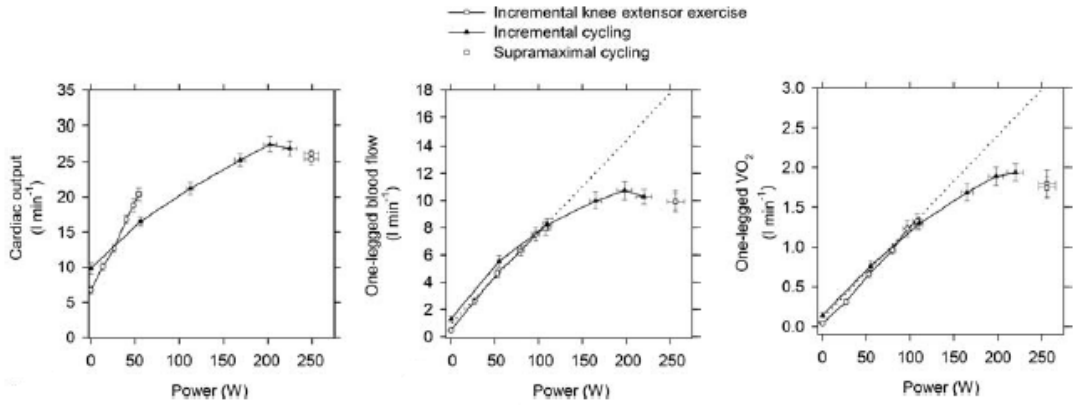

Figure 2.7: Comparison of cardiac output, one-legged blood flow, and one-legged $\dot{V}O_2$ during incremental knee extensor exercise, incremental cycling, and supramaximal cycling to exhaustion. Note the attenuation in leg blood flow and $\dot{V}O_2$ in line with reductions in cardiac output when exercise in performed at an intensity that maximally challenges the body's cardiac capacity (Reproduced from Mortensen et al 2008).

This paradoxical decrease in leg vascular conductance (or increase in vascular resistance) during severe exercise intensities is most likely mediated through neural reflexes designed to prioritise mean arterial pressure regulation when peripheral blood flow demand begins to exceed the supply capability of the heart. Evidence of this response exists through observations of an attenuation in limb blood flow when upper-body exercise is added to lower-body (Calbet *et al*, 2004; Secher and Volianitis; 2006; Calbet *et al*, 2007); an increased noradrenaline spill-over and leg constriction upon reaching severe exercise intensities in healthy humans (Pawelczyk *et al*, 1992), and the presence of unchecked limb vasodilation and subsequent hypotension during exercise in patients suffering from tetraplegia (Dela *et al*, 2003). A number of pathways have been implicated in this control, including arterial and cardiopulmonary baroreflexes (Mack *et al*, 1988; Strange *et al*, 1990), and both locomotor (Rowell and O'Leary; 1990) and respiratory muscle metaboreflexes (Harms *et al*, 1997). Although this increased sympathetic drive is present throughout all stages of exercise, its ability to increase vascular tone within skeletal muscle is predominantly over-ridden throughout light to moderate intensity exercise via locally-acting vasoactive metabolites produced during muscular contractions. The exact metabolites responsible for this hyperaemic response have yet to be

determined, but most likely include a range of mediators including nitric oxide; ATP and its derivatives ADP, AMP, and adenosine; cyclooxygenase and prostaglandins; endothelial-derived hyperpolarising factor; K^+; and reactive oxygen species (Laughlin *et al*, 2011). These most likely operate through a combination of synergistic interactions and redundant pathways where individual metabolites can act to stimulate release of or take over from various other compounds (Hellsten *et al*, 2012); whilst additional neural input from central command and chemoreflex pathways act to further fine-tune delivery so that blood flow is closely matched to metabolic demand for as long as possible. This finely-balanced competition between local metabolic dilation and central reflex mediation allows for substantial increases in blood flow to the exercising muscle whilst simultaneously redistributing blood from other compliant vascular beds such as the renal and splanchnic circulations (White and Rolf; 1948; Chapman *et al*, 1948; Rowell *et al*, 1964; Rowell *et al*, 1965; Grimby, 1965) and non-exercising limbs (Calbet *et al*, 2007). During intense whole-body exercise, however, reductions in stroke volume and cardiac output – most likely as a result of decreases in venous return to the heart and a reduced cardiac filling pressure (Bada *et al*, 2012; Munch *et al*, 2014) – challenge the maintenance of whole body arterial pressure and result in accompanying reductions in leg vascular conductance in the face of increasing oxygen demand, ultimately limiting aerobic exercise capacity. With exercise alone capable of maximally challenging cardiac pumping capacity, the added challenge of heat stress and its associated cardiovascular strain add a further obstacle to circulatory regulation under these conditions.

2.4.2 – Heat stress attenuates maximal whole-body exercise capacity

Both heat stress and exercise independently produce significant strains on the cardiovascular system for the purposes of thermoregulation and metabolism, respectively. When exercise is performed in the heat, the inefficient nature of dynamic muscular contractions results in the thermal stress experienced due to high external environmental temperatures during passive heat stress being further compounded by the substantial production of metabolic heat, with as much as 70-

80% of absolute metabolic rate being converted to heat within the tissues (González-Alonso *et al*, 2000). The need for the cardiovascular system to satisfy both an increased metabolic and thermoregulatory demand creates an additive challenge to cardiac pumping capacity that ultimately leads to an earlier decline in cardiac output as exercise intensity increases (Fig. 2.8).

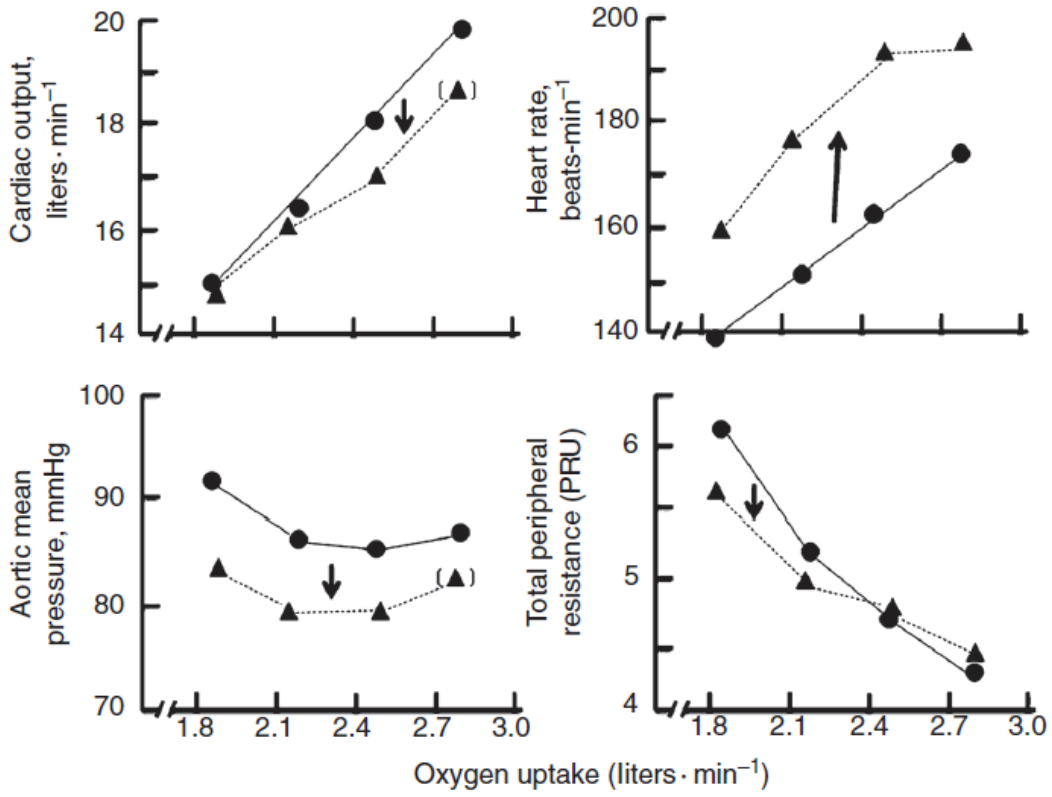

Figure 2.8: Cardiovascular responses to incremental whole-body exercise in the heat in untrained men. Peripheral redistribution of blood to satisfy both thermoregulatory and metabolic demands compromises cardiac filling and stroke volume, resulting in an elevated heart rate, decreased mean arterial pressure, and ultimately decreased cardiac output during exercise in heat stress (▲)in comparison to equivalent exercise in normothermic conditions (●) (Reproduced from Rowell et al 1966).

This decrease in cardiac output at high exercise intensities has long been postulated to predominantly occur due to the presence of an increased thermoregulatory-driven demand for skin blood flow, resulting in reductions in central blood volume, cardiac filling pressure, end diastolic volume, and ultimately a lowered stroke volume in comparison to the same exercise intensities in normothermic conditions (Rowell *et*

al, 1966; Rowell *et al*, 1969; Trinity *et al*, 2011), although this theory has not been directly scrutinised. During light to moderate intensity exercise, these decreases in stroke volume can be compensated for by reciprocal increases in heart rate and cardiac function, and indeed cardiac output is largely maintained or even increased (Rowell *et al*, 1966; Savard *et al*, 1988; Nielsen *et al*, 1990; Nybo and Nielsen; 2001; Pearson *et al*, 2011; Stöhr *et al*, 2011). As intensity increases, however, further reductions in ventricular filling time caused by heart rates approaching maximal levels compromise the heart's ability to adequately increase cardiac output (Fritzsche *et al*, 1999; Trinity *et al*, 2011). As shown in Fig. 2.9, this compromised cardiac capacity, combined with an increased adrenergic drive through circulating catecholamines (Kim *et al*, 1979) and increased muscle and skin sympathetic nerve activity (Rowell, 1990; Niimi *et al*, 1997; Crandall *et al*, 1999), acts to limit both the perfusion and maximal oxygen uptake of the exercising limbs during high intensity exercise (Rowell, 1974; González-Alonso and Calbet; 2003).

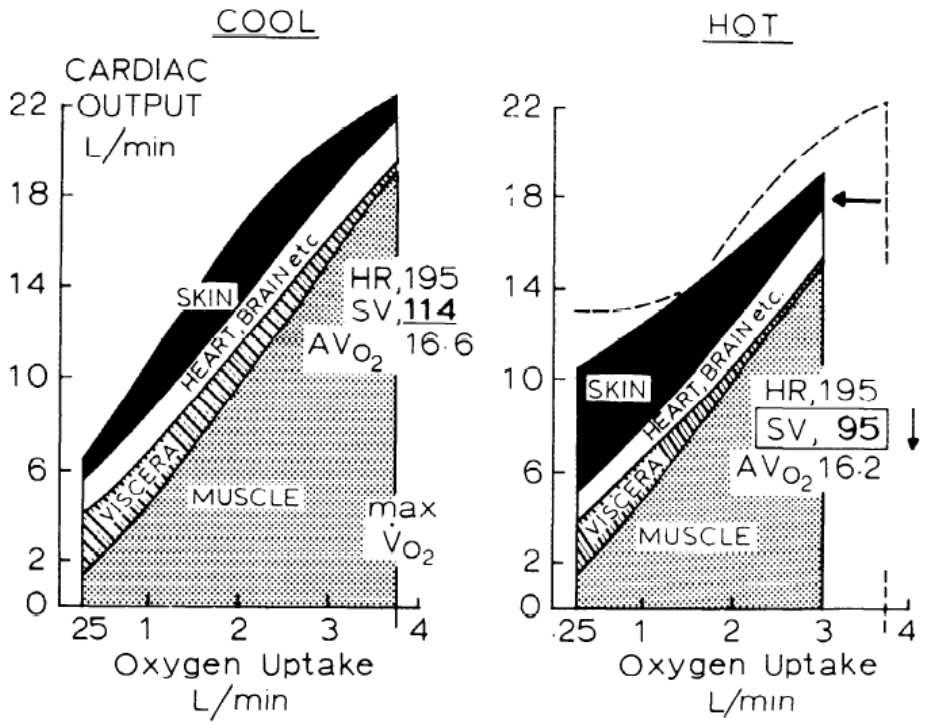

Figure 2.9: Theoretical schematic representation of blood flow distribution and overall cardiovascular responses to incremental exercise to exhaustion in both normothermic and heat-stressed conditions (Reproduced from Rowell 1974).

These responses can be exacerbated further when heat stress is combined with hypohydration; a common occurrence when fluid losses due to sweating are not adequately replaced during exercise (Sawka *et al*, 2007). A series of studies by González-Alonso and colleagues identified this decrement in performance as a result of a central limitation in blood flow delivery, with reflex increases in heart rate and systemic vascular resistance unable to compensate for the significantly greater reduction in stroke volume encountered when both fluid losses and hyperthermia were combined (González-Alonso *et al*, 1995; González-Alonso *et al*, 1997). The reversal of these detrimental effects when exercise is carried out in the supine position points to a reduction in central blood volume as the primary challenge to cardiac filling capacity and therefore stroke volume and cardiac output (González-Alonso *et al*, 1999). Interestingly, however, vascular conductance within the leg itself does not appear to change in these conditions, and this over-riding local vasodilation may therefore occur in an attempt to maintain leg blood flow for as long as possible in the face of a decreasing mean arterial pressure (González-Alonso and Calbet; 2003).

2.4.3 –Control of limb blood flow during exercise in the heat

Much like passive heat stress, the mechanisms controlling limb blood flow during exercise in the heat are complex and most likely vary depending on factors such as the muscle mass engaged, the intensity and duration of the exercise, and the severity and type of heat stress experienced. As described previously, situations in which cardiovascular capacity is pushed to its limits due to dual skin and muscle blood flow demands will ultimately result in a reduction in leg blood flow (González-Alonso and Calbet; 2003) relative to similar exercise intensities in normothermic conditions. During light to moderate exercise, however, the response is less clear. The increase in limb blood flow observed during passive resting conditions (Barcroft and Edholm; 1943; Barcroft and Edholm; 1946; Black *et al*, 2008; Keller *et al*, 2010; Naylor *et al*, 2011; Pearson *et al*, 2011; Heinonen *et al*, 2011) has been shown in some studies to be attenuated (Savard *et al*, 1988; Nielsen *et al*, 1990) and others maintained (Ferguson *et al*, 2006; Pearson *et al*, 2011). If limb blood flow is indeed attenuated

during exercise in the heat when compared to normothermic conditions, the question arises as to where these sacrifices are made; with reductions in either skin or skeletal muscle blood flow potentially comprising thermoregulatory homeostasis or metabolic processes, respectively.

Overwhelming evidence indicates that during exercise in the heat, skeletal muscle blood flow is preserved at the expense of the cutaneous circulation. Repeated studies looking at the effect of heat stress on exercising limb blood flow have found no reductions in skeletal muscle perfusion during single-legged knee extensions (Savard *et al*, 1988), two-legged cycling (Savard *et al*, 1988), or uphill walking to exhaustion (Nielsen *et al*, 1990); whilst mild intensity knee-extensions have recently been reported to in fact increase muscle perfusion (Pearson *et al*, 2011). In contrast, exercise has been shown to reflexly attenuate cutaneous vasodilation both with and without heat stress. This response is displayed in Fig. 2.10. and is characterised by a) an initial vasoconstriction upon starting exercise (Zelis *et al*, 1969; Johnson and Park; 1982; Kellogg *et al*, 1991b), b) a delayed threshold for the onset of cutaneous hyperaemia with elevations in core temperature (Johnson and Park; 1981; Taylor *et al*, 1988; Kellogg *et al*, 1991a; Smolander *et al*, 1991), and c) a plateau in skin blood flow of ~ 50% resting maximal values when a core temperature of 38 °C is reached (Brengelmann *et al*, 1977; Kellogg *et al*, 1993; Kenney *et al*, 1994). A series of studies by Kellogg and colleagues using bretylium tosylate to block sympathetic nerve release identified the initial exercise-induced vasoconstriction occurring solely through adrenergic vasoconstrictor function, whilst the delayed onset and early plateau of cutaneous hyperaemia were due to delayed and attenuated responses in reflex-mediated active vasodilation (Kellogg *et al*, 1991a; Kellogg *et al*, 1991b; Kellogg *et al*, 1993).

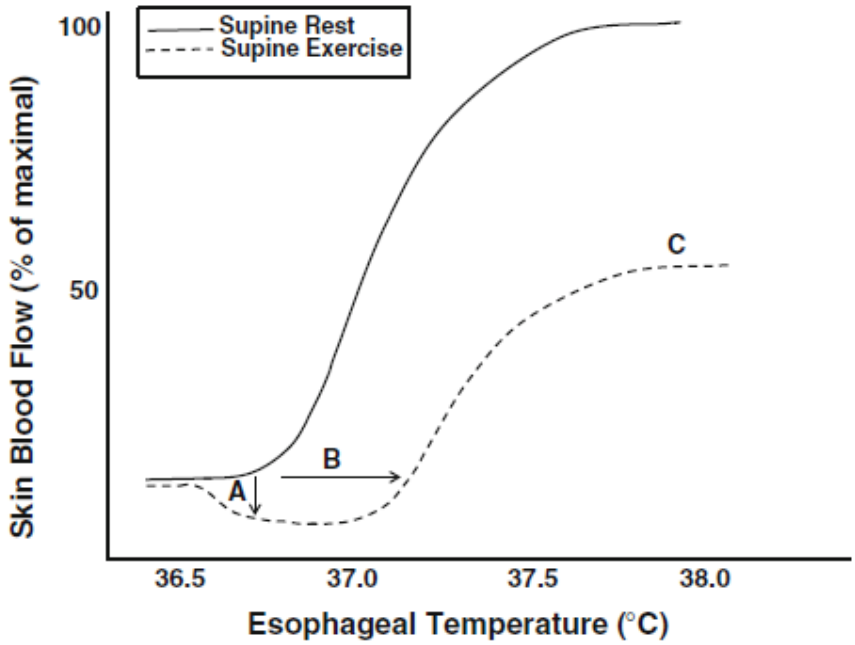

Figure 2.10: Influence on exercise on the skin blood flow response to heat stress. Dynamic exercise leads to an initial cutaneous vasoconstriction (A), delayed threshold for hyperaemia (B), and attenuated blood flow plateau (C) in comparison to exercise in normothermic conditions (Reproduced from Kenney et al 2014).

Each of these responses can be modified by numerous factors, however, including the volume of muscle mass engaged, exercise intensity, skin temperature, and initial blood flow baseline (Johnson, 2010). As such, the control mechanisms responsible for limb blood flow control most likely differ depending on a combination of these factors, and may go some way to explaining the discrepancies in previous results. Firstly, the initial vasoconstrictor response observed at the start of exercise is relatively unique in that it appears to be related to the absolute intensity of exercise being performed as opposed to the changes in relative % $\dot{V}O_2$max as is usually observed with reflex sympathetic outflow (Rowell, 1974). As a result of this fact, the performance of one-legged exercise, even at high intensities, has very little effect on the initial exercise-mediated constriction due to the low absolute workloads achieved (maximum ~100 W compared to ~400 W with two-legged cycling), and therefore the skin blood flow responses observed while using this exercise modality are lower than those seen even during low-intensity cycling (Taylor *et al*, 1990). Secondly, much

like the presence of the initial vasoconstrictor response, the delayed threshold for the onset of cutaneous vasodilation appears to be relatively unaffected at mild exercise intensities, with two-legged exercise at a workload of 125 W or less showing little to no response in comparison to resting values (Taylor *et al*, 1988). Finally, high skin temperatures can act through reflex-independent mechanisms to locally over-ride the exercise-mediated responses documented above (Bevegård and Shepherd; 1966; Taylor *et al*, 1984), thereby maintaining cutaneous vasodilation even in the face of an increased central drive favouring constriction (Pearson *et al*, 2013). Taken together, these findings could provide an explanation for the elevated leg blood flow observed by the labs of both Pearson and Ferguson, with the combination of small muscle-mass exercise and direct local heating of the leg tissues preventing any decreases in skin blood flow whilst simultaneously promoting skeletal muscle perfusion. In contrast, studies in which whole-body or intense exercise are used may limit these responses through central mechanisms described previously. For this reason, the independent effect of local tissue temperatures on the control of limb blood flow remains unclear. Also unresolved is whether the increases reported by Pearson and colleagues (2011) during light intensity exercise are maintained throughout incremental exercise to levels approaching that of an individual's peak power output. In this regard, the use of a single-legged knee extension model would allow for the attainment of near-maximal metabolic demand for skeletal muscle blood flow in the leg without the confounding influence of central limitations in flow, thereby allowing the influence of local tissue temperatures on haemodynamic responses to be investigated over a wide range of exercise intensities.

2.5 – Overall summary

Acute thermal challenges lead to marked haemodynamic adjustments in the human body in an attempt to maintain thermoregulatory homeostasis, and these can be either detrimental or beneficial to human health depending on the manner in which they are experienced. Despite over half a century of research in the area, the haemodynamic responses within the limbs during exposure to cold and heat stress and the factors responsible for their control are still poorly understood, both at rest and during

exercise. The overall aim of this thesis is to characterise these haemodynamic responses and identify the relative contributions of central vs. local thermosensitive mechanisms in limb tissue blood flow regulation. Together, these findings will help to identify not only the haemodynamic responses responsible for these potentially detrimental/beneficial outcomes, but also gain important insights into the location at which they are mechanistically controlled. The following section outlines the aims and hypotheses of the four studies comprising the thesis, following which the methods employed in each study will be described.

2.6 – Thesis aims and hypotheses

2.6.1 – Study 1: The role of local temperature alterations on haemodynamics and blood flow distribution in the resting human arm

Study Aim: To carry out a systemic investigation of the haemodynamic responses in the human arm over a wide range of physiologically relevant alterations in local tissue temperatures.

Research Hypotheses: 1) changes in whole-arm blood flow would be closely related to local temperature changes during both cooling and heating, and 2) these changes would be comprised of alterations in both skin and deep tissue (i.e., skeletal muscle) blood flow.

2.6.2 – Study 2: The role of local temperature alterations on haemodynamics and blood flow distribution in the resting human leg

Study Aim: To expand upon the previous chapter in the human arm and investigate the role of local temperature alterations on blood flow responses and distribution in the three major conduit arteries (common, superficial, and profunda femoral arteries) of the resting human leg.

Research Hypotheses: 1) during cooling, blood flow in the whole-limb (common femoral artery) and deep thigh tissue (profunda femoral artery) would decrease in line with local tissue temperatures, with heating having the opposite effect, 2) cooling and heating would lead to decreases and increases in mean shear rate in all three arteries, respectively, and 3) heating would attenuate the pro-atherogenic stimuli of oscillatory blood flow and shear stress through a combination of increased antegrade and decreased retrograde flow components.

2.6.3 – Study 3: Local temperature sensitive mechanisms, independent of systemic responses, mediate increases in limb tissue perfusion in the moderately heat stressed human at rest and during exercise

Study Aims: 1) To identify the contribution of peripheral vs. central thermosensitive mechanisms in the control of limb blood flow during heat stress by simultaneously altering leg tissue temperatures under conditions of both systemic hyperthermia and normothermia 2) to assess the effect of limb temperature on leg blood flow during one-legged knee-extensor exercise up to near maximal power output, and 3) to quantify absolute blood flow to the muscular tissue of the thigh in order to establish its contribution to whole-limb blood flow under conditions of heat stress.

Research Hypotheses: 1) limb blood flow would be primarily regulated at a peripheral level through increases in local vascular conductance, and that this response would be closely coupled to increases in local tissue and/or blood temperatures, 2) increased local temperatures would induce elevated leg blood flow throughout incremental single-legged exercise to near maximal power output, and 3) increases in skeletal muscle blood flow would contribute to the observed hyperaemic response to heat stress.

2.6.4 – Study 4: Blood flow responses, distribution, and regulation in the human leg during severe passive heat stress

Study Aims: To expand upon previous studies investigating blood flow responses and their control during localised and moderate whole-body heating, and determine whether these responses were maintained during severe heat stress up to levels approaching that of an individual's thermal tolerance.

Research Hypotheses: 1) whole-leg blood flow would continue to increase in a linear fashion from thermoneutral ambient conditions to levels of severe passive heat stress 2) these increases would be accompanied by further improvements in flow and shear rate profiles compared to isolated heating alone, and 3) blood flow regulation would differ between the deep and superficial tissues of the leg during severe heat stress.

CHAPTER 3

General Methods

3.1 – Introduction

This section outlines the general methodologies used in the following chapters. Where additional/modified procedures were used, full explanation will be given in the individual methods sections.

3.2 – Pre-test procedures

The following section highlights procedures carried out prior to the start of experimental testing.

3.2.1 – Ethical approval

Prior to all studies, ethical approval was sought and obtained from the Brunel University School of Sport and Education Ethics committee. All studies conformed to the standards laid out in the Declaration of Helsinki (Appendix I).

3.2.2 – Participants

All participants undertaking the study were young (aged 18-35) active males recruited from within the university or its surrounding areas. Following the confirmation of ethical approval, subjects were recruited via a combination of posters, internet-based advertising, and short presentations at lectures and seminars. Each subject provided written informed consent (Appendix II) prior to the start of any testing, and was made aware that they were free to leave the study at any time without reason or penalty.

3.2.3 – Anthropometry

Prior to testing, body mass of all participants was assessed to the nearest 0.1 kg whilst in a semi-nude state using a set of electronic scales, with height simultaneously assessed to the nearest centimetre using an attached stadiometer (SECA 798, Germany).

3.2.4 – Peak power test

Prior to single-legged exercise in Chapter 6, participants visited the laboratory at an earlier date in order to determine their peak power capacity on a custom-built single-legged knee extensor ergometer (Fig. 3.1). Following a short duration of sub-maximal exercise in order to become accustomed to the apparatus, participants were required to perform an incremental test to fatigue at 60 rpm, with each stage lasting 3 min and the power output being increased by 12 W between stages. The above test was carried out in both legs with the point of termination being reached when subjects could no longer maintain a rate of at least 50 rpm for 3 s.

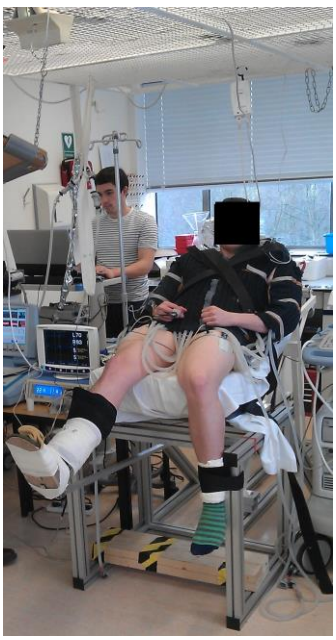

Fig. 3.1: Participant seated on the custom-built knee-extensor ergometer used for single-legged exercise in Chapter 6 (left). The right foot is securely fastened to the exercise apparatus while the left leg is secured to the frame of the machine to prevent unwanted movement. A digital display at the top left of the picture allows the participant to maintain exercise at 60 rpm throughout. The exercising leg is connected to a modified Monark cycle ergometer (right) with the addition of metal weights allowing adustments to power output within 6 W .

3.3 – Test procedures

3.3.1 – Limb blood flow

Overview

Duplex Doppler ultrasound is a commonly used technique for assessing blood flow in both healthy and abnormal human arteries and veins.

In all studies, changes in blood flow to either the arm at rest or to the leg both at rest and during one-legged knee extensor exercise were assessed using a duplex ultrasound device (Vivid 7 Dimension, GE Medical, Horton, Norway) (Fig. 3.2) with a 10 MHz linear array transducer probe (GE Medical Systems, UK).

Fig. 3.2: Vivid 7 ultrasound device used to measure limb blood flow (Reproduced from Vivid7 User Manual, GE Healthcare, UK).

A combination of arterial and venous diameter measurements from B-mode ultrasound images and time-averaged blood flow velocities from pulse-wave Doppler

were used to estimate blood flow in numerous vessels at rest, as well as in the common femoral artery during one-legged knee extensor exercise.

B-mode arterial diameter measurements

Modern ultrasound devices utilise piezoelectric crystals located in a hand-held transducer to create and propagate short pulses of ultrasonic sound waves beneath the cutaneous tissue of the human body. The application of specific voltages to the crystals within the transducer results in their vibration at unique resonance frequencies (typically between 2 and 15 MHz for diagnostic purposes), with the frequency employed a trade-off between increased spatial resolution (higher frequencies) and penetrating depth (lower frequencies). B-mode (or brightness scan) imaging allows a non-invasive method of visualising the internal structures of the human body via the propagation of these pulsed high frequency sound waves into the tissues and the detection of their subsequent return to the transducer (Fig. 3.3).

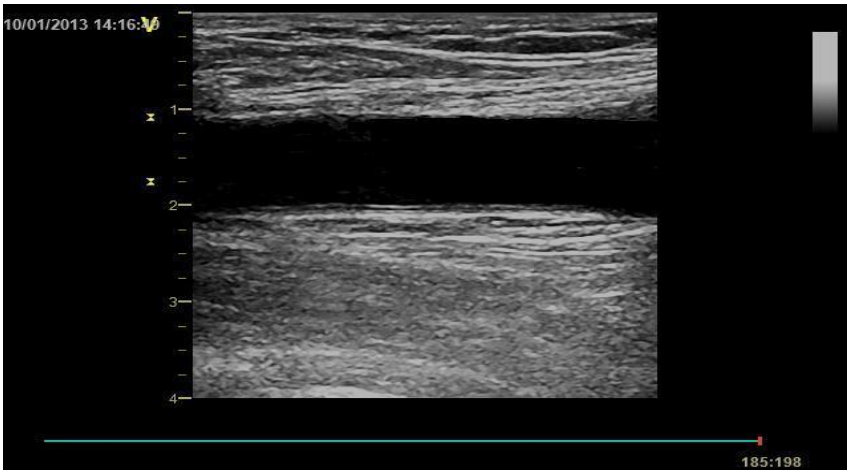

Fig. 3.3: Sample B-mode image of the common femoral artery in the human leg.

In the case of vascular measurements, ultrasonic waves commonly transmitted at frequencies of between 5 - 10 MHz are passed into the tissues via the transducer placed against the skin. Where the waves meet a large smooth interface between two media of differing acoustic impedance (such as the wall between an artery and the

surrounding skeletal muscle), some of this energy will be reflected back to the transducer in a process known as specular reflection. The interaction of the ultrasound waves with non-smooth surfaces within the tissues also leads to some reflection to the transducer, although the amplitude of the returning signal is severely attenuated due to the effects of scattering. By assuming a constant speed of sound through all tissues (1540 $m \cdot s^{-1}$), the time taken between the transmission and detection of each ultrasound pulse allows the distance of the interface to be calculated according to the equation d = tc/2; where d is the distance of the reflecting surface, t is the time taken between transmission and reception, c is the velocity of the sound wave, and the number 2 is due to the fact the pulse travels twice (once in each direction). The proportion of the signal which is reflected determines its amplitude on return, and these changes in amplitude are expressed as varying levels of brightness on a display monitor. By combining this information with the previous calculation of the interface distance, a 2D image of the different internal structures can be displayed (Fig. 3.4).

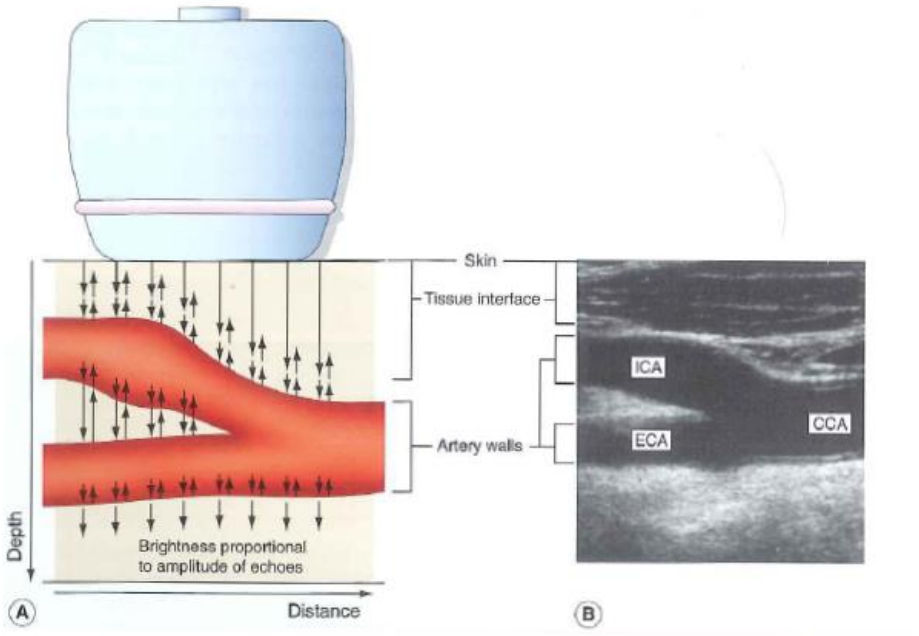

Fig. 3.4 – The reflectance of ultrasonic sound waves at the interface of two media of differing acoustic impedance can be used to create a clear 2D image of certain structures within the human body, such as the carotid bifurcation shown here (CCA/ICA/ECA = common, internal, and external carotid arteries respectively (Reproduced from Thrush and Hartshorne, 1999).

Arterial and venous diameters of all vessels in this thesis were calculated using three 2D B-mode images obtained using a 10MHz linear array transducer probe. Diameter was consistently measured during peak systole, obtained from an overlaid ECG trace (Rådegran, 1999; Mortensen *et al*, 2011) to account for changes in arterial diameter over the duration of the cardiac cycle, with the average of the three diameter measurements being used in subsequent calculations. Venous diameter was measured every 2 s over the duration of one velocity trace (16 s total) to account for diameter changes due to both cardiac and respiratory cycles. All images were taken in the longitudinal plane at a depth of between 1-2 cm (arm) and 3-5 cm (leg), with a frame rate of ~ 48 fps. Calculations were made manually by a trained investigator via the placement of callipers on the proximal and distal vessel walls (adventia/media interface) and analysed on a specialist software package (EchoPac, GE Medical, UK) on a separate laptop computer.

To assess the repeatability of the technique for accurately measuring arterial diameter, the co-efficient of variation for the measurement technique (SD/σ) was assessed in six subjects at rest across three independent time-points. The co-efficient of variation for brachial artery diameter was 1.8%; and for common, superficial, and profunda femoral arteries 0.8%, 1.4%, and 3.5%, respectively. These figures fell well inside the acceptable limits for repeatability reported in prior studies (Shoemaker *et al*, 1996; Rådegran, 1999).

Pulse-wave velocity measurements

Mean blood velocity at each time-point was measured using duplex Doppler ultrasound, allowing simultaneous B-mode images and velocity recordings to be displayed (Fig. 3.5). The Doppler effect is described as the change in an observed frequency due to the relative motion of the source and the observer, and can be used with modern-day ultrasound techniques in order to detect blood velocity within the major vessels.

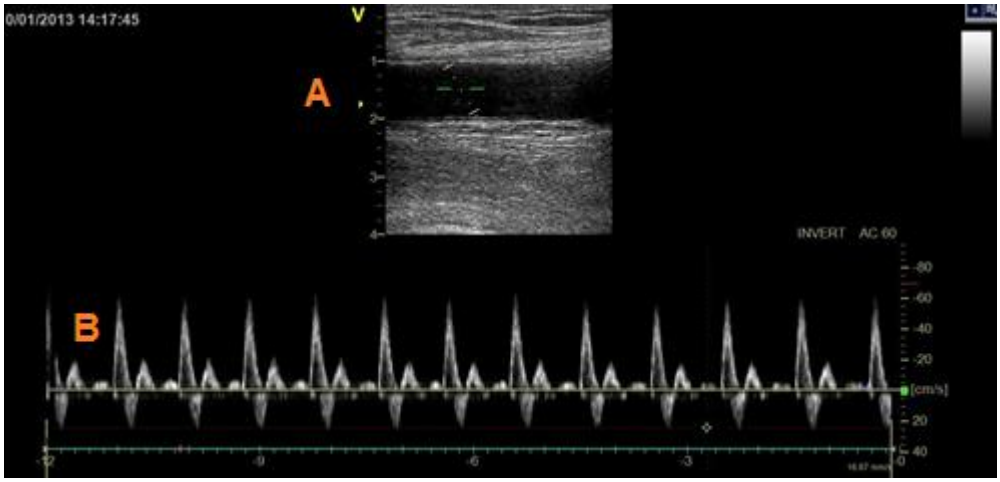

Fig. 3.5: Sample duplex pulse-wave Doppler image of the measurement of blood velocity in the common femoral artery at rest in the human leg. A = B-mode image of the common femoral artery for the purposes of vessel diameter measurements; B = Pulse-wave velocity trace showing velocity of femoral arterial blood flow over a period of 12 seconds and expressed in cm/s.

The passage of red blood cells (the moving source) through the artery results in a change of pitch in the frequency of the returning waves to the transducer (the observer), and from this information blood velocity can be determined. The equation for calculating blood velocity is shown below:

$$\Delta f = f_r - f_t = (2vf_t\cos\theta)/c$$

where Δf is the change in frequency, f_t is the transmitted frequency, f_r is the received frequency, v is the velocity of the blood, θ is the angle of insonation between the ultrasound beam and direction of blood flow, and c is the speed of sound in tissue (~1540 $m \cdot s^{-1}$). The factor of 2 is present as the Doppler effect has occurred twice. Initially, the transducer is a stationary source while the red blood cells are moving receivers of the ultrasound waves. The backscatter of ultrasound waves from the red blood cells then act as a moving source, and are detected by the stationary transducer.

As displayed by the above equation, the Doppler shift detected by the probe is affected by the angle of insonation between the ultrasound beam and the flow of the blood in the vessel, with the cosine of angles between 0° and 90° resulting in values ranging from 1 to 0. In light of this, clinical guidelines recommend insonation angles of 60° or less (Fig. 3.6) in order to minimise measurement error between samples, and as such the utmost care was taken to ensure all measurements taken during the investigations were in this range.

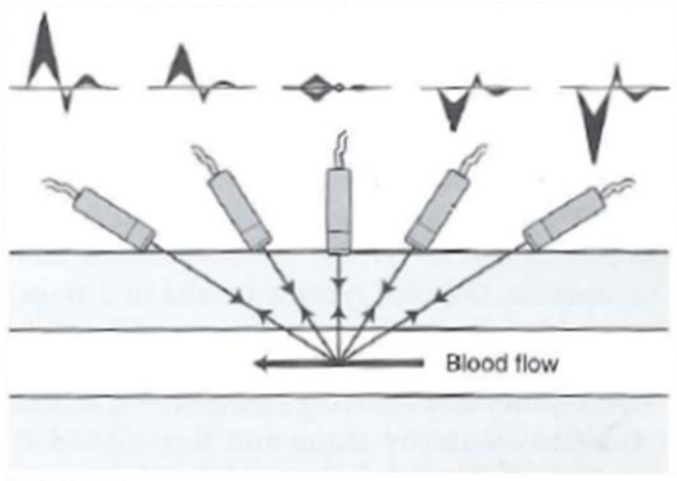

Fig. 3.6: Diagram showing amplitude of Doppler signal received when measuring at insonation angles of 60, 75, and 90°. Clinical guidelines recommend insonation angles of 60°or less at all times to minimise error in measurements (Reproduced from Thrush and Hartshorne 1999).

The presence of the cardiac cycle and the associated resistance offered by the vessel walls results in a wide-range of red blood cell velocities being present in the vessel under investigation at any one time, and these must be separated in order to accurately analyse the velocity profile of the blood over time. Spectral analysis of the returning signal using mathematical techniques such as fast Fourier transformation (FFT) allows the returning Doppler signal to be broken down into its component frequencies, with this information then being used by specialist computer software to construct consecutive spectra displaying the changes in blood velocity over time (Fig. 3.7).

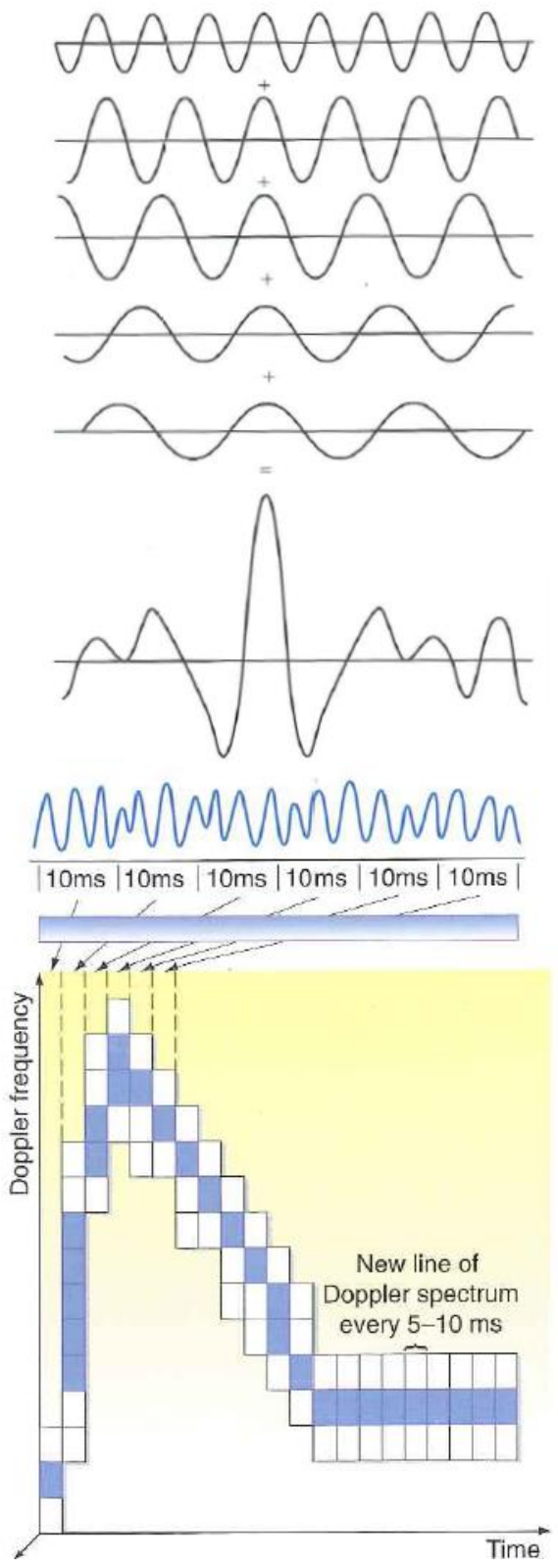

Fig. 3.7: The combined frequency of all blood velocities can be separated into its component frequencies using techniques such as fast Fourier transformation. Individual frequencies can then be plotted at specific time-points in order to create a spectrum of blood velocity over time (Reproduced from Thrush and Hartshorne 1999).

For all velocity measurements, the sample gate within the vessel was altered to cover the full width of the diameter, thereby detecting lower velocity flows near the vessel walls and preventing an overestimation of blood velocity (Rådegran, 1997). In order to prevent aliasing of the image, it was ensured that the sampling frequency was at least twice the sound wave frequency, a threshold known as the Nyquist limit (Fig. 3.8). A time-averaged mean velocity (V_{mean}) was calculated over three continuous 12-s periods (36 s total) in order to allow later calculation of blood flow.

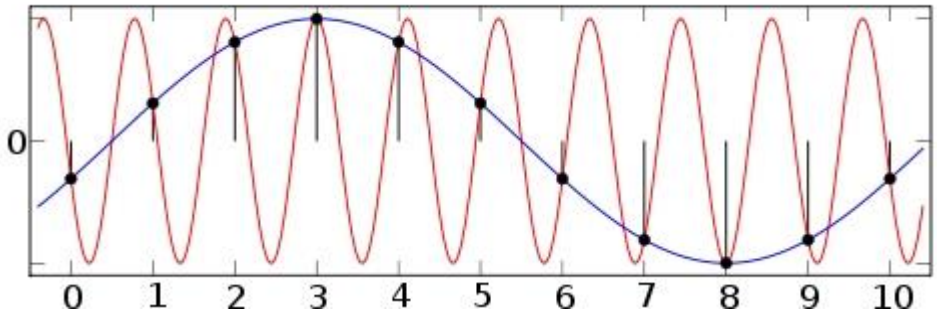

Fig. 3.8: Example of aliasing in Doppler velocity measurements. If blood flow velocities are sampled at less than twice the sound wave frequency, the measured frequency (blue sine wave) may differ from the true frequency present within the vessel (red sine wave).

Similar to the assessment of diameter measurements, the co-efficient of variation for blood flow measurements was calculated in each of the arteries at rest and in the common femoral artery during exercise. Resting values for brachial, common, superficial, and profunda femoral arterial flows were 12%, 7%, 6%, and 8%, respectively, while exercise values are displayed in Table 3.1. Co-efficient of variation in the great saphenous vein at rest was not measured due to the negligible flow values present.

Table 3.1. Blood flow co-efficient of variation during incremental single-legged exercise

Exercise Intensity (% PPO)	Co-efficient of variation (%)
20	4.7
40	3.4
60	3.7
80	3.4

Data are from four participants. %PPO, percentage peak power output.

Blood flow calculations

Blood flow through each vessel was calculated as the product of the arterial or venous cross-sectional area obtained from the average of three 2D B-mode images and the mean velocity averaged over three 12-s Doppler scans (36 s total). All values were calculated off-line using specialist ultrasound software (EchoPac, GE Medical, UK).

Blood flow was calculated in $ml \cdot min^{-1}$ using the equation:

$$BF = V_{mean} \,.\, \pi \,.\, (D/2)^2 \,.\, 60$$

Where BF is blood flow expressed in $ml \cdot min^{-1}$, V_{mean} is the time-averaged mean velocity of the blood expressed as $cm \cdot s^{-1}$, π is the mathematical constant, D is the diameter of the vessel in cm, and 60 is a constant employed to convert the units to $ml \cdot min^{-1}$.

Blood flow in the arm was measured in the brachial artery (BA), with the site of insonation lying roughly at the mid-point of the biceps brachii muscle. Flow in this vessel represents the vast majority of flow into the upper limb, with only minor contributions occurring through small collateral arteries lying proximal to the level of insonation. Lower limb arterial blood flow was measured at 3 distinct sites located distal to the inguinal ligament, namely the common femoral artery (CFA), superficial femoral artery (SFA), and profunda femoral artery (PFA), with all measurements being taken 2-3 cm from the femoral bifurcation to avoid errors in measurement due to turbulent flow (Fig. 3.9).

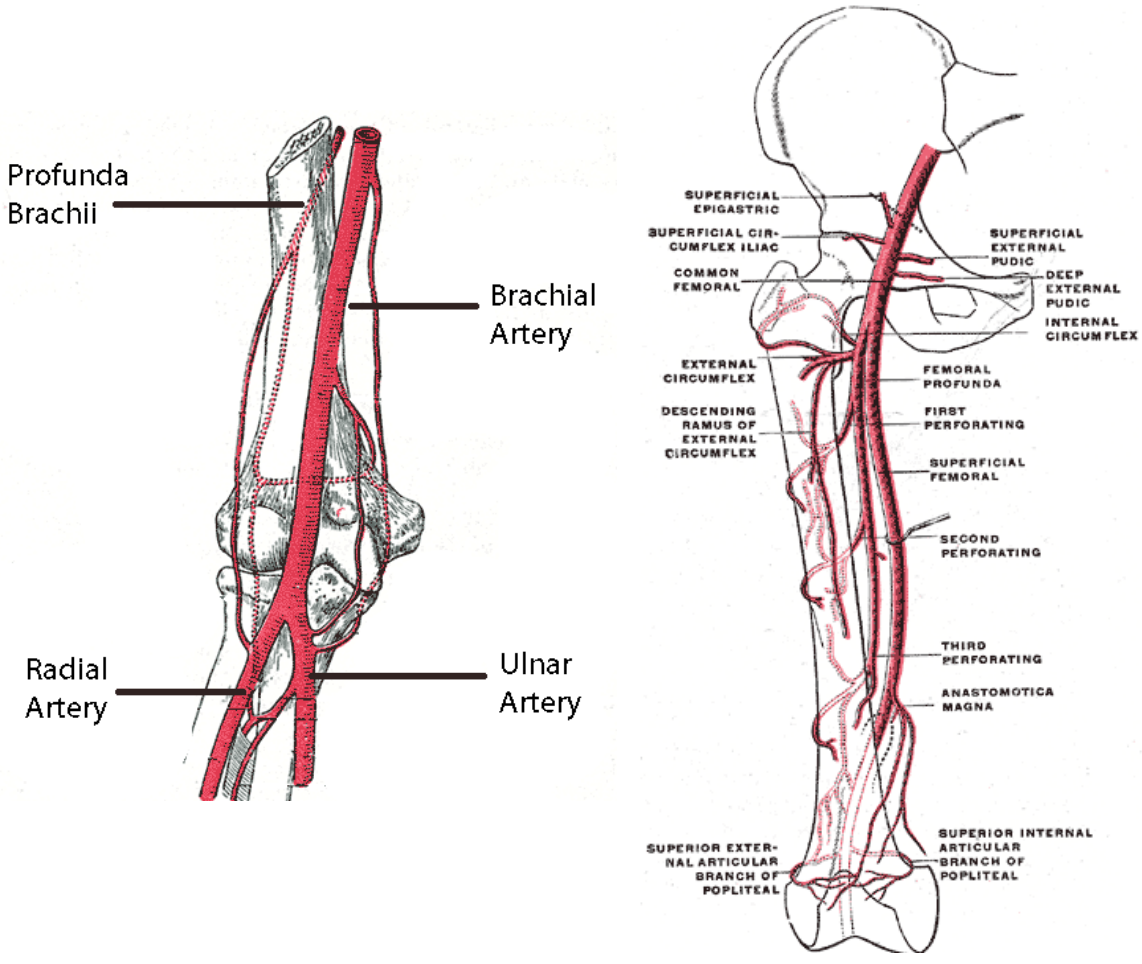

Fig. 3.9: Major arterial blood supplies of the arm (left) and leg (right) (Reproduced from Gray's Anatomy, 20th Edition, New York, 2000).

In Chapter 7, blood flow in the great saphenous vein was measured during whole-body severe passive heat-stress in order to quantify skin blood flow draining the superficial tissues of the leg. Measurements were taken immediately distal to the pre-terminal valve separating the greater saphenous vein from the saphenofemoral arch in an attempt to prevent influx of flow from accessory veins draining areas other than the leg (Fig. 3.10). Veins draining into the saphenofemoral arch commonly include the superficial circumflex iliac vein draining the trochanteric region; abdominal or epigastric subcutaneous veins draining abdominal vessels; and the superficial external pudendal veins draining the pubic and genital regions. In order for the calculation of accurate measurements of blood flow, great care was taken not to compress the vessel during probe placement in order to ensure vessel circumference remained essentially circular (Jeanneret *et al,* 2000).

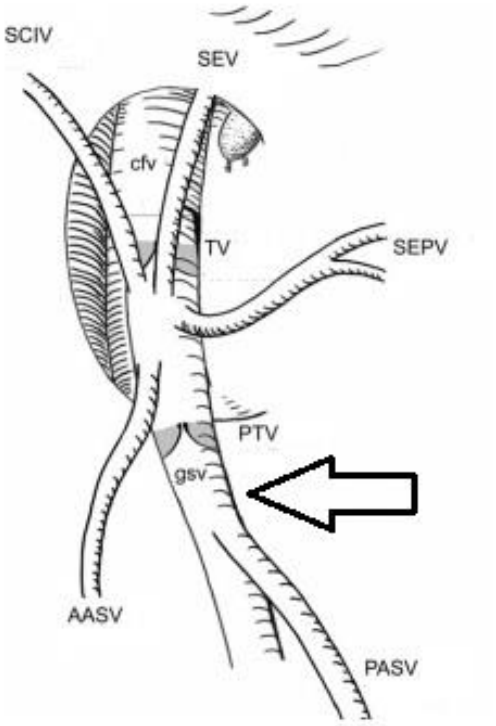

Fig. 3.10: Saphenofemoral junction located in the thigh, with arrow showing positioning of flow measurements. CFV – common femoral vein; GSV – greater saphenous vein; TV – terminal valve; PTV – preterminal valve; SCIV – superficial circumflex iliac vein; SEV – subcutaneous epigastric vein; SEPV- superficial external pudendal vein; AASV – anterior accessory saphenous vein; PASV – posterior accessory saphenous vein.

3.3.2 – Skin and intramuscular blood flow

Changes in skin and intramuscular blood flow were assessed using laser Doppler flowmetry (Periflux 4001, Jarfalla, Sweden) via a 780 nm wavelength single-point laser Doppler probe operating at a power output of 1 mW and with a fibre separation of 0.25 mm (408, Periflux, Jarfalla, Sweden). The above probe measures the total microcirculatory blood flow at a depth of ~ 0.5 – 1 mm through the propagation of near infra-red light either beneath the surface of the skin for skin blood flow or directly within skeletal muscle tissue for intramuscular blood flow. Once inside the tissue, the transmitted light is scattered and partly absorbed by the red blood cells moving within the microcirculation. Much like the previously mentioned Doppler ultrasound response, light hitting moving blood cells undergoes a frequency shift before returning to the probe. The magnitude of change in the wavelength and frequency of the returning light is directly related to the number of red blood cells present and their velocity, and as such allows the measurement of relative changes in skin blood flow over time. For skin blood flow, the probe was securely fastened to the skin overlying the flexor carpi radialis (arm) or vastus lateralis (leg) using a specialist probe holder (PH08, Periflux, Jarfalla, Sweden) and adhesive tape, ensuring the blocking of any external light that may have affected the readings detected by the flowmetry system. For intramuscular measurements, following application of lidocaine gel (1%) to minimise discomfort, a flexible fibre-optic probe was inserted 2-3 cm into the vastus lateralis muscle via a 22-gauge catheter and securely fastened to the skin surface with tape to avoid movement. All readings were recorded continuously via a data acquisition system (PowerLab 16/35, AD Instruments, US) and fed to a data acquisition software package for later analysis (LabChart 5, AD Instruments, US).

3.3.3 – Tissue oxygenation

Tissue oxygenation in the forearm (extensor carpi ulnaris) and thigh (vastus lateralis) was measured using continuous-wave near-infrared spectroscopy (NIRS; INVOS

Cerebral Oximeter, Somanetics, Troy, MI, USA). The NIRS system allows a continuous non-invasive assessment of limb tissue oxygenation through the emission of infra-red light beneath the cutaneous tissue and its subsequent detection on return by two independent detectors located 3 and 4 cm from the light source. The passage of light itself follows a 'banana-shaped' path on its journey back to the surface, with deeper penetrating signals re-emerging further from the light source. The spacing of the detectors can therefore be manipulated to determine the depth at which tissue oxygenation is sampled, and algorithms built into the NIRS system can be used to calculate the difference between the shallow skin signal (3 cm detector) and deep skeletal muscle values (4 cm detector). Upon entering the tissues, near-infrared light can be scattered, reflected or absorbed, with haemoglobin molecules exhibiting differential absorption properties depending on their oxygenation state (Fig. 3.11).

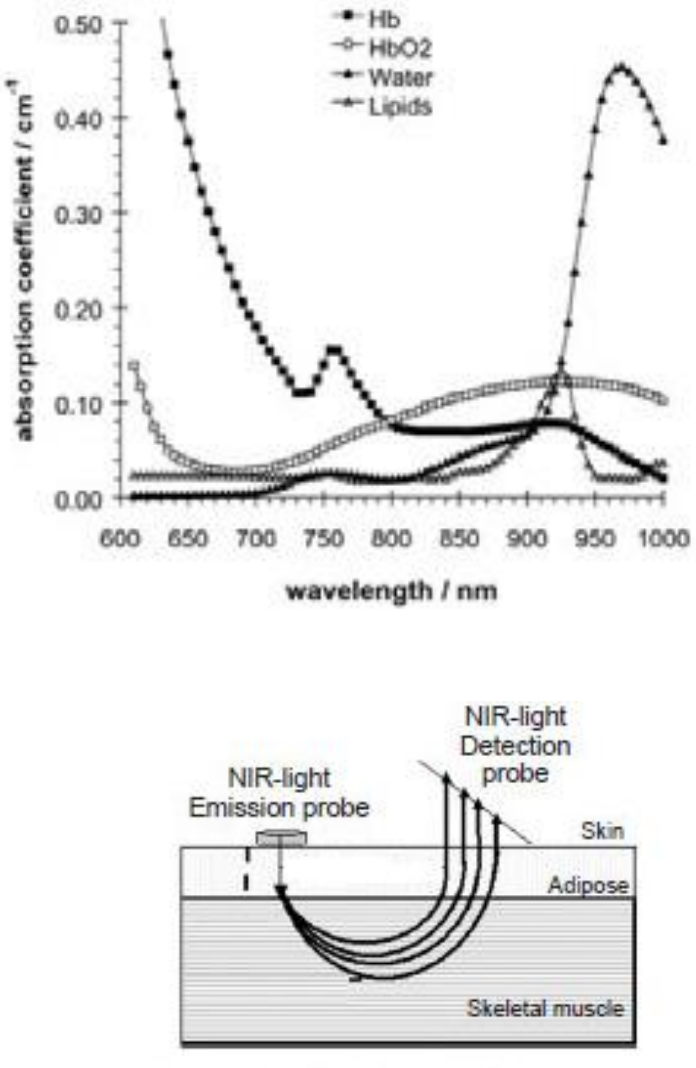

Fig. 3.11: Absorption spectra (top) and passage of light through tissues (bottom) using near-infrared spectroscopy. Note the predominant absorption of deoxyhaemoglobin (Hb) at a wavelength of ~ 730 nm and its equal absorption with oxyhaemoglobin (HbO_2) at ~ 810 nm on the left-hand diagram. On the right, the detection of deeper tissues can be achieved by sampling returning light at a greater distance from the light-emitting probe (Reproduced from INVOS User Manual, Somanetics, USA).

Oxygenated and de-oxygenated haemoglobin absorb light equally at wavelengths of ~ 810 nm, whereas at ~ 730 nm the absorption is primarily due to haemoglobin in its de-oxygenated state. By measuring the absorption at these wavelengths, the NIRS technique allows a calculation of the regional oxygen saturation of the tissues directly below the probe.

3.3.4 – Other haemodynamic measurements

The following section details the various methodologies employed for the measurement of haemodynamics during all experimental trials. Full details of the specific equipment and methods used in each of the studies will be included in their respective chapters.

Invasive blood pressure measurements

Arterial and femoral venous blood pressures were measured continuously via placement of radial arterial and femoral venous catheters, respectively. All catheters were placed by experienced clinicians from Ealing Hospital (London, UK) using the Seldinger technique (Fig. 3.12) and under the influence of local anaesthesia (Lidocaine 1%).

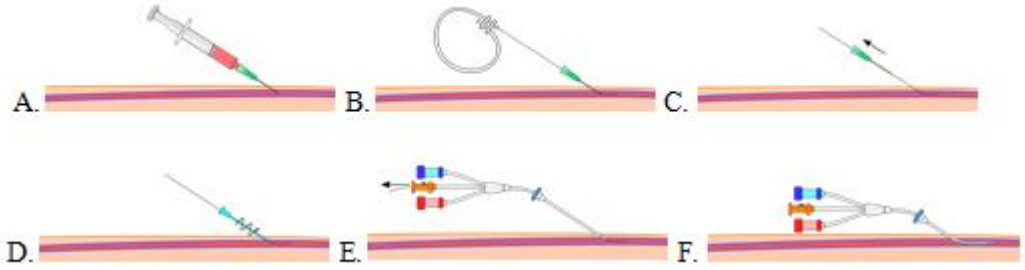

Fig. 3.12: The Seldinger technique. The vessel in question is located by the flashback of blood through the introducer needle (A). A guidewire is advanced into the vessel lumen (B) and the needle removed (C). Following a small incision with a scalpel, an 'expander' is inserted to enlarge the hole (D), following which the catheter is fed over the guidewire and into the vessel (E). The guidewire is then removed and the catheter stitched to the skin to avoid movement (F).

For arterial blood pressure measurements and blood samples, an 18 gauge catheter was placed within the radial artery of the right arm, with successful placement being confirmed through the presence of pulsatile oxygenated blood from the catheter. Following successful placement, the catheter end was capped to prevent the escape of blood and tape was applied to secure it to the skin. For femoral venous placements, double-lumen catheters (Double Lumen Catheter, 18 gauge, 16 cm; Multi-Med M2716HE, Edwards Lifesciences, USA) were inserted in a retrograde direction and under ultrasound guidance into the femoral vein of both legs at a location approximately 1-2 cm distal to the inguinal ligament, with one lumen allowing the measurement of femoral venous pressure and taking of blood samples whilst the second allowed placement of a fine-wire thermocouple to measure blood temperature from within the vessel itself (see temperature section for information on blood temperature thermocouple). Arterial and venous catheters were connected to pressure transducers located at the level of the heart (Pressure Monitoring Set, Edwards LifeSciences, Germany) before being fed through an amplifier (BPAmp, ADInstruments, Oxford, UK) to the data acquisition system (PowerLab 16/30, AdInstruments, Oxford, UK) for continuous recording.

Non-invasive blood pressure measurements

For studies where no arterial catheter was available for the direct invasive measurement of arterial blood pressure, non-invasive measurements were taken through the use of an infrared photoplethysmograph (Finometer, FMS, Netherlands). The Finometer uses the 'volume-clamp' method first pioneered by Jan Penaz in the 1970's to allow continuous non-invasive measurement of arterial pressure through a small inflatable cuff attached to the finger (Wesseling, 1990; Fig. 3.13).

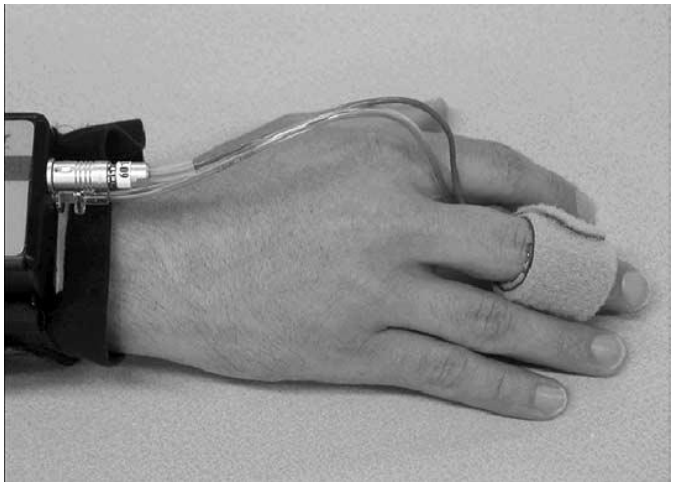

Fig. 3.13: The fitting of an infra-red photoplethysmographic cuff to the finger of the subject's hand allows a continuous non-invasive measurement of arterial blood pressure. (Reproduced from Bogert & van Lieshout 2005).

The volume-clamp method, as the name suggests, involves the detection of the arterial diameter within the finger using an infrared sensing technique and the subsequent clamping of this diameter using a rapidly responding pressure controller located within the cuff. In short, increases in intra-luminal pressure (and therefore an associated increase in diameter) during systole are detected by sensors within the cuff, which can then be rapidly inflated to a pressure to maintain arterial diameter at its predetermined unloaded 'set-point'. Conversely, decreasing pressures during diastole are met with an associated decrease in cuff pressure, again preventing changes in vessel diameter. The pressure required within the cuff to maintain vessel diameter is equal to that being generated within the vessel itself, and as such, can allow continuous accurate determination of arterial pressure using a non-invasive method (Wesseling *et al*, 1993). Potential limitations in this technique are brought about by a number of factors which influence the pressure waveform detected in the

finger. The narrowing of arteries as blood flows towards the hand leads to a significant pressure gradient within the arm, potentially distorting results gained from the cuff located at the level of the finger. In addition, the presence of reflected waves within the arteries themselves can also lead to augmentation of systolic pressure readings. These confounding factors can be minimised by using an additional forearm pressure cuff in conjunction with built-in calibration algorithms within the Finometer, allowing the reconstruction of highly accurate brachial arterial pressures from those recorded at the finger. The use of this technique has been shown to reduce differences in finger-to-brachial pressure to a standard which meets the American Association for the Advancement of Medical Instrumentation (AAMI) criteria for acceptance (Guelen *et al*, 2003), and these additional calibrations were therefore used in all studies in this thesis where the Finometer was the measurement technique of choice.

Heart rate, stroke volume, and cardiac output

Heart rate was calculated from the pulsatile rate of the arterial pressure trace measured by either the intra-arterial catheter or Finometer. Stroke volume was estimated using the Modelflow method provided as part of the BeatScope computer package (BeatScope, FMS, Netherlands). This method incorporates the participant's arterial pressure trace with their age, height and weight in order to mathematically simulate a beat-by-beat aortic flow waveform which – when integrated – allows the continuous estimation of stroke volume (Wesseling *et al*, 1993). The major determinants of systolic inflow to the aorta (i.e. stroke volume) are the aortic impedance, aortic compliance, and peripheral resistance. During each heart-beat, blood flow into the aorta from the left ventricle is opposed by an existing aortic pressure exerted by the blood already contained within the vessel (aortic impedance). The flow of blood out of the left ventricle results in a rise in this aortic pressure, and the magnitude of this pressure rise is dependent on the instantaneous flow, vessel diameter, and vessel compliance. Vessel compliance represents the aortic opposition to an increase in blood volume and is characterised by the change in volume for a given change in pressure, whilst peripheral resistance provides a measure of the ease of which blood can drain from the aorta to the subsequent peripheral beds. The

Modelflow method uses information provided on the subject's age, height and weight in order to calculate an individual pressure-area relationship for the aorta, before combining this with the recorded pressure trace readings in order to estimate beat-by-beat stroke volume. Once stroke volume has been calculated, it can be multiplied by the corresponding instantaneous heart rate in order to provide a continual estimation of cardiac output. Previous investigations have shown that the Modelflow technique correlates well with stroke volume values recorded from other well-established methods, including dye-densitometry (Matsukawa *et al*, 2004), CO_2 re-breathing (Houtman *et al*, 1999), and Doppler ultrasound (van Lieshout *et al*, 2003).

3.3.5 - Blood and plasma measurements

Blood gas variables, haemoglobin concentration, metabolites, electrolytes, and (calculated) osmolality were measured using an automated analyser (ABL 825; Radiometer, Copenhagen, Denmark) calibrated to the manufacturer's instructions. For analysis of plasma noradrenaline and adrenaline, 2 ml blood samples were collected in EDTA tubes, centrifuged, and separated before being subsequently determined using an enzyme-linked immunoassay kit (DEE6500 2-CAT, Demeditec Diagnostics GmBH, Kiel, Germany).

3.3.6 – Temperature measurements

All changes in temperature were detected using specialised thermocouples and recorded in real-time by two thermocouple meters (TC-2000, Sable Systems, Las Vegas, USA), with all data being continuously recorded via the data acquisition system mentioned previously.

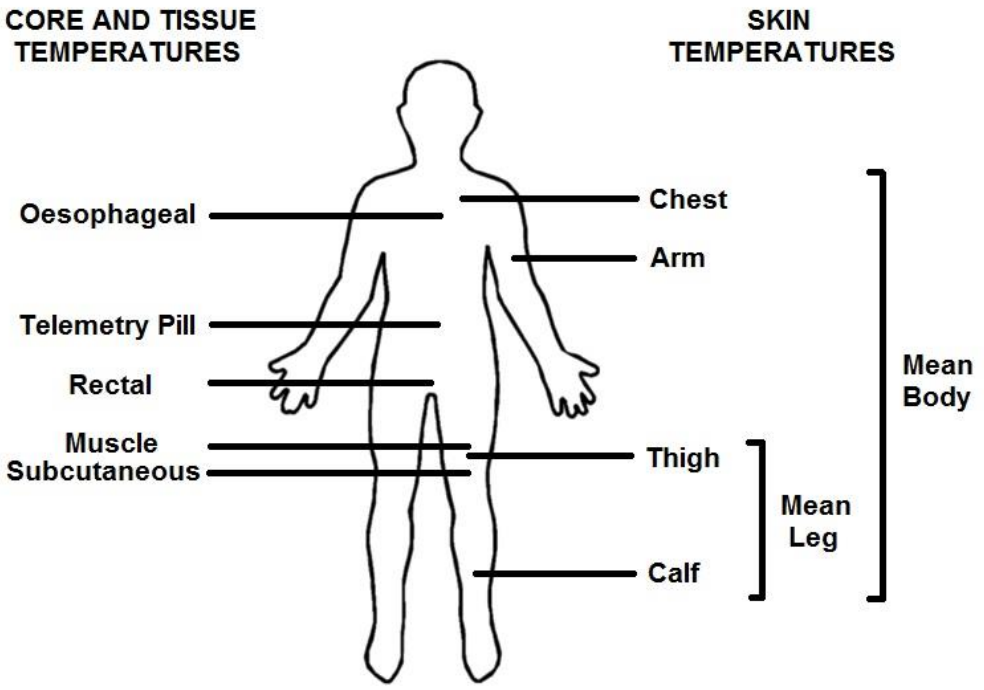

Fig 3.14 – Placement sites of numerous thermocouples for the detection of various core, tissue, and skin temperatures. Exact thermocouples used and their placement will be descrbed in more detail in each study's respective chapter.

Core temperature

Core temperature was measured using either rectal or oesophageal thermocouples (Physitemp, Clifton, NJ, USA) or a self-ingestible telemetry pill (HQInc, Palmetto, FL, US). All techniques have shown good validity and reliability, and as such were considered acceptable methods of measurement (O'Brien *et al*, 1998). Details of the chosen method for particular studies will be given in the study chapters. Oesophageal placement was conducted through the dominant nostril following intra-nasal application of 0.5 ml of 1% lidocaine. The probe was fed to a distance of ¼ standing height to sit behind the level of the left atrium (Mekjavic and Rempel; 1990). Subjects using the rectal thermocouple were instructed to self-insert the probe a minimum of 15 cm beyond the anal sphincter prior to testing, whilst those using the wireless system were required to ingest the pill at least 2-3 h before the start of the experiment.

Skin temperature

Mean skin temperature ($\overline{T}_{sk}$) was calculated using wireless data loggers (iButtons, Maxim, US) attached via transpore tape to four sites on the skin – namely the arm, chest, thigh, and calf – and weighted according to the equation: $\overline{T}_{sk}$ = 0.3(Chest + Arm) + 0.2(Thigh + Calf) (Ramanathan, 1964). $\overline{T}_{sk}$ in the experimental limbs were measured as the average of two type-t thermocouples (PhysiTemp, Clifton, NJ, US) attached to the skin with transpore tape. Both data loggers and type-t thermocouples have previously been shown to accurately and precisely track changes in skin temperature during thermal stress (Harper-Smith *et al* 2010).

Muscle temperature and subcutaneous temperatures

Muscle and subcutaneous temperatures were measured using a T-204A tissue implantable thermocouple microprobe (PhysiTemp, Clifton, NJ, US). An 18GA flexible venflon catheter (BD Infusion Therapy, Helsingborg, Sweden) was inserted into the vastus lateralis muscle at a location midway between the iliac crest and lateral aspect of the knee, and the microprobe was introduced ~ 2-3 cm beyond the thickness of subcutaneous fat for muscle measurements, or immediately under the subcutaneous tissue for subcutaneous measurements. The catheter and thermocouple were secured to the leg using transpore tape to prevent movement.

Mean leg temperature

Mean leg temperature in Chapter 7 was calculated as a ratio of skin, subcutaneous, and muscle temperatures, weighted by the volume of each of these tissue compartments in the leg (taken from Wang *et al.* 1999) and averaged to provide an indication of mean tissue temperature across the leg.

Blood temperature

Blood temperature was measured intravenously in both the antecubital vein (arm; Chapter 4) and femoral vein (leg; Chapter 6) using a fine-wire thermocouple microprobe (T-204A, Physitemp, Clifton, New Jersey, USA). More details about the insertion technique can be found in the previous section detailing catheter placements for the monitoring of blood pressure.

3.3.7 – Data and statistical analyses

All graphs were created using the commercial graphing package SigmaPlot 12.5 (Systat Software, Hounslow, London, UK). All data were analysed using a commercially available statistical software package (SPSS 20, Chicago, IL, US), with results being expressed as mean ± SEM and significance being set at a level of $P < 0.05$ unless otherwise stated.

CHAPTER 4

Study 1: The role of local temperature alterations on haemodynamics and blood flow distribution in the resting human arm

4.0 – Abstract

Haemodynamic responses to local tissue temperature changes within the human arm are incompletely understood, with data on the magnitude of change and distribution between different tissues of the limb remaining equivocal. Here, we carried out a systematic investigation of these responses over a wide physiologically-relevant range of local tissue temperatures, with the hypothesis that changes in arm blood flow would be closely associated with local temperatures alone and would occur in both skin and skeletal muscle tissues. Brachial artery blood flow, deep venous blood temperature, and blood gas parameters were measured in an experimental and contra-lateral control arm of 10 healthy males (age 21 ± 2 yr) during 1 h of passive local cooling (crushed ice) or heating (water-perfused cuff). The order of cooling and heating was randomised amongst participants and separated by at least 1 h on a single experimental visit. Systemic haemodynamics were measured throughout using infrared photoplethysmography. Blood temperature and brachial artery blood flow decreased with cooling from 34.2 ± 0.6 to 28.1 ± 1.0 °C and 98 ± 6 to 55 ± 6 ml.min^{-1}, respectively ($P < 0.05$ for both), while localised heating led to increases of 34.0 ± 0.7 to 37.0 ± 0.1 °C and 91 ± 10 to 432 ± 38 ml·min^{-1} ($P < 0.05$ for both). Systemic temperatures, haemodynamics, and contralateral limb blood flows remained essentially unchanged throughout. Changes in blood flow were closely related to venous blood temperatures throughout both cooling ($R^2 = 0.68$; $P < 0.05$) and heating ($R^2 = 0.50$; $P < 0.05$), although with the sensitivity of the response significantly higher during heating (114 ± ml·min^{-1}·°C^{-1}) compared to cooling (9 ± 1 ml·min^{-1}·°C^{-1}). Decreases in skeletal muscle tissue oxygenation and an elevated arm a-vO_2 difference during localised cooling indicated a reduction in skeletal muscle perfusion. In contrast, estimations of elevated skeletal muscle perfusion using a-vO_2 differences and the Fick equation during local heating suggested an 80% increase in perfusion during 1 h of heating, or ~ 25% of the total arm hyperaemic response. These findings indicate that changes in both skin and skeletal muscle blood flow during localised cooling and heating are mediated through local temperature-sensitive mechanisms alone, although with a significantly greater tissue perfusion responsiveness to temperature observed with heating.

4.1 – Introduction

The relationship between local tissue temperature and blood flow within the resting human arm has been extensively studied by physiologists for over half a century (Barcroft & Edholm, 1943). Despite the wealth of research conducted, numerous differences in the methods and duration of temperature manipulations have resulted in discrepancies in the overall magnitude of blood flow change and its distribution within the arm tissues. Moreover, the mechanisms responsible for its control are still not fully understood. Potential confounding factors affecting previous measurements of arm blood flow during cooling include the cold pressor reflex (Lovallo, 1975), cold-induced vasodilation (Daanen, 2003), or systemic thermogenesis (shivering; (Block, 1994), while a combination of indirect and direct temperature manipulations during previous heating studies also leads to uncertainty over the independent haemodynamic effect of localised changes in tissue temperature.

Localised cooling is generally accepted to have a powerful vasoconstrictor effect on blood vessels perfusing cooler tissues, and as such is commonly used as an intervention following physical activity or musculoskeletal injury to reduce microvascular perfusion, oedema and a subsequent inflammatory response (Bleakley *et al*, 2004). Despite compelling evidence of pronounced vasoconstriction in the cutaneous circulation in response to local cooling (Johnson and Kellogg; 2010), the impact on the underlying tissue blood flow – and therefore limb blood flow as a whole – is less clear cut. Direct assessments of whole-limb and skeletal muscle blood flow responses to cooling in the human arm are distinctly lacking, and the limited number of studies available in the literature have shown decreased (Barcroft and Edholm; 1946), unchanged (Houben *et al*, 1982) or, paradoxically, increased flow due to cold-induced vasodilation (Clarke *et al*, 1958; Ducharme and Radomski; 1990).

At the other end of the spectrum, a long-held concept with regards to localised heat stress has been that any observed increase in whole-arm blood flow is confined to the cutaneous circulation alone, implying that skeletal muscle perfusion is insensitive to changes in elevated blood and tissue temperatures. This conclusion was drawn from observations made over half a century ago, where numerous studies appeared to show that vasodilation within the human forearm was confined to the skin alone (Edholm *et al*, 1956; Roddie *et al*, 1956; Detry *et al*, 1972; Johnson *et al*, 1976). Recent evidence has challenged this prevailing idea, however, with a number of laboratories finding evidence of increased muscle and even bone and adipose tissue blood flow upon exposure to passive local heat stress when employing techniques such as radioactive microspheres (Song, 1984), Xe^{133} clearance (Keller *et al*, 2010), Positron-Emission Tomography (Heinonen *et al*, 2011), duplex Doppler ultrasound (Pearson *et al*, 2011), Near Infra-Red Spectroscopy (Okada *et al*, 2005), and laser Doppler flowmetry (Binzoni *et al*, 2012). However, none of these studies has investigated underlying tissue flow in the same forearm model as used in earlier investigations, which may potentially differ from other sites of the body due to known heterogeneous responses between limbs (Pawelczyk and Levine; 2002; Newcomer *et al*, 2004).

The aim of this study, therefore, was to carry out a systematic investigation of the haemodynamic responses in the human arm over a wide range of physiologically relevant alterations in local tissue temperatures. The following hypotheses were tested: 1) changes in whole-arm blood flow would be closely related to local temperature changes during both cooling and heating, and 2) changes in deep muscle venous oxygen content would provide evidence of alterations in both skin and deep tissue (i.e. skeletal muscle) blood flow.

4.2 - Methods

4.2.1 – Participants

Following the provision of informed written consent, nine healthy males (mean ± SD age 21 ± 2 years, height 175 ± 6 cm, and weight 74 ± 6 kg) were recruited to take part in the study. All procedures conformed to the ethical requirements laid out in the Declaration of Helsinki and were conducted following ethical approval from the Brunel University Research Ethics Committee. All participants were asked to refrain from exercise and ingestion of caffeine on the day of testing.

4.2.2 – Experimental protocol

Participants remained in the supine position throughout with both arms resting at an 80º angle to the torso, with baseline measurements commencing 30 min after the insertion of catheters and placement of monitoring equipment. For the cooling protocol, the forearm of the experimental arm was covered with a tight fitting plastic bag and encased in crushed ice in a custom built polystyrene box, with the upper arm proximal to the catheters simultaneously cooled with crushed-ice packs. The hand and the wrist area were exposed to the cold temperature reached within the box (~13°C) but were not covered with ice to avoid the cold pressor effect which could shift both blood pressure and heart rate (Mourot *et al*, 2009). Ice was kept on the arm for 60 min and measurements were taken every 10 min. For the heating protocol, a custom-built tube lined cuff was placed on the experimental arm and perfused with 50ºC water in order to raise tissue temperatures within the isolated limb. Temperature and haemodynamic measurements and blood samples for blood gases (1 ml) were taken from both arms at 10 min intervals for the entire 60 min duration of both cooling and heating, with the control arm remaining uncovered at an ambient temperature of 20-22°C. The order of cooling and heating was counterbalanced across participants and separated by a one hour resting period in order to allow tissue

temperatures and haemodynamic variables to return to baseline values following the initial intervention.

4.2.3 – Venous catheterisation

An 18-gauge, 5 cm long catheter was inserted in a retrograde direction to reside in the deep portion of an antecubital vein in both the experimental and control arms to allow for the sampling of blood draining predominantly from the muscle tissues. The placement of the catheter in this fashion has been shown to prevent the contamination of venous muscle blood with increases in skin blood flow draining through the superficial venous system of the arm (Mottram, 1955). Each catheter was continuously flushed with sterile saline throughout the protocol in order ensure patency. An additional catheter was placed in both arms within ~1 cm of the first for the insertion of a sterile implantable thermocouple microprobe (T-204F, Physitemp, Clifton, New Jersey, USA). Following insertion, the second catheter was carefully removed while the thermocouple remained in the vein and micropore tape was used to secure it in place.

4.2.4 - Temperature measurements

Core temperature was measured 15 cm past the anal sphincter muscle using a commercially available rectal probe (Thermalert, Physitemp, Clifton, New Jersey, USA). Skin thermocouples were placed on two sites on each arm (Type-t thermocouples, Grant Instruments, Cambridge, United Kingdom) and securely held in place throughout the protocol by the use of medical tape. Blood temperatures were measured using the sterile implantable thermocouples mentioned previously. Skin, rectal and blood thermocouples were connected to two 4 channel thermocouple meters (TC- 2000, Sable Systems, Las Vegas, NV, USA) and a data acquisition system (Powerlab 16/S, ADInstruments, UK).

4.2.5 - Haemodynamic measurements

Arterial blood pressure was measured non-invasively by infrared photoplethysmography (Finometer, FMS, Netherlands) via a cuff on the middle finger of the control hand. Heart rate was determined using a 3-lead ECG. Stroke volume was estimated using the ModelFlow method included with the Beatscope computer software package (Beatscope, FMS, Netherlands), with cardiac output calculated as stroke volume x heart rate following corrections for age, height, and weight (Wesseling *et al*, 1993). Brachial artery blood flow was measured using duplex Doppler ultrasound equipped with a 10 MHz linear probe (Vivid 7 Dimension, GE Medical, Horton, Norway), and was calculated using the equation Brachial Artery Blood Flow ($ml \cdot min^{-1}$) = $V_{mean} \times \pi \times (D/2)^2 \times 60$: where V_{mean} is time-averaged mean velocity ($cm \cdot s^{-1}$), π is the mathematical constant, D is vessel diameter (cm), and 60 is a conversion factor to convert to $ml \cdot min^{-1}$.

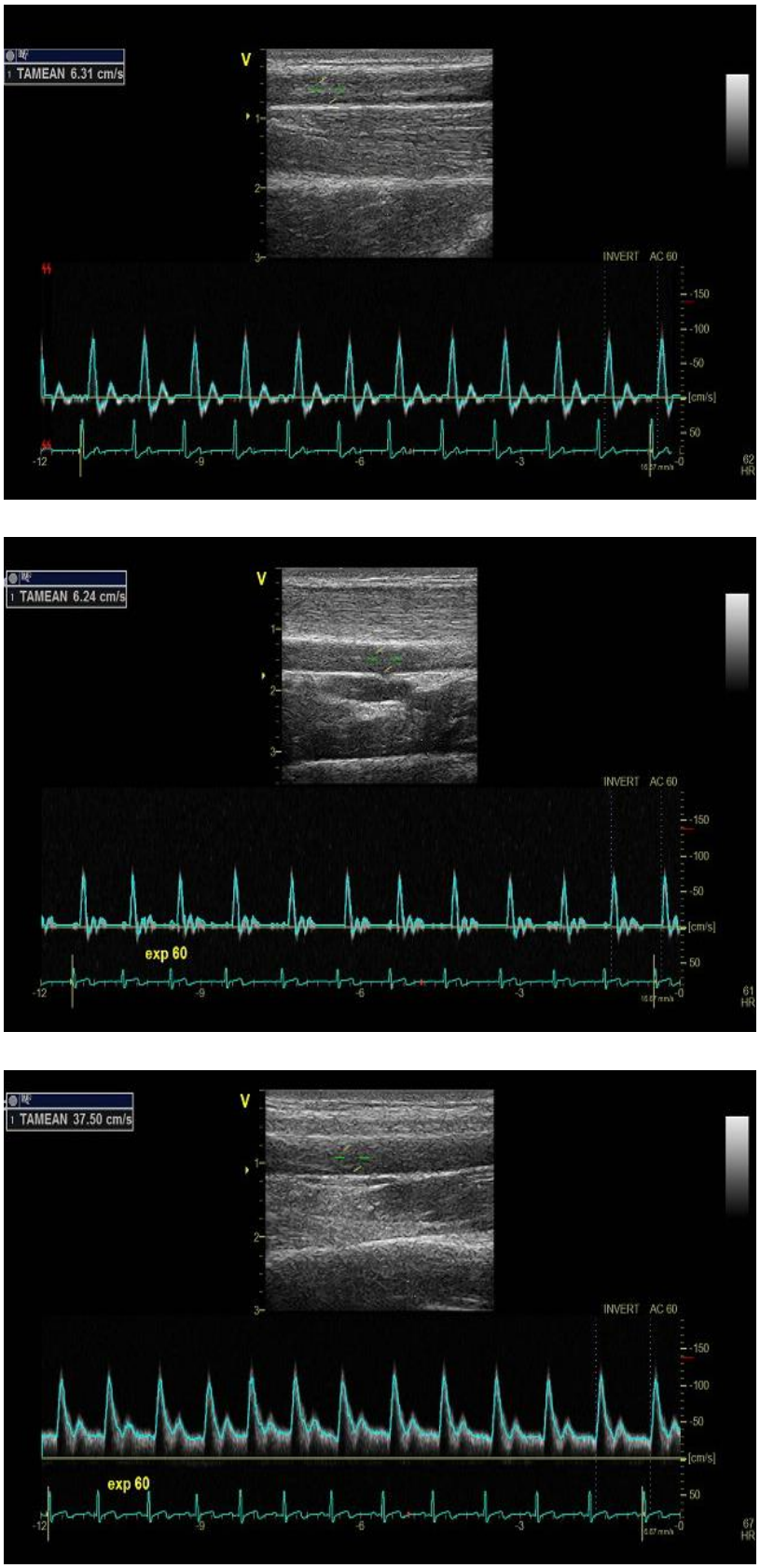

Fig. 4.1 – Typical brachial artery Doppler ultrasound traces during normothermia (top), cooling (middle) and heating (bottom). Note the significant increase in both peak and mean blood velocity during localised heating, despite an unchanged heart rate.

Brachial artery vessel diameter was determined at peak systole from three 2D B-mode images in the longitudinal view at ~70 frames per second. Mean blood velocity (V_{mean}) was measured using continuous pulsed-wave Doppler at a frequency of 4.4 MHz, at an insonation angle of 60º and the sample volume extended to cover the entire vessel lumen. Blood velocity was average over 3 consecutive 12 s profiles (36 s total). Skin blood flow (SkBF) was measured via laser-Doppler flowmetry (Periflux Flowmetry System, Jarfalla, Sweden) on the ventral surface of the both forearms (i.e. above the flexor carpi radialis). Tissue oxygenation in the main muscle of the forearm (extensor carpi ulnaris) was measured using near-infrared spectroscopy (NIRS; INVOS Cerebral Oximeter, Somanetics, Troy, MI, USA), with each optode pad taped to reduce interference from external light sources. Arm and systemic vascular conductance were calculated as brachial arterial blood flow/MAP and $\dot{Q}$/MAP, respectively. Brachial artery shear rate was calculated according to the formula SR = 4 x (TAMV/D) where SR is shear rate, TAMV is time-averaged mean velocity ($cm \cdot s^{-1}$) and D is vessel diameter (cm) (Newcomer *et al*, 2008).

Fig. 4.2 – Experimental set-up showing measurement of brachial artery blood flow in a supine individual using duplex Doppler ultrasound.

4.2.6 - Blood sampling for blood gases and metabolites

For the measurement of blood gas variables, haemoglobin, glucose and lactate concentrations, 1 ml of blood was drawn into pre-heparinized PICO syringes every 10 min and measured using an automated analyser (ABL 825 M Flex, Radiometer, Denmark).

4.2.7 – Data analysis

Blood flow and arterial diameters were recorded using the techniques described previously, burned to DVD, and analysed on specialist software installed on a stand-alone laptop (EchoPac, GE Medical, UK). All other data were collected via a data acquisition system (Powerlab 16S, AD Instruments, UK) and fed to a desktop computer data acquisition software package (LabChart 5, AD Instruments, UK). All analysed data were stored in a PC-based computer spreadsheet software programme (Excel, Microsoft, US).

4.2.8 – Statistical analysis

A one and two-way repeated measures ANOVA was used to test for differences within and between arms, with Holm-Bonferroni *post-hoc* testing employed to identify the time-points at which changes occurred once a significant overall effect was found. Multiple regression for within-subject repeated measures was used for the analysis of the relationship between blood flow and temperatures (Bland and Altman, 1995). All statistical analyses were carried out using SPSS (Version 20, IBM, Armonk, US) with results expressed as mean ± SEM. Significance is set at $P < 0.05$.

4.3 – Results

4.3.1 – Temperature responses

Core temperature was maintained at ~37 ºC throughout both interventions and remained stable until the end of the experimental protocol. With isolated arm cooling, arm $\overline{T}_{sk}$ decreased from 29.9 ± 1.1 to 16.3 ± 1.1 ºC while T_b decreased from 34.2 ± 0.6 to 28.1 ± 1.0 ºC ($P < 0.05$ for both). In contrast, isolated heating of the arm led to increases in $\overline{T}_{sk}$ and T_b of 29.8 ± 0.4 to 39.5 ± 0.4 ºC and 34.0 ± 0.7 to 37.0 ± 0.1 ºC, respectively ($P < 0.05$ for both). $\overline{T}_{sk}$ and T_b in the control arm remained unchanged throughout both interventions ($P = 0.22$, 0.82, 0.31, and 0.35 for cold and hot $\overline{T}_{sk}$ and T_b, respectively).

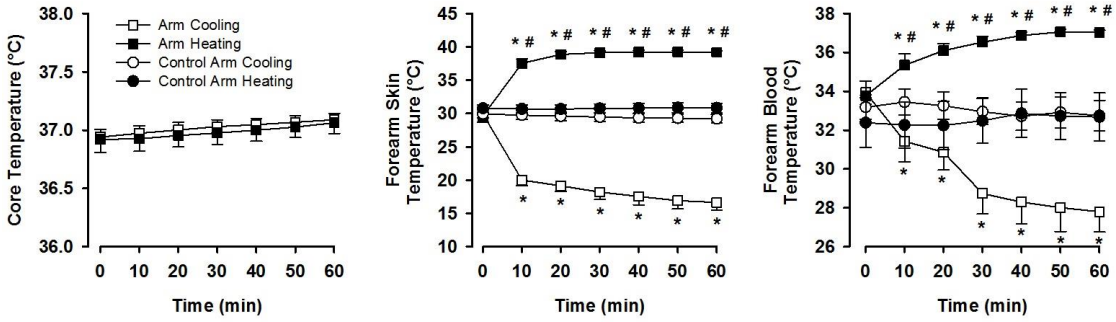

Figure 4.3: Temperature responses to localised cooling and heating. Core, forearm skin, and forearm deep venous blood temperatures during 60 min of localised cooling and heating of the limb. Data are mean ± SEM for nine participants (n=7 for blood due to catheterisation problems in two participants). * Significantly different from baseline. # Significantly different from cooled arm; $P < 0.05$.

4.3.2 – Arm and systemic haemodynamic responses

Localised cooling of the experimental arm steadily decreased brachial artery blood flow and conductance from 98 ± 6 to 55 ± 6 ml·min^{-1} and 0.9 ± 0.1 to 0.5 ± 0.1 ml·min^{-1}·mmHg^{-1}, respectively ($P < 0.05$). These changes in flow were solely due to a decreased brachial arterial time-averaged mean blood velocity (12 ± 2 to 7 ± 1 cm·s^{-1}) ($P < 0.05$), as brachial arterial diameter remained unchanged throughout (0.41 ± 0.01 cm). A small but significant drop in conductance and blood flow was also observed in the contralateral control arm at the end of the 60 min, although this equated to a decrease in flow of only ~ 10 ml·min^{-1} in absolute terms. Skin blood flow displayed a small but significant drop over the initial 30 min of cooling before levelling off at a value close to zero (7 ± 3 to 2 ± 1 LDU; $P < 0.05$), while mean shear rate through the conduit artery roughly halved from 132 ± 18 to 72 ± 8 s^{-1}. The decreased flow with cooling was accompanied by an increase in (estimated) a-vO_2 difference of blood draining the skeletal muscle ($P < 0.05$) as a result of the gradual decrease in venous O_2 content after 20 min ($P < 0.05$).

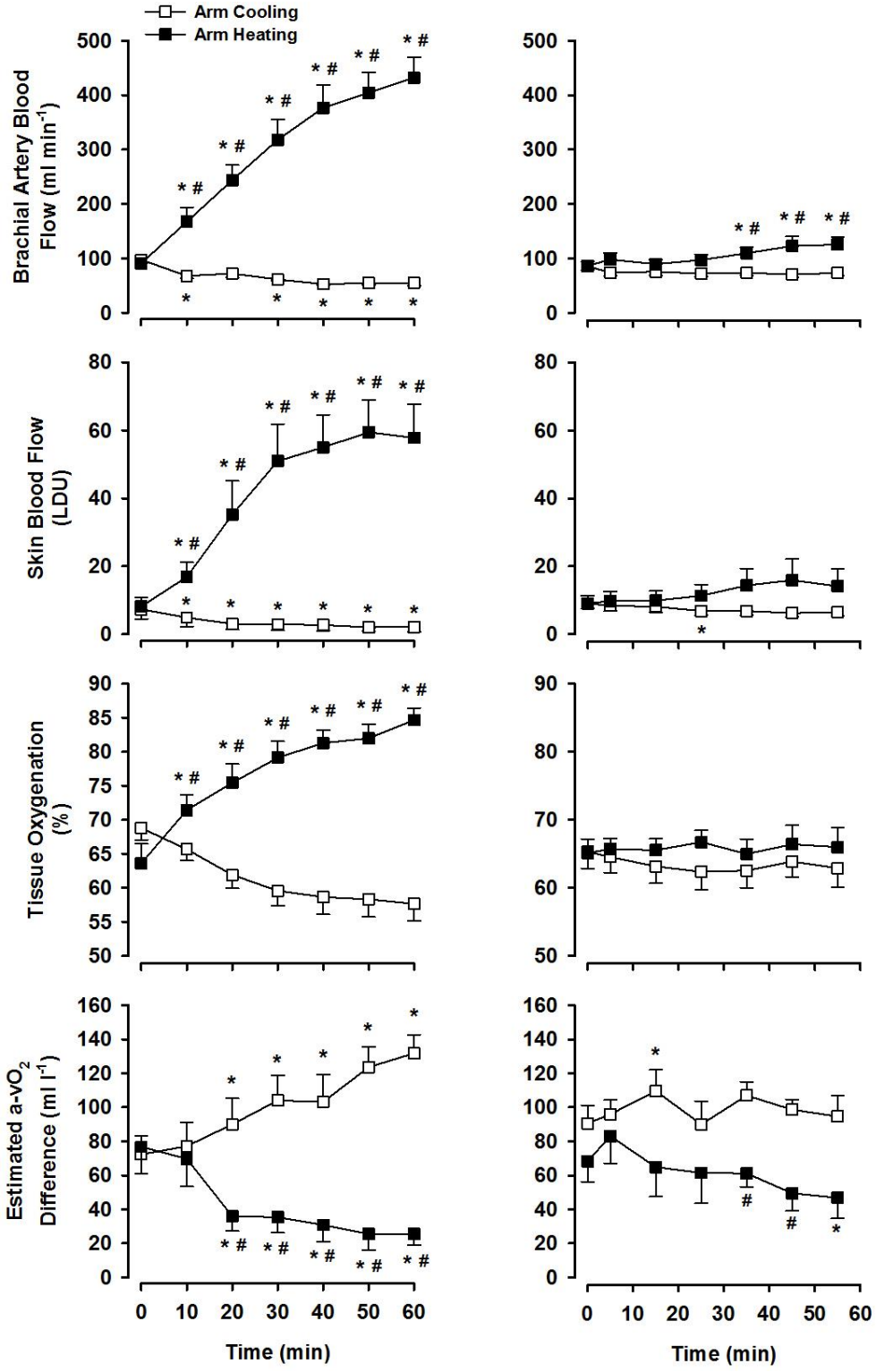

Figure 4.4: Haemodynamic responses to localised cooling and heating. Brachial artery blood flow, skin blood flow, tissue oxygenation, and estimated a-vO_2 difference during localised cooling and heating in the experimental arm (left column) and opposing control arm (right column). Data are mean ± SEM for nine participants (n= 7 for tissue oxygenation and n= 4 for control arm a-vO_2 difference due to equipment and catheterisation limitations). * Significantly different from baseline. # Significantly different from cooled arm; $P < 0.05$.

Venous O_2 saturation and PO_2 in the experimental arm also decreased with cooling (60 ± 5 to 33 ± 4% and 29 ± 2 to 13 ± 2 mmHg, respectively; $P < 0.05$), while tissue oxygenation measured by NIRS followed a similar pattern in decreasing from 69 ± 2% to 58 ± 3% ($P < 0.05$). No changes in haemoglobin concentration, pH, lactate or plasma osmolality were observed in either arm.

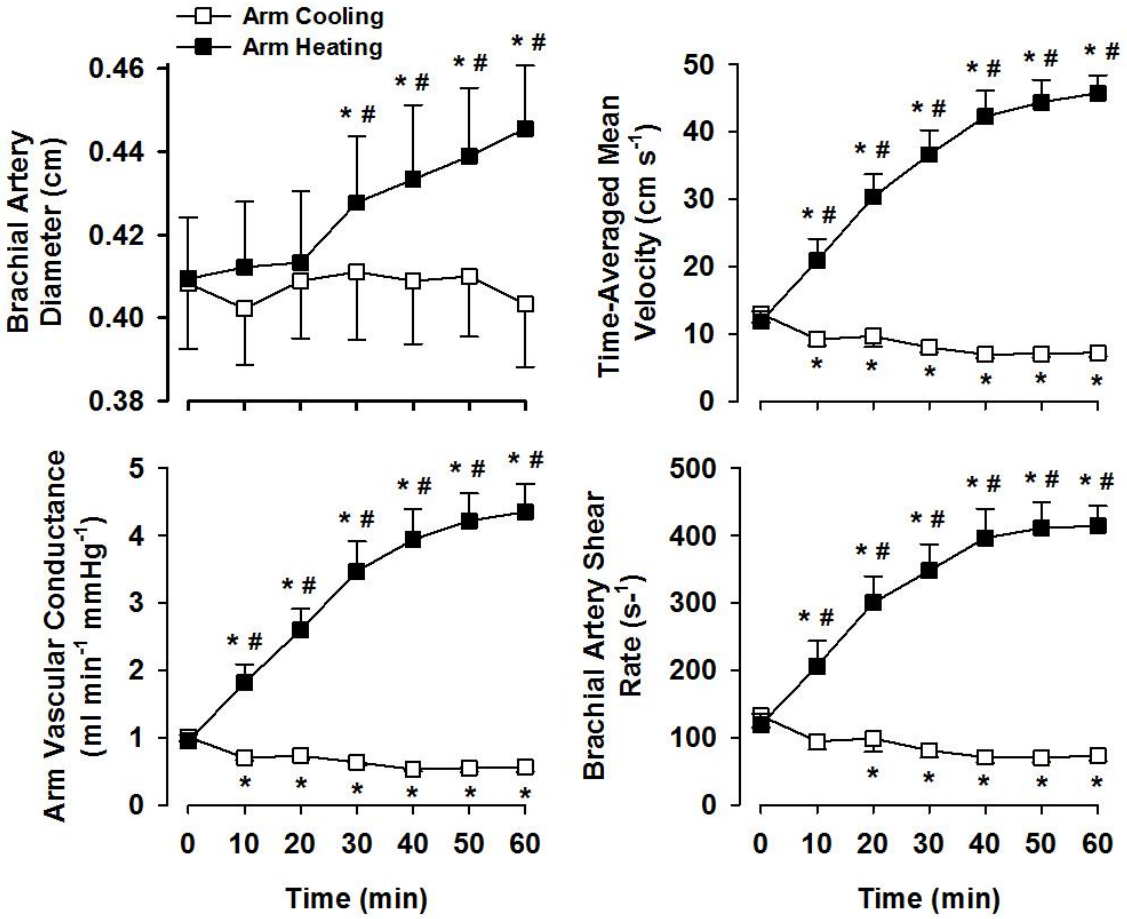

Figure 4.5: Brachial artery haemodynamic responses to localised cooling and heating. Diameter, time-averaged mean velocity, vascular conductance, and shear rate in the brachial artery during localised cooling and heating. Data are mean ± SEM for nine participants. * Significantly different from baseline. # Significantly different from cooling; *P* < 0.05.

Localised heating of the arm led to substantial increases in brachial artery blood flow and conductance from 91 ± 10 to 432 ± 38 ml·min^{-1} and 0.9 ± 0.1 to 3.9 ± 0.6 ml·min^{-1}·mmHg^{-1}, respectively (P < 0.05 for both). In contrast to the cooling protocol, these changes in flow were the result of increases in both brachial artery blood velocity (12 ± 2 to 46 ± 3 cm·s^{-1}) and diameter (0.41 ± 0.01 to 0.45 ± 0.01 cm; P < 0.05 for both), accompanied by significant increases in mean brachial artery shear rate from 119 ± 17 to 417 ± 30 s^{-1}.

Blood flow in the control arm showed a small increase over the course of the 60 min from 86 ± 8 to 125 ± 14 ml·min^{-1}. Skin blood flow significantly increased over the first 30 min of heating before plateauing for the remainder of the intervention (8 ± 3 to 57 ± 10 LDU; P < 0.05). Increased forearm flow was accompanied by a decline in a-vO_2 difference in both the experimental and control arms, whilst augmenting venous O_2 saturation, PO_2 and O_2 content in the experimental arm (55 ± 8 to 79 ± 4%, 27 ± 3 to 48 ± 7 mmHg and 114 ± 15 to 159 ± 9 ml·l^{-1}, respectively; P < 0.05). Tissue oxygenation steeply inclined after the first 10 min of heating before rising gradually until the end of the intervention (64 ± 3% to 85 ± 2%; P < 0.05) in the experimental arm, but remained unchanged in the control arm (65 ± 2% to 66 ± 3%). Other measured blood variables such as haemoglobin concentration, pH, lactate and osmolality were not affected by the elevated venous blood temperatures produced by the heating protocol. Systemic responses with passive arm heating were unchanged during the first 40 min, although small increases in $\dot{Q}$, heart rate, stroke volume and MAP were observed from 50-60 min. The systemic responses with cooling only affected the systolic and diastolic pressures.

Table 4.1. Blood variable responses during heating and cooling in the experimental and control arm

			Time (min)						
			0	10	20	30	40	50	60
Hb ($g \cdot l^{-1}$)	Heating	Experimental	144±3	144±3	143±4	141±4	143±3	142±3	143±4
		Control	144±5	145±2	145±6	144±6	143±6	145±5	145±4
	Cooling	Experimental	147±3	146±3	145±3	146±3	148±2	149±2	149±2
		Control	149±2	150±3	150±3	145±6	150±3	150±2	150±3
O_2 Sat (%)	Heating	Experimental	55±8	61±11*	77±5*#	75±6*#	76±6*#	82±5*#	79±4*#
		Control	66±5‡	61±7	66±7	66±7†	67±4†	70±5†	75±5*†
	Cooling	Experimental	60±5	52±4	49±7*	40±5*	40±5*	31±4*	33±4*
		Control	55±4	51±3	47±5*	51±5	45±3	49±2	49±5
PO_2 (mmHg)	Heating	Experimental	27±3	35±7	42±3*#	45±7*#	45±6*#	51±6*#	48±7*#
		Control	33±3‡	31±4	33±4	34±5	32±2†	35±4	38±4*
	Cooling	Experimental	29±2	23±1*	21±2*	16±2*	16±2*	14±2*	13±2*
		Control	26±2	25±1	23±2	25±1	22±1	24±1	24±1
Ct_vO_2 ($ml \cdot l^{-1}$)	Heating	Experimental	114±15	124±22	156±12*#	148±13*#	151±11*#	164±9*#	159±9*#
		Control	134±8‡	125±14*	132±15*	132±14*†	133±10*†	141±13†	149±11*†
	Cooling	Experimental	123±10	106±6	99±14*	81±10*	82±10*	65±8*	69±8*
		Control	112±7	104±5	96±9*	101±9	92±4	100±3	101±10
PCO_2 (mmHg)	Heating	Experimental	46±2	46±2	42±3	45±2†	45±2#	43±2	44±2
		Control	45±2	46±2	43±2	42±2	42±2	43±2	41±2†
	Cooling	Experimental	45±2	43±2*	41±3	39±2*	39±2*	41±2	40±2
		Control	45±2	46±2	46±2	44±3	47±2	46±2	46±2
pH	Heating	Experimental	7.41±0.01	7.41±0.01	7.41±0.01	7.40±0.01	7.40±0.01	7.40±0.01	7.40±0.01
		Control	7.40±0.02	7.40±0.01	7.44±0.03	7.41±0.01	7.40±0.01	7.40±0.01	7.42±0.01
	Cooling	Experimental	7.41±0.01	7.44±0.02	7.44±0.01	7.47±0.02	7.47±0.02	7.45±0.01	7.44±0.01
		Control	7.41±0.01	7.40±0.01	7.40±0.01	7.40±0.01	7.40±0.01	7.40±0.02	7.40±0.02
Lactate ($mmol \cdot l^{-1}$)	Heating	Experimental	1.1±0.1	1.0±0.1	1.0±0.1	0.9±0.1	0.8±0.1	0.8±0.1	0.8±0.1
		Control	1.3±0.1	1.2±0.1	1.1±0.1	0.9±0.2	1.0±0.1	1.0±0.1	1.0±0.2
	Cooling	Experimental	1.1±0.1	1.2±0.1	1.1±0.1	1.1±0.1	1.1±0.1	1.1±0.1	1.1±0.1
		Control	1.3±0.1	1.3±0.1	1.3±0.1	1.3±0.1	1.3±0.1	1.2±0.1	1.3±0.2
Osmolality ($mOsm \cdot kg^{-1}$)	Heating	Experimental	280±1	279±2	280±2	280±1	279±2	279±2	279±2
		Control	281±1	280±2	281±2	281±2	282±2	281±2	281±2
	Cooling	Experimental	280±2	280±2	281±2	280±2	280±2	280±1	279±2
		Control	282±1	282±1	282±1	283±2	282±1	282±1	281±1

Values are means ± SEM for 8 participants (n= 4 for control arm variables due to catheterisation problems in four participants). * Significantly different from baseline # Different from respective experimental cooling † Different from respective cooling control; $P < 0.05$.

4.3.3 – Relationship between local blood temperature and arm blood flow

Localised cooling in the experimental arm displayed a strong linear relationship with blood temperature (R^2 = 0.68; P < 0.01), with modest decreases in blood flow of ~ 40 ml·min^{-1} closely coupled to a 6 °C decrease in temperature over the 1 h cooling intervention. The absence of any alterations in systemic haemodynamics resulted in this being achieved through changes in local vascular conductance alone (1.0 to 0.5 ml·min^{-1}·mmHg^{-1}; P < 0.05), a response which was once again closely related to the temperature of blood draining the arm (R^2 = 0.60; P < 0.01). In contrast to the small linear decrease in blood flow when lowering temperatures below resting levels, localised arm heating led to a marked increase in arm blood flow when temperatures were raised above baseline values. This increase was also achieved through changes in local vascular conductance alone, with both blood flow and conductance once again being closely related to their corresponding blood temperatures (R^2 = 0.50 for both; P < 0.01). No significant relationships were observed in the control arm for either heating or cooling, with both temperatures and blood flow remaining close to baseline levels throughout.

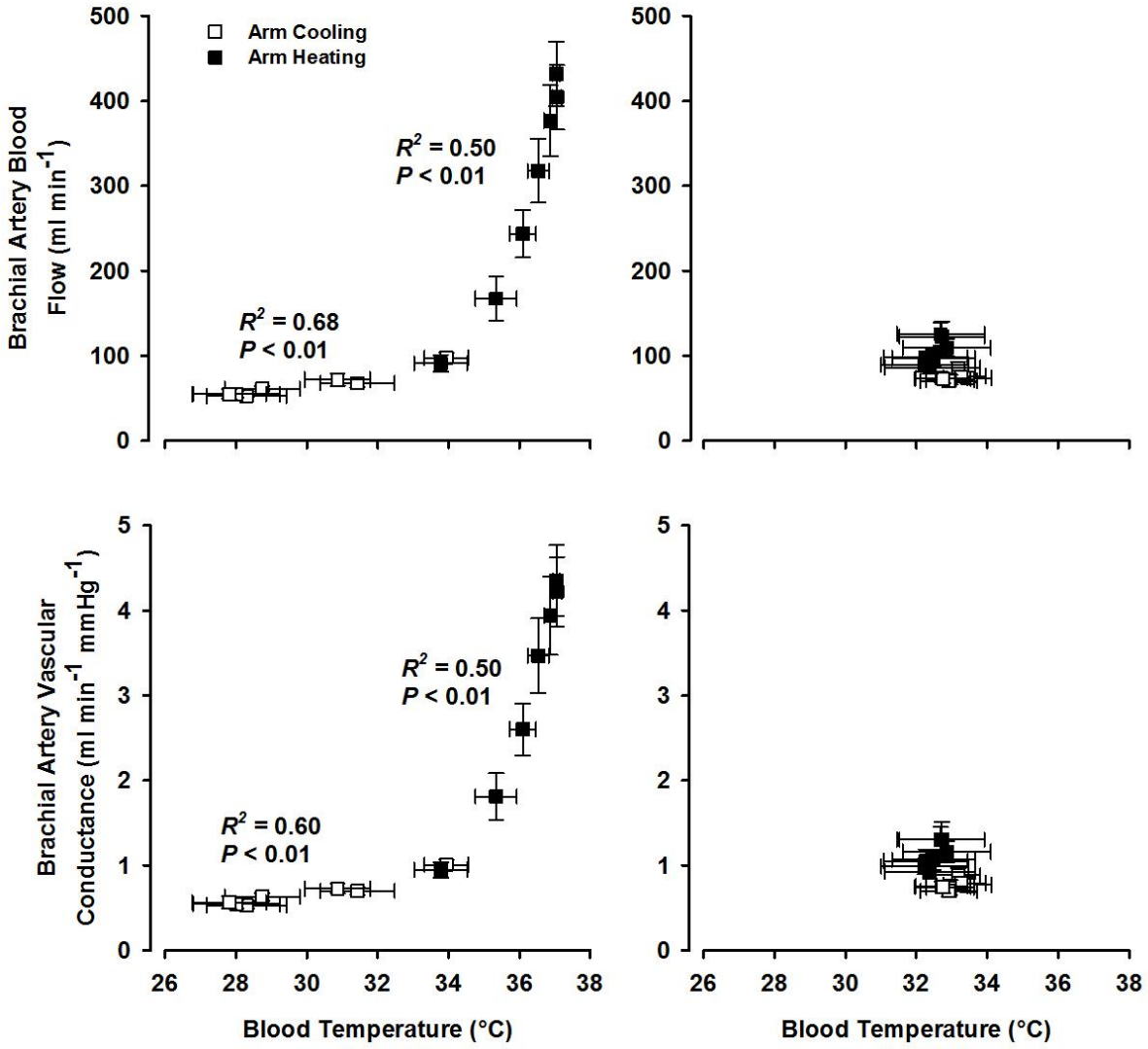

Figure 4.6: Relationship between brachial artery blood flow, conductance, and deep venous blood temperature during localised cooling and heating in the experimental arm (left column) and control arm (right column). Data are mean ± SEM for nine participants (n=4 for control arm variables due to catheterisation issues prior to testing). Corresponding R^2 values are calculated from individual data multiple regression analyses as described in prior statistical methodology.

Table 4.2. Systemic responses during arm heating and cooling

		Time (min)						
		0	10	20	30	40	50	60
Cardiac output ($l \cdot min^{-1}$)	Heating	5.7 ± 0.5	5.9 ± 0.5	5.9 ± 0.5	5.7 ± 0.5	5.8 ± 0.5	6.0 ± 0.6*****	6.3 ± 0.5*****
	Cooling	5.8 ± 0.5	5.7 ± 0.4	5.9 ± 0.5	5.9 ± 0.5	5.9 ± 0.5	6.1 ± 0.4	5.8 ± 0.6
Heart Rate (bpm)	Heating	62 ± 3	61 ± 3	62 ± 3	62 ± 3	63 ± 3	64 ± 4	67 ± 3*
	Cooling	63 ± 3	60 ± 2*****	60 ± 3*****	60 ± 3	61 ± 3	61 ± 3	60 ± 3
Stroke Volume (ml)	Heating	91 ± 7	92 ± 6	91 ± 6	91 ± 6	90 ± 6	93 ± 6	94 ± 6
	Cooling	91 ± 6	93 ± 6	96 ± 6	96 ± 7	96 ± 7	98 ± 5	95 ± 8
Mean Arterial Pressure (mmHg)	Heating	93 ± 4	93 ± 3	95 ± 4	92 ± 4	95 ± 4	95 ± 4	98 ± 2*****
	Cooling	97 ± 4	96 ± 5	98 ± 4	99 ± 4	101 ± 4	101 ± 4	100 ± 2
Systolic Blood Pressure (mmHg)	Heating	126 ± 5	127 ± 5	127 ± 5	123 ± 5	129 ± 5	129 ± 5	133 ± 4*****
	Cooling	133 ± 5	131 ± 6	137 ± 5	137 ± 5	137 ± 6	140 ± 6*****	145 ± 6*****
Diastolic Blood Pressure (mmHg)	Heating	71 ± 5	72 ± 4	72 ± 4	72 ± 6	74 ± 5	73 ± 4	76 ± 4*****
	Cooling	75 ± 3	75 ±4	77 ± 3	76 ± 3	78 ± 4	79 ± 3	82 ± 3*****
Systemic Vascular Conductance ($ml \cdot min^{-1} \cdot mmHg^{-1}$)	Heating	63 ± 7	64 ± 6	63 ± 6	64 ± 7	61 ± 6	64 ± 7	66 ± 6
	Cooling	59 ± 4	59 ± 4	60 ± 5	60 ± 5	60 ± 5	60 ± 4	59 ± 5

Values are means ± SEM for 8 participants. * Different from zero; $P < 0.05$.

4.4 – Discussion

The primary aim of this study was to systematically assess the relationship of haemodynamic responses in the human arm to changes in local temperature. In addition, we sought to provide evidence that changes in flow occurred in various tissues within the limb as opposed to the skin alone.

4.4.1 – Magnitude and distribution of arm blood flow in response to changes in local tissue temperatures

The magnitude of change in blood flow to the skin and skeletal muscle tissues during localised cooling and heating remains poorly characterised. Here, cooling of the limb led to modest yet significant decreases in whole-arm blood flow over 60 min, with final values being 44% below those recorded at baseline - broadly agreeing with previous studies (Barcroft and Edholm; 1943; Thorsson *et al*, 1985; Taber *et al*, 1992; Karunakara *et al*, 1999; Yanagisawa *et al*, 2007; Gregson *et al*, 2011). Doppler ultrasound measurements of conduit artery diameter identified this response as being confined to the downstream resistance vessels, as brachial artery diameter remained unchanged throughout. The observed vasoconstrictor response appeared to occur in vessels perfusing both skin (as measured by laser Doppler flowmetry) and underlying deep tissue (predominantly skeletal muscle) as evidenced by increases in deep tissue a-vO_2 difference, decreases in venous blood PO_2 and O_2 saturation, and decreases in NIRS-derived tissue oxygenation (tSO_2 -15%). Although a limited number of studies have shown decreased muscle perfusion in the human leg following localised cold stress (Thorsson *et* al, 1985; Yanagisawa *et al*, 2007; Gregson *et al*, 2011), previous studies specifically assessing muscle blood flow in the forearm have shown a skeletal muscle vasodilatory response (Clarke *et al*, 1958; Ducharme and Radomski; 1990), presumably through the initiation of a hunting response within the deep tissues of the limb. Differences between these previous studies and the current one are most likely down to the duration of cooling involved, with the observation of the cold-induced vasodilation occurring between 1 and 3 h after the initiation of cooling (Ducharme

and Radomski; 1990), long after the end of the 1 h protocol employed here. Interestingly, although no participant in the current study exhibited any overall increase in arm blood flow throughout the cooling trial, one subject showed an initial 41% decrease in brachial artery blood flow over the initial 40 - 50 min of the test before gradually increasing back to starting levels by the end of the 60 min. The higher blood flow observed in this participant was accompanied by an attenuated temperature drop of deep venous blood (3 ºC decrease vs. average 7 ºC decrease), suggesting an increased blood flow and heat influx in the deep tissues of the arm, as $\overline{T}_{sk}$ and blood flow remained consistently low. This and previous results suggest the possibility of the vasoconstrictor effect of localised cold stress in the human arm being partially reversed over longer durations, possibly to prevent cold damage and/or preserve oxygen delivery to the tissues.

As expected, localised heating led to an increase in brachial artery blood flow from 90 to 430 ml·min^{-1} over the course of 1 h. In contrast to cooling of the tissues, this response was due to a combination of both increased blood velocity due to downstream peripheral vasodilation as well as a direct vasodilatory effect on the brachial artery itself (10% diameter increase after 1 h), findings which are in agreement with previous studies investigating brachial artery diameter and blood flow under numerous heating protocols (Black *et al*, 2008; Green *et al*, 2010; Padilla *et al*, 2011; Simmons *et al*, 2011; Carter *et al*, 2014). Although the magnitude of flow increase in the arm is generally accepted, its distribution to different tissues within the limb is still unclear. Early studies on the response of forearm muscle blood flow to heat stress are mixed, with various studies showing increases (Barcroft *et al*, 1947; Rowell *et al*, 1970), decreases (Rowell, 1977), or no change (Edholm *et al*, 1956; Roddie *et al*, 1956; Roddie *et al*, 1957; Detry *et al*, 1972; Johnson *et al*, 1976). A common methodology in all studies (with the exception of Johnson *et al*, 1976) was the use of a whole-body or indirect (e.g. foot immersion) protocol to raise forearm temperature. More recent studies in which tissues have been directly heated using water-perfused suits or microwave diathermy display the ability of the skeletal muscle vasculature to dilate in direct response to increased temperatures (up to 15-fold increase in flow when tissue temperature rapidly raised to 45 ºC) (Sekins *et al*, 1984; Song, 1984; Giombini *et al*, 2007; Heinonen *et al*, 2011). Raising the blood

temperature from 34 to 37 ºC in the current experiment was associated with a 5-fold increase in whole-arm blood flow matched by an inverse decrease in a-vO_2 difference of blood draining from the deep tissues of the forearm, suggesting a reciprocal increase in skeletal muscle perfusion. While it has been shown that blood draining from these deep vessels is independent of blood draining the forearm skin (Mottram, 1955; Roddie *et al*, 1956), the possibility exists that blood flow draining the hand may have affected the venous oxygen content if the catheter was positioned within the radial or ulnar veins as opposed to the interosseous vein (Mottram, 1955). If this were the case, however, the a-vO_2 difference measured in blood draining the combined tissues of the hand and deep muscles of the arm could allow calculations of hand and deep tissue blood flow through the use of the Fick Principle and an assumption of an unchanged $\dot{V}O_2$ from baseline measurements. This calculation would result in a blood flow of 310 ml·min^{-1} for these tissues alone (i.e. the whole of the limb with the exception of the skin of the forearm). With a maximum recorded whole-limb blood flow of 430 ml·min^{-1} in the current study, this would leave a forearm skin blood flow of 120 ml·min^{-1}. A near identical protocol by Padilla *et al*. (2011) also using Doppler ultrasound reported a brachial artery flow of ~300 ml·min^{-1} after 60 min of heating the forearm alone, with blood flow to and from the hand eliminated through the use of an arterial cuff occlusion at the wrist. Assuming a similar response was observed in the previous study when compared to the present one, this would equate to 120 ml·min^{-1} skin blood flow and an underlying muscle blood flow of 180 ml·min^{-1}, roughly 80% higher than baseline and in agreement with increases in calf muscle blood flow measured using PET (+85%; Heinonen *et al*, 2011). In order to try to elucidate this response further, muscle tissue oxygenation was simultaneously measured via NIRS. Despite significant increases in tissue O_2 saturation (+30%), recent papers have cast doubt on the validity of these data due to significant interference from skin blood flow increases (Hirasawa *et al*, 2014), rendering the NIRS data as a marker of changes in both skin and deep tissue, rather than just tissue alone.

4.4.2 – Relationship between local blood temperature and arm blood flow

Central reflex responses such as a cold-pressor activated increases in perfusion pressure during cooling (Lovallo, 1975) or reflex cutaneous vasodilation during whole-body or indirect heating (Barcroft *et al*, 1947; Edholm *et al*, 1956; Roddie *et al*, 1956; Detry *et al*, 1972; Rowell, 1977) can alter both local and systemic haemodynamics, making it difficult to isolate the local haemodynamic response to changes in local tissue temperatures. With this in mind, the current study design allowed the manipulation of local temperatures alone whilst minimising the risk of these potential confounding central effects. With the exception of a small increase in heart rate and cardiac output in the final 10 min of heating; all other systemic temperatures, haemodynamic responses and blood metabolites remained essentially unchanged throughout both interventions, suggesting that alterations in arm tissue flow were unaffected by central input.

Changes in brachial artery blood flow were closely associated with changes in blood temperature throughout both cooling and heating, although with a significantly higher rate of increase at temperatures above normal resting values (~ 34 ºC). These results agree with those from the classical study of Barcroft and Edholm (1943) in which forearm blood flow was measured using venous occlusion plethysmography whilst $\overline{T}_{sk}$ was clamped at different levels using forearm water immersion (range 15 – 45 ºC). A novel aspect of the current study was the simultaneous measurement of brachial artery blood flow and deep venous blood temperature throughout each intervention, with results displaying a close coupling between the two over the entire duration of both cooling and heating. In the present study, despite $\overline{T}_{sk}$ being effectively clamped at 18 and 39 ºC during cooling and heating, respectively, the slow nature of heat transfer within the limb resulted in gradual changes in blood temperature of - 6 ºC and + 3 ºC over the 1 h of cooling and heating, respectively. The presence of essentially unchanged systemic responses, including perfusion pressure, throughout the majority of both protocols demonstrated that these changes in flow were exerted solely through changes in arm vascular conductance, which in turn closely followed changes in blood temperature perfusing the vessels. These

findings suggest that local tissue or intravascular temperature-sensitive mechanisms act to regulate whole-arm blood flow in response to localised cold or heat stress, although the sensitivity of this response is significantly higher at temperature above that of resting values (129 ± 34 vs. 6 ± 2 $ml \cdot min^{-1} \cdot °C^{-1}$ for heating and cooling, respectively).

4.4.3 – Mechanisms of blood flow control

With numerous *in vitro* preparations generally showing little to no direct effect of temperature changes on smooth muscle contractility (Vanhoutte and Shepherd; 1970; Vanhoutte and Shepherd; 1970; Ives *et al*, 2011), it would appear that temperature-sensitive mechanisms acting indirectly on local vascular tone are the most likely regulators of flow in response to cold or heat stress. Basal tone in the resting human arm is predominantly mediated through adrenergic control, with minimal input from nitric oxide sources. The decrease in skin blood flow observed here during localised cooling is most likely mediated through a translocation of α_2-receptors from vascular smooth muscle (Bailey *et al*, 2004; Bailey *et al*, 2005) alongside an attenuation in the release of the vasodilator signal nitric oxide (Yamazaki *et al*, 2006). Although as yet unproven, it is likely that skeletal muscle vessels may be controlled by similar processes, alongside factors such as increased muscle sympathetic nerve activity (Mizushima *et al*, 1998).

The substantial increases in arm blood flow when temperatures are elevated above resting values are most likely due to a withdrawal of this adrenergic control combined with additional heat-sensitive vasodilator stimuli (Keller et al 2010). Elevations in skin blood flow during localised heat stress are mediated through the release of mediators such as substance P and neurokinan A via an afferent axon reflex (Holzer, 1998; Minson *et al*, 2001), in combination with augmented NO release through eNOS pathways (Kellogg *et al*, 1999) and increased shear stress (Padilla *et al*, 2011). The failure of the NO blocker L-NAME to completely abolish this response, however, plus the lack of any known temperature-sensitive

vasodilatory pathways within the skeletal muscle, means that other factors must contribute to the overall changes observed in arm blood flow. One recent mechanism proposed by this laboratory is the temperature-dependent release of the vasodilator ATP from erythrocytes in response to changes in blood temperature. ATP has been shown not only to be a potent dilator within skeletal muscle vasculature (González-Alonso *et al*, 2002), but also to be released in a temperature-dependent manner both *in vitro* (Kalsi and González-Alonso; 2012) and *in vivo* (Pearson *et al*, 2011; Kalsi et al unpublished results). Indeed, the increased rate of ATP release *in vitro* closely matches increases in blood flow in the current study, with a rapid increase in ATP release observed when blood temperature is raised above normal resting arm temperatures (~ 34 ºC). With its sympatholytic properties (Rosenmeier *et al*, 2004) and known effects on skeletal muscle tissue, temperature-induced ATP release could provide a potential mechanism for increased blood flow in the deep tissues of the arm during heating.

4.4.4 – Limitations

The inability to determine the precise placement of the deep venous catheter during the current study meant that it was not possible to identify which of the deep venous vessels the sampling line resided in during cooling or heating. With previous work by Mottram (1955) identifying possible contamination of the deep venous blood with that of the hand when the catheter resides in the radial or ulnar veins, it is possible that venous oxygen contents in the present study may have been affected by returning skin blood flow of the hand. Although this posed no problems during the cooling protocol due to the extremely low levels of skin blood flow present, the large increases in skin blood flow during heating may have influenced results reported here. Despite this, estimations of forearm skin and muscle blood flow could still be made using the Fick Equation, with results showing a similar relative increase in forearm muscle blood flow compared to recent work in the human calf (Heinonen *et al*, 2011). Future studies using a wrist occlusion cuff during sampling would minimise this problem and help to confirm results reported here. The NIRS system employed here to detect muscle tissue oxygenation is claimed to disregard any

contribution of skin blood flow through the use of dual-spaced optodes and built-in mathematical algorithms. Despite these claims, recent research has clearly shown a strong influence of cutaneous blood flow on validity of results (Hirasawa *et al,* 2014), and as such the values reported here are likely to represent both skin and muscle oxygenation during heating.

4.4.5 – Conclusions

Blood flow and vascular conductance in the human arm are closely coupled to changes in blood temperature during cold and heat stress, suggesting the regulation of local vascular tone by temperature-sensitive mechanisms located within the vasculature itself or surrounding tissues. Localised cooling leads to decreases in both skin and deep tissue blood flows, although longer periods may at least transiently reverse this process. Local heating results in significant increases in arm blood flow, with at least 25% of this increase due to increases in arm muscle blood flow. These results clarify the haemodynamic responses of the human arm to localised cooling and heating, and agree with recent work in the human leg showing the contribution of skeletal muscle to these increases in flow.

CHAPTER 5

Study 2: The role of local temperature alterations on blood flow responses and distribution in the resting human leg

5.0 – Abstract

Changes in local tissue temperatures have previously been shown to alter skin and muscle tissue perfusion and blood flow profiles in the human forearm. Less is known of the haemodynamic responses occurring during equivalent temperature manipulations in the leg, which may differ due to known heterogeneity between limb vascular responses. Leg tissue temperatures in 7 males (age 22 ± 1 yr) were altered over 1 h at rest through the use of frozen gel packs (cooling) or a water perfused trouser (heating). Core, skin and deep muscle (T_m) temperatures were measured throughout. Haemodynamic alterations in 3 major arteries of the leg (common, superficial, and profunda femoral arteries: CFA, SFA, and PFA) were assessed using duplex Doppler ultrasound, with the contralateral leg providing control measures. Systemic haemodynamic responses were measured non-invasively using infrared plethysmography. Systemic and control leg values were essentially unchanged throughout 1 h of both cooling and heating. Localised cooling led to a small yet significant decrease in blood flow to all three vessels, including the major arterial supply of the thigh skeletal muscle (20-40 ml·min^{-1} or ~ 20-30 %; $P < 0.05$), while heating led to significantly greater increases (120-510 ml·min^{-1} or 160 – 270 %; $P < 0.05$). These changes were related to local tissue temperatures during both cooling (R^2 = 0.28, 0.41, and 0.45) and heating (R^2 = 0.38, 0.53, and 0.55 for CFA, SFA, and PFA, respectively), although with an increased sensitivity at temperatures above resting baseline values (7 *vs.* 119 ml·min^{-1}·°C^{-1} for cooling and heating, respectively, in CFA). Alongside increases in tissue perfusion, localised heating led to reductions in oscillatory shear index in all three arteries (final values ~ 0.1 for all), mediated through a combination of increased antegrade and decreased retrograde flow and shear rates (PFA increased antegrade only). This study is the first to characterise the relationship between local tissue temperatures and leg haemodynamic responses over a wide-range of physiologically relevant temperatures. In addition, novel evidence is provided that localised heating attenuates pro-atherogenic blood profiles in all three major arteries perfusing the leg tissues and as such may offer a potential mechanism for non-pharmacological improvements in vascular health.

5.1 – Introduction

Local temperature manipulations alter both skin and skeletal muscle blood flow in the human arm (Chapter 4). These manipulations are often used for therapeutic benefit in both sports-related injury and clinical settings, with changes in local tissue blood flow, perfusion, and vessel wall shear responses all being cited as potential beneficial mechanisms (Bleakley *et al*, 2004; Padilla *et al*, 2011). Human forearm models, such as that used in the previous chapter, are often used to study these responses due to their ease of flow measurement, easy access for arterial and venous blood sampling, and their large surface-to-volume ratio – permitting wide-ranging temperature manipulations (Barcroft and Edholm; 1943). However, heterogeneity exists between the upper and lower limbs, with the human leg showing an attenuated vasodilator responsiveness (Newcomer *et al*, 2004), augmented vasoconstrictor responsiveness (Pawelczyk and Levine; 2002), significantly lower shear rate exposure (Wu *et al*, 2004; Newcomer *et al*, 2008), and increased risk of atherosclerosis and peripheral arterial disease than that of the arm (Kroger *et al*, 1999). For this reason, the use of temperature-induced haemodynamic changes within the arm to characterise corresponding responses within the leg is potentially unreliable.

Localised tissue cooling is a commonly prescribed recovery method following strenuous exercise or tissue injury in the lower limb, with benefits such as decreased tissue perfusion, decreased oedema, and attenuated inflammatory responses claimed to occur, in part, due to local vasoconstrictor responses within the tissues (Bleakley *et al*, 2004). Despite the wide adoption of this practice, however, scientific studies investigating absolute changes in blood flow have returned conflicting results, with muscle and limb flows previously having been shown to decrease (Thorlacius *et al*, 1998; Yanagisawa *et al*, 2007; Peiffer *et al*, 2009; Gregson *et al*, 2011), increase (Clarke *et al*, 1958; Ducharme and Radomski; 1990), or remain unchanged (Fiscus *et al*, 2005; Selkow *et al*, 2011; Ives *et al*, 2011). Fewer studies still have reliably assessed the relationship between decreased local tissue temperatures and its associated haemodynamic changes within the deep tissues of the limb, with the

possibility of a decrease in skin blood flow alone potentially resulting in little to no change in the underlying targeted tissues following musculoskeletal injury.

Localised tissue heating has been proposed to aid recovery following tissue fatigue or injury, and could also provide potential vascular benefits in clinically-relevant populations through mechanisms of increased tissue perfusion (Keller *et al*, 2010; Pearson *et al*, 2011; Heinonen *et al*, 2011), improved blood, oxygen and substrate delivery (Slivka *et al*, 2012), and anti-atherogenic flow profiles (Padilla *et al*, 2011; Simmons *et al*, 2011). Increased forearm tissue temperatures have been shown to increase blood flow up to 3-4 fold, despite little to no change in core temperature or cardiac strain (Simmons *et al*, 2011; Padilla *et al*, 2011; Carter *et al*, 2014). In addition, evidence of an attenuation of oscillatory blood flow within the brachial artery has been proposed as a potential stimulus to enhance endothelial function and health through the prevention of pro-atherogenic retrograde flows and shear stimuli (Thijssen *et al*, 2009). Despite the significantly increased risk of arterial dysfunction in the leg in comparison to the upper body (Kroger *et al*, 1999), no study to date has systematically investigated the relationship between local tissue temperatures and blood flow responses and distribution within this limb.

The current study, therefore, aimed to expand upon the previous chapter in the human arm and investigate the role of local temperature alterations on blood flow responses and distribution in the three major arteries of the resting human leg (common, superficial, and profunda femoral arteries). We hypothesised that 1) during cooling, blood flow in the whole-limb (common femoral artery) and deep thigh tissue (profunda femoral artery) would decrease in line with local tissue temperatures, with heating having the opposite effect, 2) cooling and heating would lead to decreases and increases in mean shear rate in all three arteries, respectively, and 3) heating would attenuate the pro-atherogenic stimuli of oscillatory blood flow and shear stress through a combination of increased antegrade and decreased retrograde flow components.

5.2 – Methods

5.2.1 – Participants

Following completion of a medical questionnaire and the provision of informed written consent, seven healthy males (age 22 ± 1 yr; height 179 ± 2 cm; weight 72 ± 4 kg) were recruited to participate in the study. All participants were recreationally active, and abstained from alcohol, caffeine, and strenuous exercise in the 24 h leading up to the day of testing. All procedures in this experiment conformed to the Declaration of Helsinki and were approved by the Brunel University School of Sport and Education Research Committee.

5.2.2 – Experimental protocol

Each subject visited the laboratory on two occasions to allow for isolated cooling or heating of a single leg in a counterbalanced design, with subjects positioned at rest in a semi-recumbent position for the duration of the experiment. Following instrumentation and baseline measurements, each experimental protocol consisted of a 1 h intervention period in which the tissue temperature of the left leg was either lowered or raised while the opposing leg and rest of the body remained in normothermic conditions. The order of each intervention was randomised amongst subjects, with at least 7 days between each visit. For the isolated cooling protocol, decreases in leg tissue temperature were achieved through the use of commercial frozen gel packs (KoolPak, Warwickshire, UK) strapped to the leg and fully encased in thick insulation to prevent heat exchange with the surrounding atmosphere.

Figure 5.1: Experimental set-up showing participant resting in normothermic conditions in a semi-recumbent position while the left leg is passively heated through a water-perfused cuff. In a separate visit, frozen gel packs were used in an otherwise identical set-up in order to elicit local tissue cooling.

To avoid cold injury to the skin whilst cooling, the frozen gel packs were wrapped in a gauze cover and applied intermittently over the 1 h period for 20 min at a time followed by 10 min recovery. Tissue temperature during the heating protocol was raised via the use of a custom-designed trouser leg connected to a water circulator (Julabo F-34, Seelbach, Germany) and perfused with 50°C water. The trouser leg surrounded the entire leg and foot and was insulated in an identical fashion to the cooling intervention. Throughout both protocols, the legs were rested on a flat surface to prevent muscle activation. Following baseline measurements, blood flow in the common, superficial and profunda femoral arteries of the experimental leg were measured at times points 5, 15, 25, 35, 45, and 55, with control blood flow in the opposing common femoral artery being measured on 30 and 60 min. All other temperature and haemodynamic variables were averaged over 30 s at time points corresponding to the blood flow measurements.

5.2.3 – Temperature measurements

Core temperature was measured via self-insertion of a rectal thermocouple (Physitemp, Clifton, NJ, USA) prior to the start of the trial. Mean skin temperature ($\overline{T}_{sk}$) was measured as described previously. Muscle temperature (T_m) was measured in the mid-portion of the vastus lateralis via the insertion of a tissue implantable thermocouple microprobe (Physitemp T204-A, Clifton, NJ, USA) to a depth of 2-3 cm. Rectal and muscle thermocouples were connected to a 4 channel thermocouple meter (TC- 2000, Sable Systems, Las Vegas, NV, USA) and a data acquisition system (Powerlab 16/S, ADInstruments, UK).

5.2.4 – Haemodynamic measurements

Arterial blood pressure was measured non-invasively by infrared photoplethysmography (Finometer, FMS, Netherlands) via a cuff on the middle finger of the right hand. Heart rate was determined using a 3-lead ECG. Stroke volume was estimated using the ModelFlow method included with the Beatscope computer software package (Beatscope, FMS, Netherlands), with cardiac output calculated as stroke volume x heart rate following corrections for age, height, and weight (Wesseling *et al*, 1993). Common, superficial, and femoral arterial blood flows were measured using duplex Doppler ultrasound equipped with a 10 MHz linear probe (Vivid 7 Dimension, GE Medical, Horton, Norway), and calculated using the equation. Blood Flow ($ml \cdot min^{-1}$) = V_{mean} x π x $(D/2)^2$ x 60: where V_{mean} is time-averaged mean velocity ($cm \cdot s^{-1}$), π is the mathematical constant, D is vessel diameter (cm), and 60 is a conversion factor to convert to $ml \cdot min^{-1}$. Vessel diameter was determined at peak systole from three 2D B-mode images in the longitudinal view at ~70 frames per second. Time-averaged mean blood velocity (V_{mean}) was measured using continuous pulsed-wave Doppler at a frequency of 4.4 MHz, at an insonation angle of 60º and the sample volume extended to cover the entire vessel lumen. Blood velocity was averaged over 3 consecutive 12 s profiles (36 s total). To further characterise the haemodynamic profile of each of the arteries during cooling and heating; mean, antegrade, and retrograde blood flows were also calculated by

substituting their respective mean velocities into the equation. Shear rates were calculated using the formula SR = (4 x V_{mean}) / D: where SR is shear rate, V_{mean} is mean, antegrade or retrograde blood velocity, and D is vessel diameter. Oscillatory shear index was calculated using the equation OSI = (SR_{ret}) / (SR_{ant} + SR_{ret}): where OSI is oscillatory shear index, SR_{ret} is mean retrograde shear rate, and SR_{ant} is mean antegrade shear rate. The OSI calculation provides a dimensionless variable that indicates the relative contribution of antegrade and retrograde flow (and therefore shear) profiles over the duration of a cardiac cycle, and ranges from 0 (pure antegrade flow) to 0.5 (pure oscillatory flow). Leg and systemic vascular conductance were calculated as common femoral arterial blood flow/MAP and $\dot{Q}$/MAP, respectively.

5.2.5 – Data analysis

Blood velocities and diameters were recorded using the techniques described previously, burned to DVD, and analysed on specialist software installed on a stand-alone laptop (EchoPac, GE Medical, UK). All other data were collected via a data acquisition system (Powerlab 16S, AD Instruments, UK) and fed to a desktop computer data acquisition software package (LabChart 5, AD Instruments, UK). All analysed data were stored in a PC-based computer spreadsheet software programme (Excel, Microsoft, US).

5.2.6 – Statistical analysis

A one and two-way repeated measures ANOVA was used to test for differences within and between legs, with Holm-Bonferroni *post-hoc* testing employed to identify the time-points at which changes occurred once a significant overall effect was found. Multiple regression for within-subject repeated measures was used for the analysis of the relationship between blood flow and temperatures (Bland and Altman, 1995). All statistical analyses were carried out using SPSS (Version 20, IBM, Armonk, US) with results expressed as mean ± SEM. Significance is set at $P < 0.05$.

5.3 – Results

5.3.1 – Temperature responses

Core temperature was maintained at ~37 ºC throughout both interventions and remained stable until the end of the experimental protocol. With isolated leg cooling, leg $\overline{T}_{sk}$ decreased from 29.2 ± 0.4 to 20.4 ± 1.4 ºC while T_m decreased from 34.9 ± 0.3 to 29.5 ± 0.6 ºC ($P < 0.05$ for both). In contrast, isolated heating of the leg led to increases in $\overline{T}_{sk}$ and T_m of 31.1 ± 0.4 to 38.9 ± 0.6 °C and 34.5 ± 0.5 to 36.8 ± 0.1 °C, respectively ($P < 0.05$ for both).

5.3.2 – Leg and systemic haemodynamic responses

Isolated leg cooling led to modest decreases in blood flow to all three arteries from the 40 min timepoint to the end of the cooling protocol (192 ± 9 to 155 ± 15, 93 ± 13 to 75 ± 12, and 74 ± 15 to 42 ± 7 ml·min^{-1} or 19%, 19% and 43% in the common, superficial, and profunda femoral arteries, respectively; $P < 0.05$ for all). These effects were exerted solely by a decreased downstream vascular conductance and subsequent reduction in mean blood velocity ($P < 0.05$ for both), as arterial diameters remained unchanged throughout ($P > 0.05$ for all). Isolated leg heating led to significant increases in blood flow to the common (250 ± 21 to 764 ± 75 ml·min^{-1}; 3-fold increase), superficial (129 ± 14 to 458 ± 67 ml·min^{-1}; 3.5-fold increase), and profunda femoral arteries (84 ± 13 to 216 ± 53 ml·min^{-1}; 2.5-fold increase; $P < 0.05$ for all). These responses were also elicited by vascular conductance changes downstream of the site of measurement, as arterial diameters remained unchanged throughout ($P > 0.05$ for all). In the contralateral control leg, no significant changes in flow were observed during either the cooling or heating protocol ($P > 0.05$), although there was a small tendency for blood flow to increase by the end of 1 h of heating (0.40 to 0.45 l·min^{-1}).

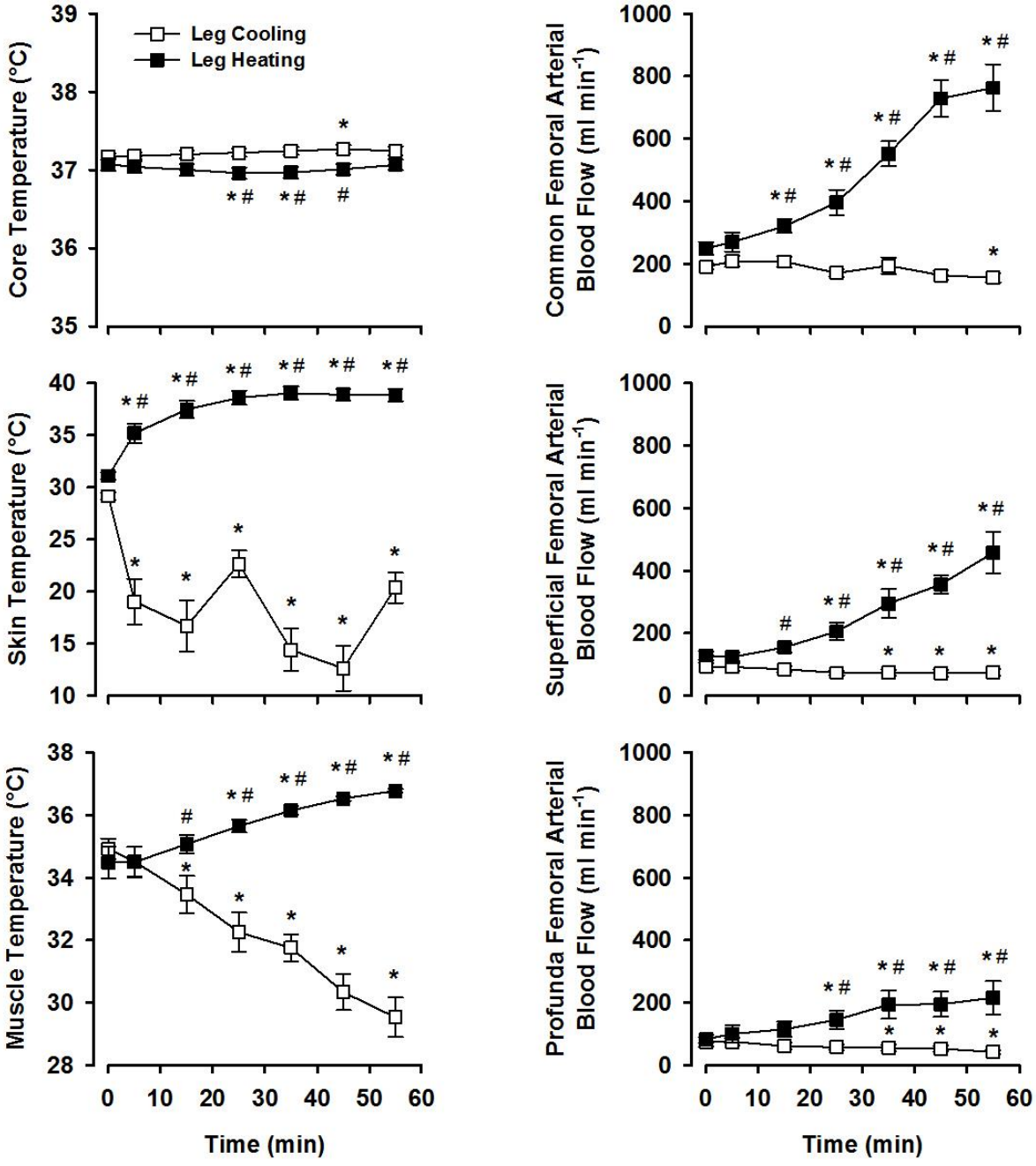

Figure 5.2: Temperature (left) and blood flow (right) responses during 1 h of isolated leg cooling or heating. Data are mean ± SEM for seven subjects. * Significantly different from first 5 min. # Significantly different from cooled leg; $P < 0.05$.

At the systemic level, there were no significant changes in haemodynamic variables during either the cooling or heating protocol, with the exception of small yet significant increase in arterial blood pressure during the cooling protocol.

Table 5.1. Systemic haemodynamic responses to isolated cooling and heating at rest

Variable	Time (min)					
	Cooling			Heating		
	0	30	60	0	30	60
$\dot{Q}$ ($l \cdot min^{-1}$)	5.7± 0.1	5.6± 0.2	6.1± 0.3	6.2± 0.5	6.3± 0.7	6.4± 0.5
HR ($beats \cdot min^{-1}$)	63 ± 4	60 ± 6	65 ± 4	65 ± 8	72 ± 4	72 ± 4
SV (ml)	91 ± 4	93± 4	94± 3	95 ± 5	88 ± 8	89 ± 7
MAP (mmHg)	76 ± 1	87 ± 3*	83 ± 3	79 ± 6	77 ± 5	79 ± 4
SBP (mmHg)	116 ± 2	129 ± 5	130 ± 4*	121 ± 8	107 ± 6	115 ± 4
DBP (mmHg)	56 ± 1	65 ± 4*	62 ± 3*	58 ± 5	55 ± 3	59 ± 4
SVC ($ml \cdot min^{-1} \cdot mmHg^{-1}$)	75± 2	64± 3*	73± 1	78 ± 3	82± 2	81 ± 3

Data represent mean ± SEM for 5 subjects only due to data collection issues associated with signal acquisition during infrared photoplethysmography.. SVC calculated as $\dot{Q}$/MAP. * Significantly different from baseline; $P < 0.05$.

5.3.3 – Relationship between local muscle temperature and leg blood flow

At rest, blood flow displayed a linear relationship with quadriceps muscle temperature in all three arteries during both the cooling and heating interventions, with a stronger relationship during cooling observed in the superficial and profunda femoral arteries (R^2 = 0.41 and 0.45, respectively; $P < 0.01$ for both) than common

femoral artery (R^2 = 0.28; P < 0.01). Heating of the leg resulted in R^2 values of 0.55, 0.53, and 0.38 for common, superficial, and profunda femoral arteries, respectively (P < 0.01 for all). Similar to results from the previous chapter in the human arm, localised heating led to a marked increase in leg blood flow when temperatures were raised above baseline values. The absence of any alterations in systemic haemodynamics and arterial blood pressure resulted in this being associated with changes in local vascular conductance alone (P < 0.05).

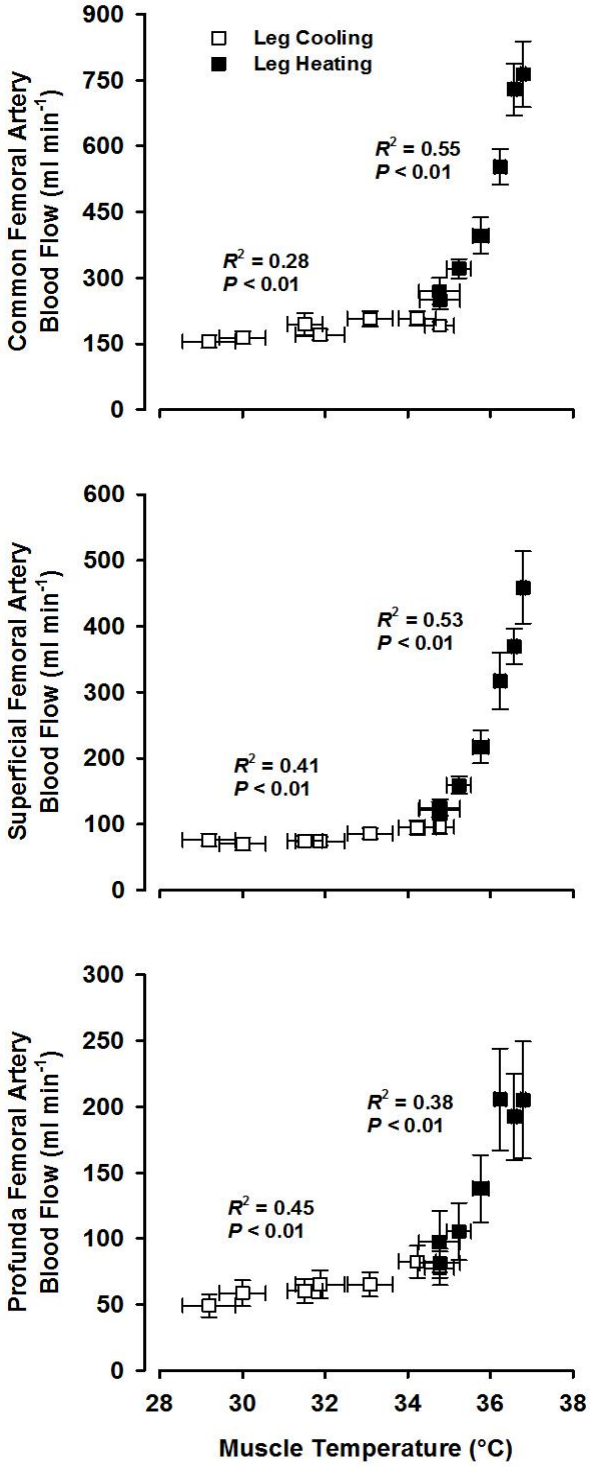

Figure 5.3: Relationship between blood flow and muscle temperature in the common, superficial and profunda femoral arteries during isolated leg cooling and heating. Data are expressed as mean ± SEM for seven subjects. Corresponding R^2 values are obtained from individual multiple regression analyses as described in statistical methodology.. * Significantly different from baseline; $P < 0.05$.

5.3.4 – Flow profile and shear rate responses

No significant changes in mean, antegrade, or retrograde shear were observed following the 1 h of isolated cooling, and therefore oscillatory flow index was not altered ($P > 0.05$ for all). In contrast, mean shear rate significantly increased in all three arteries over the 1 h of isolated heating ($P < 0.05$) due to a combination of both increased antegrade and decreased retrograde shear over the duration of the heating intervention (PFA increased antegrade only). As a result of this altered flow profile, oscillatory shear index significantly decreased from baseline values in the common (0.30 ± 0.02 to 0.10 ± 0.02), superficial (0.34 ± 0.01 to 0.12 ± 0.03), and profunda femoral artery (0.21 ± 0.03 to 0.10 ± 0.01; $P < 0.05$ for all). Despite significantly different flows in each of the arteries at the end of the heating intervention, the presence of different arterial diameters within the three vessels resulted in no significant differences in final heated mean and antegrade shear rates (100 – 130 s^{-1} for all; $P > 0.05$).

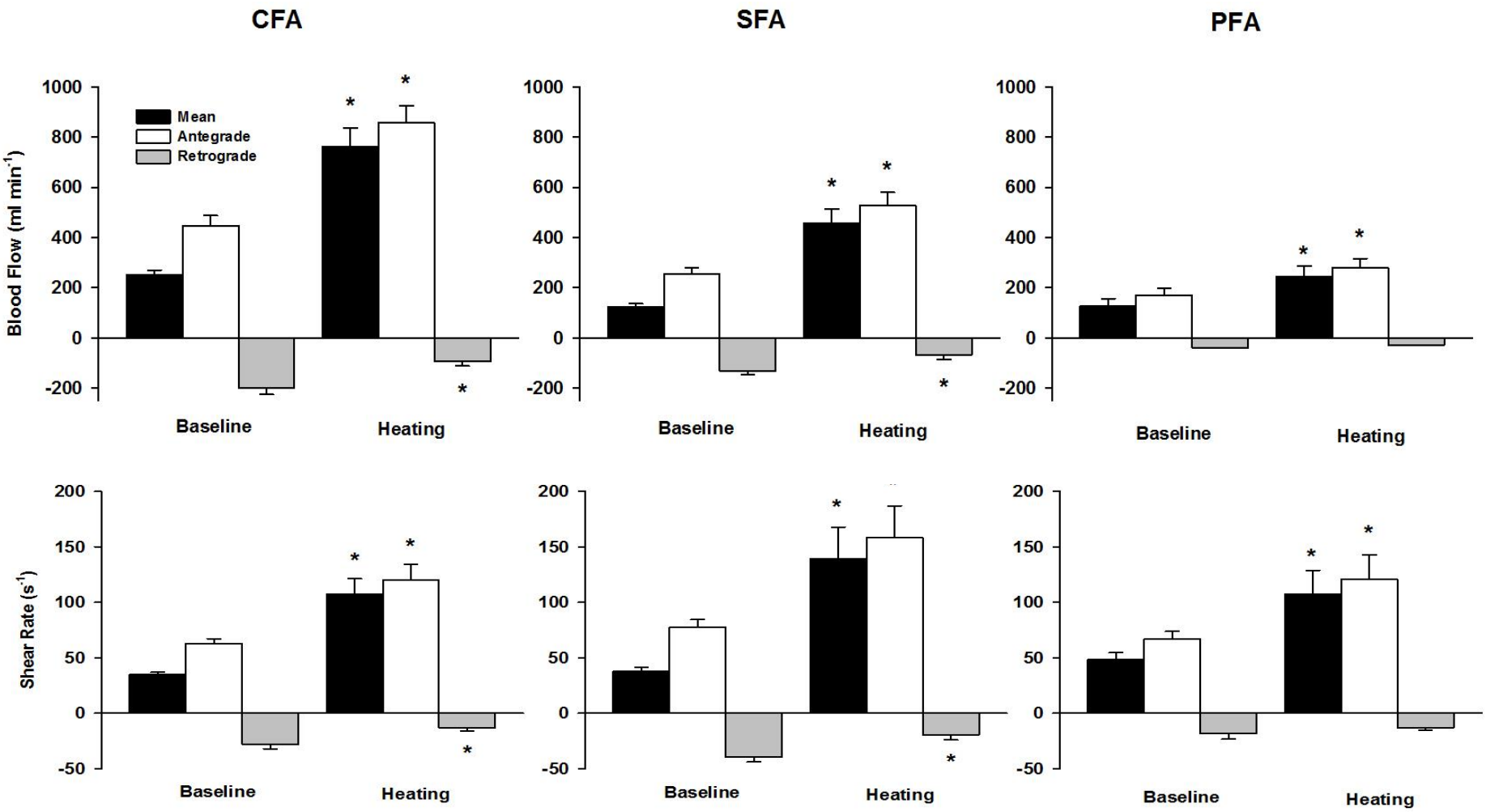

Figure 5.4: Mean, antegrade, and retrograde blood flow (top) and shear rate (bottom) profiles in the common (CFA), superficial (SFA), and profunda (PFA) femoral arteries at baseline and following 1 h of isolated leg heating. Data are mean ± SEM for seven subjects. * Significantly different from baseline; $P < 0.05$.

Table 5.2: Arterial diameters and flow profiles during isolated leg heating

		Time (min)						
		0	5	15	25	35	45	55
Diameter (cm)	CFA	0.83 ± 0.01	0.83 ± 0.02	0.84 ± 0.01	0.83 ± 0.02	0.83 ± 0.01	0.84 ± 0.02	0.84 ± 0.02
	SFA	0.67 ± 0.02	0.66 ± 0.02	0.66 ± 0.02	0.67 ± 0.02	0.67 ± 0.02	0.67 ± 0.02	0.68 ± 0.02
	PFA	0.61 ± 0.04	0.60 ± 0.04	0.60 ± 0.04	0.60 ± 0.04	0.60 ± 0.04	0.60 ± 0.04	0.61 ± 0.04
V_{mean} ($cm \cdot s^{-1}$)	CFA	7.4 ± 0.4	7.9 ± 0.9	9.6 ± 0.6*	11.6 ± 1.3*	16.1 ± 1.0*	20.9 ± 1.7*	22.6 ± 2.7*
	SFA	6.2 ± 0.6	6.1 ± 0.6	7.8 ± 0.6	10.4 ± 1.1*	15.4 ± 2.0*	18.3 ± 1.9*	22.6 ± 3.9*
	PFA	5.0 ± 0.6	6.3 ± 1.5	7.3 ± 1.7*	9.1 ± 1.8*	12.8 ± 2.5*	12.9 ± 2.8*	13.6 ± 3.*3
V_{ant} ($cm \cdot s^{-1}$)	CFA	13.2 ± 0.8	14.0 ± 1.0	14.6 ± 1.0	16.3 ± 1.3*	19.9 ± 1.2*	23.8 ± 1.7*	25.3 ± 2.6*
	SFA	12.7 ± 1.1	12.9 ± 1.2	13.5 ± 1.0	15.9 ± 1.4*	19.7 ± 2.2*	21.1 ± 1.9*	25.9 ± 3.9*
	PFA	9.7 ± 1.0	10.2 ± 1.4	10.5 ± 1.6	13.1 ± 1.7*	15.4 ± 2.6	16.6 ± 2.3	17.0 ± 2.8*
V_{ret} ($cm \cdot s^{-1}$)	CFA	5.9 ± 0.8	6.1 ± 0.8	5.0 ± 0.6	4.7 ± 0.8*	3.7 ± 0.8	2.9 ± 0.6*	2.8 ± 0.6*
	SFA	6.5 ± 0.7	6.8 ± 0.7	5.6 ± 0.8	5.5 ± 1.0	4.3 ± 0.9*	2.8 ± 0.7*	3.2 ± 0.8*
	PFA	2.5 ± 0.5	2.9 ± 0.5	1.6 ± 0.2	2.4 ± 0.3	1.7 ± 0.2	1.8 ± 0.3	1.8 ± 0.2
SR_{mean} (s^{-1})	CFA	34.9 ± 1.9	37.4 ± 4.8	45.7 ± 3.4*	55.0 ± 6.9*	76.2 ± 5.5*	97.3 ± 9.1*	107.1 ± 14.5*
	SFA	37.8 ± 3.7	37.5 ± 4.4	48.2 ± 4.5*	62.7 ± 6.8*	93.6 ± 12.7*	112.4 ± 14.4*	138.9 ± 28.8*
	PFA	35.7 ± 5.5	44.5 ± 10.8	53.8 ± 12.9	65.8 ± 14.0*	90.1 ± 18.9*	94.2 ± 23.6	98.4 ± 25.0*
SR_{ant} (s^{-1})	CFA	62.9 ± 4.3	66.1 ± 5.6	69.6 ± 5.7	76.8 ± 6.9	93.9 ± 6.9*	111.1 ± 9.8*	120.1 ± 14.3*
	SFA	77.6 ± 6.7	79.9 ± 8.8	83.0 ± 7.8	96.2 ± 9.1*	119.9 ± 14.4*	129.6 ± 15.1*	159.4 ± 28.6*
	PFA	66.7 ± 7.1	72.1 ± 10.6	75.0 ± 13.0	93.0 ± 13.5*	108.1 ± 19.7*	118.7 ± 20.9*	120.7 ± 22.1*
SR_{ret} (s^{-1})	CFA	28.0 ± 4.1	28.7 ± 4.0	23.9 ± 3.1	21.8 ± 3.7*	17.7 ± 3.7	13.8 ± 3.2*	13.0 ± 2.8*
	SFA	39.8 ± 4.3	42.4 ± 4.8	34.8 ± 5.0	33.4 ± 5.9	26.3 ± 5.4*	17.2 ± 4.0*	19.5 ± 4.7*
	PFA	18.5 ± 4.7	21.4 ± 4.7	11.5 ± 2.1	17.8 ± 3.2	12.3 ± 2.1	13.4 ± 2.4	13.0 ± 2.3
OSI	CFA	0.30 ± 0.02	0.30 ± 0.02	0.25 ± 0.01*	0.22 ± 0.03*	0.15 ± 0.03*	0.11 ± 0.02*	0.10 ± 0.02*
	SFA	0.34 ± 0.01	0.35 ± 0.01	0.29 ± 0.02*	0.25 ± 0.03*	0.18 ± 0.03*	0.12 ± 0.03*	0.12 ± 0.03*
	PFA	0.21 ± 0.03	0.23 ± 0.03	0.13 ± 0.01*	0.16 ± 0.02	0.14 ± 0.04	0.11 ± 0.03*	0.10 ± 0.01*

Data represent mean ± SEM for 7 subjects. V_{mean}, time-averaged mean velocity; V_{ant}, time-averaged antegrade velocity; V_{ret}, time-average retrograde velocity; SR_{mean}, mean shear rate; SR_{ant}, antegrade shear rate; SR_{ret}, retrograde shear rate; OSI, oscillatory shear index. * Significantly different from first 5 min; $P < 0.05$.

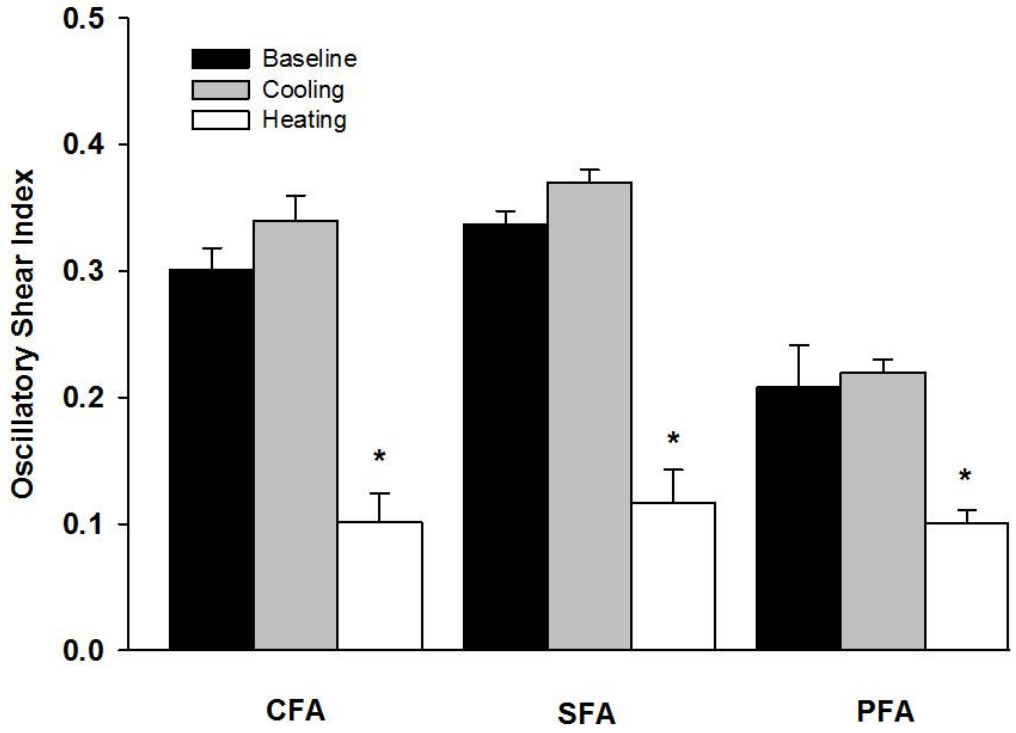

Figure 5.5: Oscillatory shear index in the common (CFA), superficial (SFA), and profunda (PFA) femoral arteries at baseline and following 1 h of isolated leg cooling and heating. Data are mean ± SEM for seven subjects. * Significantly different from baseline; $P < 0.05$.

5.4 – Discussion

This study sought to characterise the magnitude of blood flow changes and their relationship to local temperature changes in the resting human leg during isolated cooling and heating of the limb. Similar to the previous study, changes in whole-limb and skeletal muscle blood flow were associated with changes in local tissue temperature during both local cooling and heating, although with a greater sensitivity when local temperatures were raised above resting baseline values. Novel findings were that 1) one hour of localised whole-limb cooling resulted in no significant decrease in skeletal muscle blood flow within the 20 min time frame commonly quoted for the treatment of soft tissue injuries, with significant decreases only observed following 40 min of cooling 2) in contrast, localised heating led to significant increases in both whole-limb and skeletal muscle blood flow in tandem with a decrease in oscillatory shear index, suggesting a potential use of localised heating as a therapeutic benefit for vascular health. These results expand upon the haemodynamic changes reported in the human arm in the previous study and are necessary due to the potential presence of vascular heterogeneity between the upper and lower limbs.

5.4.1 – Relationship between local muscle temperature and leg blood flow

The presence of essentially unchanged core temperatures and central haemodynamics throughout both protocols ensured that any variations in leg blood flow with isolated leg cooling and heating were associated with local physiological processes and mechanisms sensitive to changes in tissue temperature. Changes in leg blood flow (common femoral artery) were closely related to changes in local tissue temperature during 1 h of both isolated leg cooling and heating. However, similar to responses within the arm in the previous chapter, the sensitivity of the relationship altered dramatically at temperatures above resting baseline values, with a marked rise in leg blood flow occurring at skin and muscle temperatures above 30 and 34.5 °C, respectively.

Isolated cooling of the leg led to a modest decrease in leg blood flow of ~ 19% at the end of the 1 h intervention, equating to an absolute decrease in flow of 35 $ml \cdot min^{-1}$ or 6.5 $ml \cdot min^{-1} \cdot °C^{-1}$. These findings of decreased whole-limb blood flow are in agreement with cooling studies in the forearm (Barcroft and Edholm; 1946), leg (Yanagisawa *et al*, 2007; Gregson *et al*, 2011), and in isolated muscle samples (Thorlacius *et al*, 1998), in which decreases in local tissue temperature have been shown to exert the expected vasoconstrictor effect on the immediate vasculature. We have expanded upon these results by attempting to characterise changes between the deep and superficial tissues of the thigh *in vivo* through the assessment of profunda femoral artery (major supplier of deep tissue of the thigh) and superficial femoral artery (major supplier of thigh skin and lower leg) blood flow changes. Both superficial and profunda femoral arterial blood flows steadily decreased throughout the protocol and were significantly lower by ~ 20 $ml \cdot min^{-1}$ and 25 $ml \cdot min^{-1}$, respectively, at the end of the cooling intervention. This 35% decrease in blood flow supplying thigh skeletal muscle occurred in line with decreasing surrounding muscle tissue temperatures ($R^2 = 0.45$; $P < 0.01$), and are of a similar relative magnitude to that reported in the deep tissues of the arm in Chapter 4 (assuming that increases in a-vO_2 difference during cooling in the prior study occurred due to reciprocal decreases in deep tissue blood flow). This relatively minor absolute decrease in deep tissue blood flow in combination with its slow onset (no change within the first 35 min) raises questions over the contribution of decreased tissue perfusion as a direct therapeutic effect of cooling in deep tissues, with the majority of cooling modalities being implemented for a maximum of 20 min at a time. Whilst the intermittent application of ice packs in the current study resulted in cyclical alterations in leg skin temperature, underlying skeletal muscle temperature steadily decreased throughout the 1 h cooling intervention; a response most likely due to the rewarming of subcutaneous and skin regions from both deep tissue and surrounding environmental heat sources (Enwemeka *et al*, 2002).

A novel observation in the current study was a decrease in skeletal muscle blood flow after 1 h in line with decreases in deep muscle temperatures and independent to changes in overlying superficial temperatures and haemodynamics, providing direct evidence for the efficacy of repeated intermittent ice applications as an effective

method for blood flow reductions in deep muscle tissues. Although previous studies have shown contrasting decreases in perfusion within the commonly-quoted 20 min timeframe, how much of this effect is due to the hydrostatic effect of water immersion (Gregson *et al*, 2011), the weight of ice packs on superficial tissues (Taber *et al*, 1992; Yanagisawa *et al*, 2007), or the superficial site of blood flow measurement (e.g. ankle; Taber *et al*, 1992) remains unclear. Results from this study suggest that the local effects of cold temperatures alone are insufficient to decrease skeletal muscle blood flow in this timeframe in a large muscle volume such as the thigh.

Isolated heating led to increases in blood flow to all three arteries of the leg, with the significant increases observed in the deep tissues of the thigh once again occurring in line with an increase in surrounding muscle temperature measured throughout the 1 h intervention (R^2 = 0.38; P < 0.01). These findings suggest a coupling between regulation of perfusion with progressive changes in local tissue temperature, findings which agree with recent studies reporting increases in leg muscle blood flow during heat stress (Keller *et al*, 2010; Pearson *et al*, 2011), and its regulation through local temperature-sensitive mechanisms alone (Heinonen *et al*, 2011). The preferential distribution of blood flow to the superficial femoral artery (3.5-fold increase) over the profunda femoral artery (2.5-fold increase) displayed a differential magnitude of change in vascular tone in each of these vessels, with a combined increase in vascular conductance in the skin and deep tissues supplied by the superficial femoral artery most likely responsible for a greater demand in blood flow than the deep tissues supplied the profunda femoral artery alone. In contrast to vascular control in the arm, however, these changes were solely due to alterations in conductance downstream from the major conduit arteries, as all three arterial diameters remained unchanged throughout. This finding is unsurprising in the common femoral artery given that arterial diameter in this particular vessel has been shown to remain unchanged even during the high flow and shear conditions during high-intensity single-legged exercise (Rådegran, 1997). However, an increased blood flow following arterial occlusion has been shown in numerous studies to cause a nitric-oxide dependent flow-mediated dilation in the superficial femoral artery (Gaenzer *et al*, 2001; Kooijman *et al*, 2008); a response which may have also been expected here

given the significant increases in flow during heating. With post-ischaemic hyperaemia producing transient peak increases in superficial femoral artery flow up to 3 fold higher than recorded here, it is possible that the level of heating achieved was of an insufficient stimulus to lead to arterial dilation in the conduit artery itself.

5.4.2 – Effect of local cooling and heating on blood flow profiles and shear rates

Whilst increased antegrade blood flows and shear rates previously been shown to have long-term benefits for vascular function and health (Tinken *et al*, 2009; Tinken *et al*, 2010), the presence of retrograde blood flows and the subsequent oscillatory shear stress they place on the endothelium have been indicated as a stimulus for vascular dysfunction and pro-atherogenic conditions in both the brachial (Thijssen *et al*, 2009) and superficial femoral arteries (Schreuder *et al*, 2014). Increased temperatures in the human forearm have recently been shown to significantly attenuate retrograde and oscillatory shear rates within the brachial artery through increases in downstream vasodilation and vascular conductance (Simmons *et al*, 2011; Padilla *et al*, 2011), potentially providing implications for local heating as a mechanism for improving vascular health. No studies to date, however, have investigated the effects of local tissue temperature changes on flow profiles in the resting leg, which is surprising given that blood flow in the lower limbs exhibits a lower resting shear rate (Wu *et al*, 2004; Newcomer *et al*, 2008), higher retrograde flow component and oscillatory shear stress (Newcomer *et al*, 2008; Schreuder *et al*, 2014), and higher incidence of vascular dysfunction than that recorded in the arm (Kroger *et al*, 1999).

No significant changes were observed in antegrade or retrograde shear rates in any artery during 1 h of isolated cooling in the present study, and therefore oscillatory shear index remained unchanged (~ 0.30; $P < 0.05$). In contrast, we show here for the first time that 1 h of localised leg heating increases mean shear rate, decreases retrograde flow and shear, and attenuates oscillatory shear index in all three arteries

of the leg to levels similar to that present in the atherosclerotic-resistant resting human arm. With low shear rates and high oscillatory flow potentially implicated in the presence of endothelial dysfunction and development of atherosclerosis (Caro *et al*, 1969), haemodynamic changes resulting from local heating may potentially prove beneficial in improving vascular health in clinically-relevant populations. Recent research showing the ability of local heat stress to attenuate decreases in FMD following lower-body negative pressure further support this possibility (Thijssen *et al*, 2014), but more research is required at both an acute and chronic level to provide further understanding of any potential benefits.

5.4.3 – Limitations

Blood flow through the profunda femoral artery was used in the present study to attempt to characterise tissue perfusion in the deep tissues of the thigh alone. Despite the vast majority of flow through this vessel supplying this vascular bed, there exists the presence of small tributary arteries supplying the skin of the back of the thigh that may have affected current results (Hupkens *et al*, 2013). Although it is not possible to completely discount the fact that some of the blood flow changes in this vessel were in fact supplying the skin, a number of observations suggest that these changes were minimal. During cooling, the rotation of ice packs resulted in cyclical variations in skin temperature (and presumably skin blood flow) over the 1 h intervention, but a continual steady decrease in muscle temperature. In line with these responses, the strong correlation between muscle temperature and profunda femoral arterial flow (R^2 = 0.45) was attenuated somewhat in the common and superficial femoral arteries, potentially due changes in skin blood flow supplied by these vessels. During heating, the compressive effect of the weight of the leg on vessels supplying the back of the thigh has been shown to limit skin blood flow in the region supplied by profunda femoral tributaries (Heinonen *et al*, 2011), further suggesting a minimal contribution to the observed increases in profunda femoral artery flow.

While results from this study have characterised blood flow responses to local temperature changes in young healthy males, and suggested that local heating could have potential benefits for vascular health; further interventions are required to 1) assess whether the magnitude of change observed here is sufficient to elicit either acute or chronic adaptations within the vasculature, and 2) to assess whether these responses are also present within clinically-relevant/aged populations.

5.4.4 – Conclusions

Leg tissue blood flow is closely related to changes in local tissue temperature during both cooling and heating, revealing the presence of local temperature-sensitive mechanisms in its regulation. Absolute decreases in deep tissue blood flow during cooling appear minimal and show no significant differences within the first 35 min, raising questions as to the effectiveness of ice pack treatment on decreasing deep tissue perfusion following musculoskeletal injury. Isolated heating leads to significant increases in flow and shear rates to all three arteries supplying the leg, although with a preferential distribution to the superficial over the profunda femoral artery. These changes in flow are accompanied by a significant alteration in oscillatory shear profiles due to a combination of increases in antegrade flow (all arteries) and accompanying decreases in retrograde flow (common and superficial femoral arteries only). These results suggest that isolated leg heating may have the potential to provide multiple vascular benefits through both an increased tissue perfusion and the establishment of anti-atherogenic flow profiles.

CHAPTER 6

Study 3: Local temperature-sensitive mechanisms, independent of systemic responses, mediate increases in limb tissue perfusion in the moderately heat-stressed human at rest and during single leg exercise

6.0 – Abstract

Limb tissue and systemic blood flow increase with heat stress in resting and exercising humans, but the underlying mechanisms remain poorly understood. Here, we tested the hypothesis that heat-stress mediated increases in limb tissue blood flow, including that to skeletal muscle, are primarily mediated by local temperature-sensitive mechanisms. Leg and systemic temperatures and haemodynamics were measured at rest and during incremental single-legged knee extensor exercise in 15 male participants exposed to 1 h of either systemic passive heat stress with simultaneous cooling of a single leg (n = 8) or isolated leg heating or cooling (n = 7). Systemic passive heat stress increased core, skin and heated leg blood (T_b) temperatures, cardiac output and heated leg blood flow (0.6 ± 0.1 l·min^{-1} in common femoral artery), the latter due partly to downstream increases in profunda femoral arterial flow (0.13 ± 0.02 l·min^{-1}; all $P < 0.05$). In the cooled leg, however, leg tissue blood flow remained unchanged throughout ($P > 0.05$). Increased profunda femoral arterial flow was closely related to T_b ($R^2 = 0.50$; $P < 0.01$), which is partly attributed to increases in tissue $\dot{V}O_2$ ($R^2 = 0.55$; $P < 0.01$). During isolated limb heating and cooling, leg blood flows were equivalent to those found during systemic heat stress ($P > 0.05$), despite unchanged systemic temperatures and haemodynamics. During incremental exercise, heated leg blood flow was consistently maintained ~ 0.6 l·min^{-1} higher than that in the cooled leg ($P < 0.01$), with blood flow and vascular conductance in both legs showing a strong correlation with their respective local blood temperature ($R^2 = 0.77$ and 0.63, $P < 0.05$). We conclude that local temperature-sensitive mechanisms, independent of systemic temperature and haemodynamic responses, directly influence limb tissue blood flow regulation both at rest and during small-muscle mass exercise in the heat-stressed human. Furthermore, increases in perfusion in hyperthermic skeletal muscle may be in part mediated through local metabolic vasodilatation.

6.1 – Introduction

Upon exposure to acute heat stress, numerous cardiovascular adjustments occur in the human body in order to redistribute blood from the core to the peripheral tissues, thereby aiding heat dissipation to the surrounding environment. At a systemic level, the increased demand for blood flow to the skin and outer extremities to dissipate heat is predominantly achieved through significant increases in cardiac output from ~ 6 $l \cdot min^{-1}$ to values as high as 12 $l \cdot min^{-1}$ (Rowell *et al*, 1969; Rowell *et al*, 1970; Minson *et al*, 1998; Stöhr *et al*, 2011; Pearson *et al*, 2011). These increases in cardiac output are primarily mediated through a significantly increased heart rate, with stroke volume remaining relatively stable (Rowell *et al*, 1969; Minson *et al*, 1998) due to an augmented ejection fraction (Stöhr *et al*, 2011) in the face of reduced central blood volume (Crandall *et al*, 2008), end-diastolic volume (Stöhr *et al*, 2011) and mean arterial pressure (Rowell *et al*, 1969; Minson *et al*, 1998). At the peripheral level, increased tissue perfusion in the extremities is well-documented and has been shown in the forearm (Edholm *et al*, 1956; Roddie *et al*, 1956; Detry *et al*, 1972; Johnson *et al*, 1976; Black *et al*, 2008; Green *et al*, 2010; Carter *et al*, 2014), leg (Keller *et al*, 2010; Pearson *et al*, 2011; Heinonen *et al*, 2011), and head (Ogoh *et al*, 2013). It remains unknown, however, whether the observed increases in cardiac output drives the increased perfusion evident in the extremities, or whether tissue blood flow responses to heat stress are determined by local temperature-sensitive mechanisms.

Numerous recent studies have provided compelling evidence that changes in peripheral tissue perfusion can be controlled by local regulatory mechanisms both at rest and during exercise. Femoral arterial infusions of the potent vasodilator ATP in the resting human leg have been shown to cause equivalent increases in cardiac output, leg blood flow and leg oxygen delivery to those achieved during single-legged exercise, despite an unchanged central venous pressure and lack of muscle metabo- and mechanoreflex stimuli (González-Alonso *et al*, 2008). Additionally, studies using right atrial-pacing to manipulate the heart-rate response to exercise in dogs (Guyton *et al*, 1962; Guyton, 1968; Shepherd *et al*, 1973) and more recently humans (Bada *et al*, 2012; Munch *et al*, 2014) have shown that the tight coupling

between oxygen demand and blood flow in skeletal muscle during exercise is chiefly regulated at a peripheral level through local metabolic-sensitive mechanisms, with systemic adjustments such as the exercise-induced increase in heart rate occurring as a secondary response in order to regulate cardiac output. Whilst these studies provide strong evidence of local haemodynamic and metabolic regulation of both peripheral and systemic blood flow, it remains to be determined whether thermoregulatory control of tissue perfusion is similarly regulated through local temperature-sensitive mechanisms during thermal homeostatic challenges. If true the combination of metabolic and temperature stimuli during the additive stresses of exercise and heat stress should result in an elevated leg blood flow to satisfy the demands for both metabolism and thermoregulation. The previous chapter of this thesis (Chapter 5) has shown increases in leg blood flow of up to 0.6 $l \cdot min^{-1}$ when exposed to localised heat stress in resting conditions. Despite this significantly increased flow at rest, numerous studies have found no changes between normothermia and hyperthermia when performing single-leg knee extensions (Savard *et al*, 1988; Ferguson *et al*, 2006), two-legged cycling (Savard *et al*, 1988) or uphill walking (Nielsen *et al*, 1990); whilst intense exercise in the heat with the added stress of dehydration causes the opposite effect with a decreased flow to the limb (González-Alonso *et al*, 1998). In contrast, more recent work from our laboratory has shown an elevated leg blood flow when performing single-legged knee extensions in the heat at a mild exercise intensity (Pearson *et al*, 2011). However, whether these increases were due to a combination of local and systemic haemodynamic alterations and whether the response is maintained at higher exercise intensities remains to be determined.

Within the peripheral circulation, the distribution of blood and its regulation within the different tissues of the limb remain poorly characterised and understood. With early research using a forearm model demonstrating that the hyperaemic response to heat stress was confined to the circulation of the skin alone (Edholm *et al*, 1956; Roddie *et al*, 1956; Detry *et al*, 1972), changes in skin blood flow are often assumed to represent whole-limb blood flow (Minson, 2010). Recent research in the lower limb, however, strongly supports an increase in skeletal muscle blood flow contributing to the hyperaemic response to heat stress (Keller *et al*, 2010; Pearson *et al*, 2011; Heinonen *et al*, 2011), although as yet no quantification of absolute flow

rates to this tissue have been reported. Unlike skin blood flow, which has been shown to increase in the arm during indirect heating of the lower-body (Edholm *et al*, 1956), skeletal muscle tissue in the leg appears to respond solely to direct heating and be closely linked to increases in local tissue temperature (Pearson *et al*, 2011; Heinonen *et al*, 2011). Therefore, the relative contributions of local vs. central factors to the overall regulation of whole-leg blood flow during exposure to heat stress (i.e. inclusive of all tissues as opposed to simply local sites on the skin) is still unresolved, as is the distribution of blood to different vascular beds within the limb.

The aims of this study, therefore, were three-fold. Firstly, by using two separate within-subjects contralateral limb models, we sought to identify the contribution of peripheral vs. central thermosensitive mechanisms in the control of limb blood flow during heat stress by systematically altering leg tissue temperatures under conditions of both systemic heat stress and normothermia. Secondly, we aimed to assess the effect of limb temperature on leg blood flow during one-legged knee-extensor exercise up to near maximal power output, and finally, to quantify absolute blood flow to the muscular tissue of the thigh in order to establish its contribution to whole-limb blood flow under conditions of heat stress. We hypothesised that 1) limb blood flow would be primarily regulated at a peripheral level, and that this response would be closely coupled to increases in local tissue and/or blood temperatures, 2) increased local temperatures would result in elevated leg blood flow throughout incremental single-legged exercise to near maximal power output, and 3) an increase in skeletal muscle blood flow, beyond that of skin blood flow, would contribute to the observed hyperaemic response to heat stress.

6.2 – Methods

6.2.1 – Ethical approval

Informed written consent was obtained from each participant prior to commencing the study. All procedures were approved by the Brunel University London Research Ethics Committee (RE04-11) and conformed to the *Declaration of Helsinki.*

6.2.2 – Participants

Fifteen healthy males (age 23 ± 4 years; height 177 ± 4 cm; weight 73 ± 7 kg) were recruited to participate in two studies. Participants abstained from alcohol, caffeine, and strenuous exercise in the 24 h leading up to the day of testing.

6.2.3 – Experimental protocols

Two separate studies were conducted to investigate the role of local vs. systemic effects of heat stress on lower limb tissue blood flow, its distribution and potential underlying mechanisms at rest and during exercise. In study 1, leg and systemic haemodynamic responses, temperatures, and blood variables were measured throughout 1 h of passive whole-body heat stress with single leg cooling before subsequent incremental single-leg knee extensor exercise was performed with both the cooled and heated legs (n=8) (Fig. 6.1). Participants were passively heated through the use of a custom-built suit perfused with 50 °C water and fitted to the entire upper body and right (heated) leg. Blood and tissue temperatures of the contralateral (cooled) leg were prevented from increasing via the application of frozen gel packs before being wrapped in an insulating blanket. Following the 1 h intervention, incremental single-leg knee extensor exercise (3 min stages) was performed with the cooled leg at 20, 40, 60, and 80% peak power output (65 ± 3 W;

determined during an earlier visit and identical for both left and right legs). Exercise was carried out on a custom-built modified Monark ergometer, with power outputs controlled within 6 W via an increased resistance on the flywheel following the application of metal weights. Following a 20 min rest period, the exercise protocol was then repeated with the heated leg at the same exercise intensities. In a follow up study (Study 2; n=7), the isolated effects of limb heating and cooling were investigated by measuring leg and systemic haemodynamic and temperature responses in two separate visits involving 1 h of either isolated leg heating or cooling (Fig. 6.1). Following each intervention, single-leg knee extensor exercise was carried out as previously described for Study 1. Each laboratory visit for Study 2 was separated by at least one week and the order of heating and cooling was counterbalanced among participants.

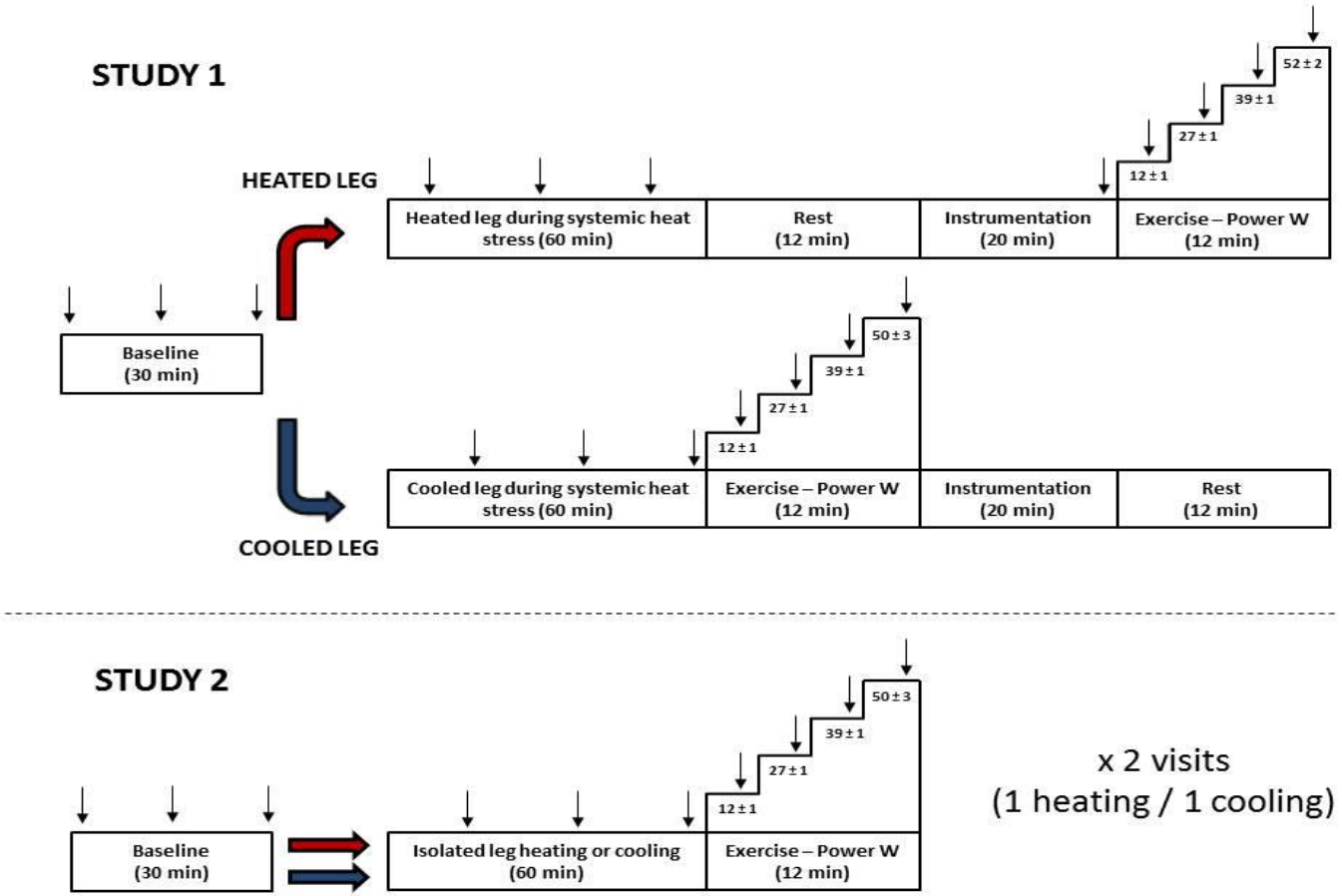

Figure 6.1. Sequence of the experimental protocol. In study 1, participants were exposed to 1 h of passive whole-body heating through the use of a water-perfused suit, following 30 min of resting baseline measurements. The suit was designed to cover the entire body with the exception of the left leg, which was surrounded with frozen gel packs in order to cause isolated cooling of the limb. Immediately after the 1 h resting intervention, single-legged incremental knee-extensor exercise was carried out with either the cooled or heated limb, with each exercise protocol separated by at least 20 min. In study 2, participants visited the laboratory on two occasions in order to have a single leg heated or cooled for 1 h, followed by an identical exercise bout to that carried out in study 1. The order of heating and cooling was counterbalanced between visits. Arrows denote timing of measurements.

6.2.4 – Instrumentation of participants

In Study 1, participants reported to the laboratory at 8 am following ingestion of their usual breakfast. Upon arrival, participants rested in the supine position to allow the ultrasound-guided placement of one double-lumen femoral intravenous catheter into each leg (Double Lumen Catheter, 18 gauge, 16 cm; Multi-Med M2716HE, Edwards Lifesciences, USA) and one radial intra-arterial catheter into the right wrist under local anaesthesia (1% Lidocaine). Both femoral venous catheters were inserted approximately 1-2 cm distal to the inguinal ligament and advanced in a retrograde direction (15 cm) to reside in the deep portion of the femoral vein. Following successful placement, a fine-wire tissue implantable thermocouple (PhysiTemp T-204A, Clifton, NJ, US) was advanced through the distal lumen of both femoral catheters in order to measure deep blood temperature within the femoral vein. Participants were then moved to the main experimental laboratory and seated in a semi-recumbent position on the single-leg knee extensor ergometer (modified Monark ergometer; custom-built) with their legs rested on a table in front. Participants were fitted with a water-perfused suit (pipe diameter 5mm and 9mm for jacket and legs, respectively) for the manipulation of body temperature, designed to cover the entire upper-body and right leg of the participant with the left leg remaining exposed to allow the application of ice packs for isolated limb cooling. The suit was connected to a thermostatically-controlled water circulator (Julabo F-34, Seelbach, Germany) to allow the constant perfusion of 50 °C water (rate 0.9 $l.min^{-1}$) throughout the experimental protocol. Ice packs (KoolPak, Warwickshire, UK) were secured to the cooled leg with Velcro strapping, with each pack being replaced after 30 min to prevent increases in leg temperature. Participants were permitted to drink *ad libitum* throughout the protocol to prevent any confounding factors caused by dehydration. In Study 2, participants were seated on the knee-extensor ergometer in ambient conditions (20-22 °C) and exposed to 1 h of isolated leg heating (single water-perfused leg cuff as mentioned before) or leg cooling (ice packs), with the contralateral leg acting as a control. In both studies, participants wore the water-perfused suit/leg cuff and ice-packs throughout the entire duration of both rest and exercise protocols. Core, muscle (measured by fine-wire thermocouple inserted 2-3 cm into the vastus lateralis), and skin temperatures; haemodynamic

responses; and blood and plasma parameters were measured according to the procedures described below.

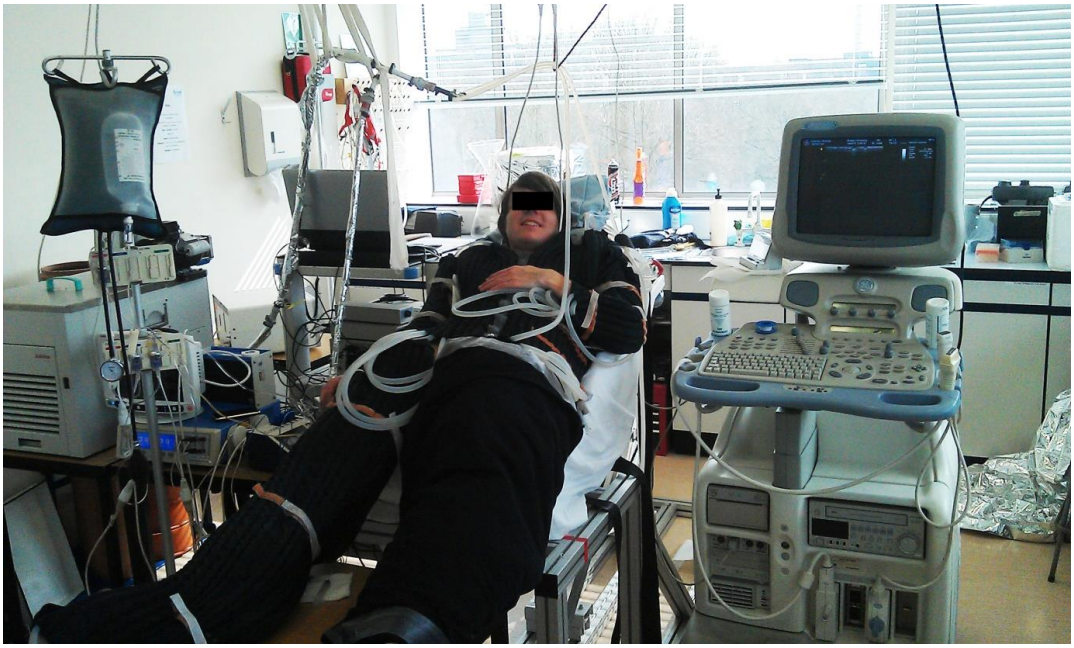

Figure 6.2. Experimental set-up showing participant resting in a semi-recumbent position on the knee-extensor ergometer while passively heated through a water-perfused suit. The left leg is surrounded in frozen gel packs and covered in an insulting blanket to prevent increases in local tissue temperature.

6.2.5 – Temperature measurements

Core temperature (T_c) was measured via the ingestion of a wireless telemetry pill (HQInc, Palmetto, FL, USA; Study 1) or the self-insertion of a rectal thermocouple 15 cm beyond the anal sphincter (PhysiTemp, Clifton, NJ, US; Study 2). Mean body skin temperature ($\overline{T}_{sk}$) was calculated as a weighted mean using wireless temperature sensors (iButtons, Maxim, CA, US) attached to the arm, chest, thigh, and calf (Fig. 3.13); with relative contributions calculated according to the formula $\overline{T}_{sk} = 0.3(T_{chest} + T_{arm}) + 0.2(T_{thigh} + T_{calf})$ (Ramanathan, 1964). Blood temperature (T_b) in Study 1 was measured using the fine-wire thermocouples mentioned previously. Methodological limitations prevented the measurements of T_b in Study 2 due to the

invasive nature of the measurement technique. However, measurements of muscle temperature (T_m) obtained at a tissue depth of 2-3 cm allowed the assessment of the relationship between local tissue temperatures and blood flow. Leg skin temperature ($T_{sk\ leg}$) was recorded via type-t thermocouples (PhysiTemp, Clifton, NJ, USA) and calculated as the average of thigh and calf measurements. All temperature inputs were fed through a thermocouple meter (TC-2000, Sable Systems, US) for continuous measurement throughout the protocol.

6.2.6 – Haemodynamic measurements

Leg blood flow (LBF) was measured both at rest and during single-legged exercise in the common femoral artery (CFA) using a duplex Doppler ultrasound device (Vivid 7 Dimension, GE Medical, Horton, Norway) with a 10 MHz linear array transducer probe (GE Medical Systems, UK). All measurements were taken at least 2 cm above the bifurcation into the superficial and profunda femoral arteries in order to minimise disruptions to measurements due to turbulent flow. Blood flow through the vessel was calculated as the product of the average arterial cross-sectional area obtained from three 2D B-mode images and the mean velocity averaged over three 12 s Doppler scans (36 s total). Arterial diameter was consistently measured at peak systole (Rådegran, 1999), identified by an overlaid ECG trace. LBF was calculated in $ml \cdot min^{-1}$ using the equation: $LBF = V_{mean} \times \pi \times (D/2)^2 \times 60$ where V_{mean} is the time-averaged mean velocity of the blood expressed as $cm \cdot s^{-1}$, π is a mathematical constant, D is the diameter of the vessel in cm, and 60 is a constant employed to convert the units to $ml \cdot min^{-1}$. During the resting protocol, blood flow was also measured in both the superficial and profunda femoral arteries (SFA and PFA, respectively) to characterise the distribution of flow to different portions of the leg.

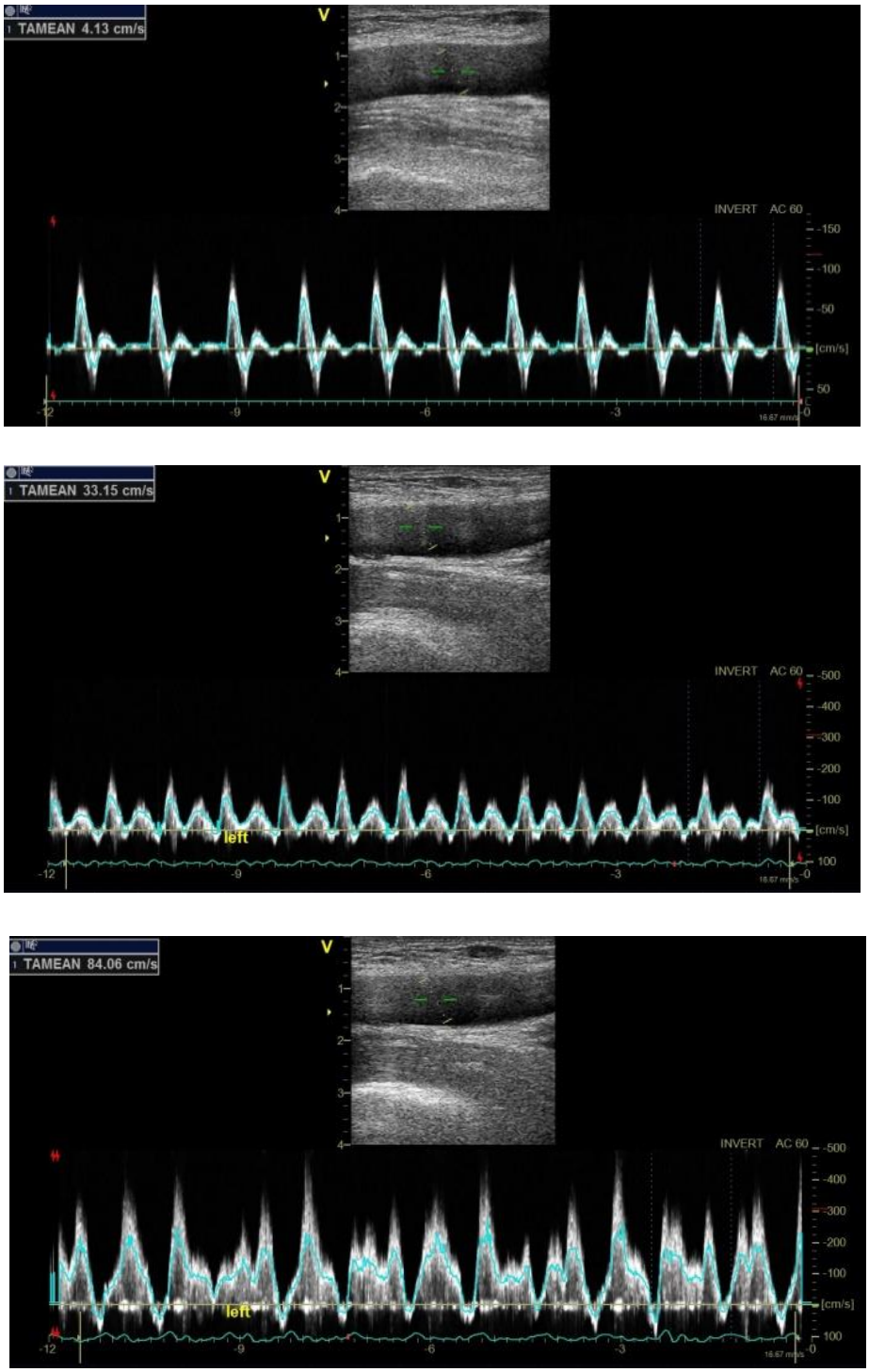

Figure 6.3. Typical common femoral artery Doppler ultrasound traces at rest (top) and during single-legged knee extension exercise at 20% (middle) and 80% (bottom) peak power output.

Changes in skin blood flow (SkBF) during resting conditions were assessed non-invasively using laser Doppler flowmetry (Periflux 4001, Jarfalla, Sweden) via a 780 nm wavelength single-point laser Doppler probe (408, Periflux, Jarfalla, Sweden) fastened securely above the vastus lateralis muscle of each leg. In Study 1, mean arterial and femoral venous pressures (MAP and FVP, respectively) were measured directly from the radial and femoral venous catheters using pressure transducers at the level of the heart and leg (Pressure Monitoring Set, Edwards LifeSciences, Germany) connected to two amplifiers (BPAmp, ADInstruments, Oxford, UK) and fed to a data acquisition system (PowerLab 16/30, AdInstruments, Oxford, UK), allowing perfusion pressure of the leg to be calculated. MAP in Study 2 was measured non-invasively using infrared photoplethysmography (Finometer, FMS, Netherlands).

In both studies, cardiac output ($\dot{Q}$) was calculated as heart rate x stroke volume, with stroke volume estimated using the ModelFlow method (Beatscope, FMS, Netherlands) following corrections for participants' age, sex, mass and height (Wesseling *et al*, 1993). Leg and systemic vascular conductances were calculated as CFA/perfusion pressure and $\dot{Q}$/MAP, respectively. Leg O_2 delivery to each leg was calculated as LBF x arterial O_2 content, while leg a-vO_2 difference was calculated for both heated and cooled legs using the difference between arterial O_2 content and heated and cooled femoral venous O_2 contents, respectively. Due to the positioning of the sampling catheter beyond the saphenofemoral junction, whole-leg $\dot{V}O_2$ was calculated using the following modified two-component Fick equation: whole-leg $\dot{V}O_2$ = [(LBF-GSVBF) x (a-v_{fdeep} O_2 difference)] + [GSVBF x (a-v_{sk} O_2 difference)], where LBF is whole-leg blood flow, GSVBF is great saphenous vein blood flow (estimated using comparable data; Chiesa *et al*, unpublished), a-v_{fdeep} O_2 difference is the difference between radial arterial and deep femoral O_2 content, and a-v_{sk} O_2 difference is the difference between radial arterial and great saphenous vein oxygen content (the latter of which was calculated using [Hb] and PO_2 measurements from the present study combined with estimates of superficial venous oxygen saturation from a previous study (Detry *et al*, 1972).

6.2.7 - Blood parameters

Arterial and femoral venous blood samples (1 ml each) were drawn into pre-heparinised syringes and analysed immediately for blood gas variables, haemoglobin, electrolytes, lactate, and glucose (ABL 800 FLEX, Radiometer, Copenhagen, Denmark) with values corrected to blood temperatures measured simultaneously from the site of sampling in each vessel and the analyser calibrated at regular intervals in accordance with manufacturer guidelines. Additional arterial and femoral venous blood samples from both legs were collected in 2 ml syringes and transferred to EDTA tubes, centrifuged and separated. Plasma adrenaline and noradrenaline were subsequently determined using an enzyme-linked immunoassay kit (DEE6500 2-CAT, Demeditec Diagnostics GmbH, Kiel, Germany).

6.2.8 – Statistical analysis

A one- and two-way repeated measures ANOVA was used to test for differences within and between legs, with Holm-Bonferroni *post-hoc* testing employed to identify the time-points at which changes occurred once a significant effect was found. Differences between studies were assessed using an independent samples two-way ANOVA with similar *post-hoc* testing. Multiple regression for within-subject repeated measures was used for the analysis of the relationship between blood flow and blood gas variables and temperatures (Bland and Altman, 1995). All statistical analyses were carried out using SPSS (Version 20, IBM, Armonk, US) with results expressed as mean ± SEM. Significance is set at $P < 0.05$.

6.3 – Results

6.3.1 – Haemodynamic responses to altered leg temperature during systemic heat stress

Resting responses

Full temperature responses are shown in Table 6.1. Systemic heating resulted in a 0.5 ± 0.1 °C increase in T_c over the 1 h resting intervention, with an associated rapid and significant increase in $\overline{T}_{sk}$ from 32 to ~ 38 °C. Heated leg $\overline{T}_{sk}$ was significantly increased throughout to ~38 °C, whilst the corresponding T_b steadily increased from 36.3 ± 0.3 to 37.4 ± 0.3 °C; $P < 0.05$; Fig. 6.4D) over 1 h. In contrast, cooled leg $\overline{T}_{sk}$ decreased rapidly from 29 to ~ 17 °C; $P < 0.05$, and remained at this level for the duration of the test, whilst T_b also decreased to a level significantly below baseline (36.5 ± 0.2 to 35.7 ± 0.4 °C; $P < 0.05$). Consequently, $\overline{T}_{sk}$ and T_b were significantly higher in the heated than the cooled leg from 40 to 60 min (~ 12 and 1.7 °C higher, respectively; $P < 0.05$).

Table 6.1. Temperature and haemodynamic responses to systemic and leg heating combined with leg cooling at rest and during exercise

	Systemic and Leg Heating with Single Leg Cooling		Exercise Cooled Leg		Exercise Heated Leg	
Systemic Variables	Start	End	Start	End	Start	End
T_c (°C)	37.2 ± 0.1	37.7 ± 0.1*	37.7 ± 0.1	37.8 ± 0.1	37.9 ± 0.1	38.0 ± 0.1
$\overline{T}_{sk}$ (°C)	32.0 ± 0.2	38.6 ± 0.2*	38.4 ± 0.1	38.3 ± 0.2	37.5 ± 0.1	37.6 ± 0.1
Heart Rate (beats min^{-1})	69 ± 3	94 ± 4*	91 ± 4	150 ± 9*	93 ± 5	149 ± 9*
Stroke Volume (ml)	100 ± 7	90 ± 7	85 ± 7	86 ± 8	81 ± 7	88 ± 6
SBP (mmHg)	164 ± 9	132 ± 6*	134 ± 6	219 ± 10*	133 ± 10	196 ± 11*
DBP (mmHg)	78 ± 2	71 ± 2*	73 ± 4	102 ± 5*	72 ± 6	89 ± 4*
Leg Variables						
$\overline{T}_{sk}$ (°C)						
Heated Leg	28.9 ± 0.9	38.2 ± 0.9*#	-	-	36.3 ± 0.5	36.3 ± 0.6#
Cooled Leg	29.1 ± 0.3	17.3 ± 0.2*	19.9 ± 1.4	23.8 ± 0.6*	-	-
T_b (°C)						
Heated Leg	36.3 ± 0.3	37.4 ± 0.3*#	-	-	37.4 ± 0.2	37.7 ± 0.2*#
Cooled Leg	36.5 ± 0.2	35.7 ± 0.4*	35.7 ± 0.4	36.3 ± 0.4*	-	-
SkBF (AU)						
Heated Leg	8 ± 2	69 ± 11*#	-	-	-	-
Cooled Leg	9 ± 2	25 ± 8	-	-	-	-

Values are mean ± SEM for eight participants for all variables except heart rate, stroke volume (n=7) and core and blood temperatures (n = 6). Missing values are due to equipment failures or catheterisation issues prior to the experiment. * Significantly different from baseline. # Significantly different from cooled leg; $P < 0.05$.

In the heated leg, blood flow through the common femoral artery showed a steady and significant increase throughout the duration of the 1 h intervention period (Fig. 6.4A) and in line with increases in femoral venous blood temperature draining the leg (R^2 = 0.74: P < 0.01; Fig. 6.7A), with final values being ~ 3-fold higher than baseline (0.30 ± 0.03 to 0.88 ± 0.08 l·min^{-1}; P < 0.01). Similarly, blood flow through the superficial and profunda femoral arteries also displayed significant increases throughout, with the magnitude of increase being greater to the superficial artery (4.5-fold increase; 0.12 ± 0.01 to 0.54 ± 0.06 l·min^{-1}; Fig. 6.4B) compared to the profunda artery (2-fold increase; 0.11 ± 0.02 to 0.24 ± 0.03 l·min^{-1}; Fig. 6.4C). In the isolated cooled leg, no significant changes in flow were observed in any artery at the end of the 1 h intervention (P > 0.05). SkBF in the heated leg increased from 8 ± 2 to 69 ± 11 AU (P < 0.01), whilst in the cooled leg values were not significantly different from baseline (final value 25 ± 8 AU; P > 0.05).

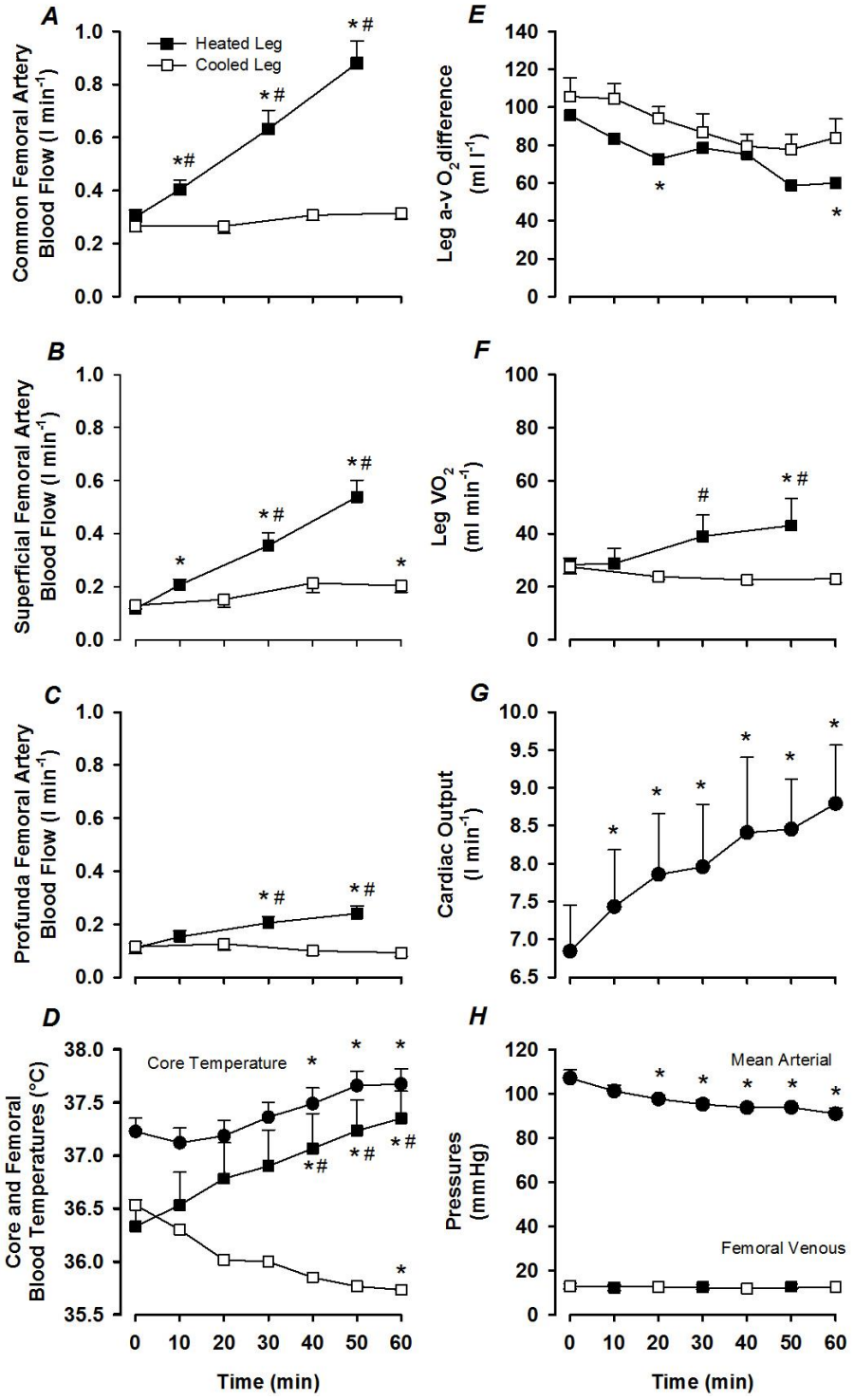

Fig. 6.4. Haemodynamic and temperature responses to systemic and leg heat stress combined with leg cooling. Leg and systemic haemodynamic and temperature responses over 1 h of passive heat stress with simultaneous isolated cooling of a single leg. Values are mean ± SEM for eight participants except for cardiac output (n=7) and core temperature (n=6). Missing values are due to equipment failures or catheterisation issues prior to the experiment. * Significantly different from baseline. # Significantly different from comparable measurement in the cooled leg; $P < 0.05$.

At the systemic level, $\dot{Q}$ increased ~ 1.9 l·min^{-1} after 1 h of heating ($P < 0.05$; Fig. 6.4G), with the response owed solely to increases in heart rate (69 ± 3 to 94 ± 4 beats·min^{-1}; $P < 0.01$) as stroke volume remained unchanged. A steady decrease in MAP over the hour (107 ± 4 to 91 ± 3 mmHg; $P < 0.01$; Fig. 6.4H) accounted for a decreased perfusion pressure to both legs, as femoral venous pressures remained unchanged throughout (~ 12 mmHg; Fig. 6.4H). Blood haemoglobin, arterial O_2 content, osmolality, and electrolytes remained unchanged in both legs throughout the resting protocol (Table 6.2). In contrast, an unchanged arterial O_2 content coupled with a significantly increased leg blood flow resulted in an increased O_2 delivery to the heated compared to the cooled leg (end values 185 ± 23 vs. 64 ± 5 ml·min^{-1}). Leg $\dot{V}O_2$ was significantly elevated in the heated leg after 1 h (28 ± 3 to 43 ± 1 ml·min^{-1}; $P < 0.05$; Fig. 6.4F), despite a decrease in the a-vO_2 difference of blood draining from the deep veins of the thigh skeletal muscles (87 ± 12 to 60 ± 8 ml·l^{-1}; $P < 0.05$; Fig. 6.4E). Cooled leg $\dot{V}O_2$ remained unchanged throughout ($P > 0.05$). In both the heated and the cooled legs, glucose uptake and lactate exchange remained stable over the 1 h thermal protocols and thus no significant differences were observed between legs at any time point, despite the significant differences in flow. Arterial and venous plasma noradrenaline concentrations were also stable over the 1 h thermal intervention in the heated and cooled legs (mean range values of 3-4 nmol.l^{-1}). However, arterial adrenaline decreased from 1.7 ± 0.8 to 0.8 ± 0.2 nmol.l^{-1} ($P < 0.05$) over the 1 h, while femoral venous values in the heated and cooled leg remained stable (Table 6.2). Multiple regression analyses displayed strong linear relationships between femoral venous blood temperature and both profunda femoral artery blood flow and tissue $\dot{V}O_2$ over the full range of temperatures in the cooled and heated legs ($R^2 = 0.50$ and 0.55 for T_b vs. PFA and $\dot{V}O_2$, respectively; $P < 0.01$ for both).

Table 6.2. Blood variable responses to systemic and leg heating combined with leg cooling at rest

		Time (min)						
		0	10	20	30	40	50	60
Hb ($g \cdot l^{-1}$)	a	147 ± 3	147 ± 3	145 ± 3	148 ± 3	147 ± 4	149 ± 4	149 ± 5
	vh	147 ± 3	143 ± 4	145 ± 3	147 ± 3	149 ± 4	150 ± 4	150 ± 4
	vc	146 ± 3	145 ± 3	147 ± 4	149 ± 4	149 ± 4	149 ± 4	151 ± 3
O_2 Sat. (%)	a	98 ± 0.1	98 ± 0.1	98 ± 0.3	98 ± 0.1	98 ± 0.1	98 ± 0.2	98 ± 0.2
	vh	53 ± 6	62 ± 5*	66 ± 4*	64 ± 4	67 ± 5*	65 ± 5	70 ± 4*
	vc	50 ± 6	56 ± 6	60 ± 5	62 ± 5	65 ± 5	62 ± 3	60 ± 5
PO_2 (mmHg)	a	99 ± 3	101 ± 3	109 ± 6	103 ± 2	112 ± 6	100 ± 4	105 ± 5
	vh	30 ± 3	35 ± 3	37 ± 3	36 ± 3	39 ± 4	38 ± 3	41 ± 4
	vc	28 ± 3	31 ± 3	32 ± 3	32 ± 2	36 ± 4	31 ± 2	33 ± 3
CtO_2 ($ml \cdot l^{-1}$)	a	200 ± 4	198 ± 4	197 ± 3	200 ± 4	199 ± 4	201 ± 5	201 ± 5
	vh	106 ± 11	121 ± 12*	132 ± 8*	130 ± 9	136 ± 12	134 ± 13	146 ± 11*
	vc	100 ± 11	111 ± 12	122 ± 13	127 ± 11	134 ± 12	128 ± 10	127 ± 13
PCO_2 (mmHg)	a	39 ± 1	37 ± 3	40 ± 1	41 ± 1	40 ± 1	40 ± 1	40 ± 1
	vh	49 ± 2	48 ± 1	48 ± 1	48 ± 1	47 ± 1	48 ± 1	47 ± 1
	vc	50 ± 2	49 ± 2	48 ± 2	47 ± 2	46 ± 2	46 ± 2	47 ± 2
pH	a	7.43 ± 0.01	7.42 ± 0.01	7.43 ± 0.01	7.43 ± 0.01	7.43 ± 0.01	7.43 ± 0.01	7.42 ± 0.01
	vh	7.39 ± 0.01	7.39 ± 0.01	7.39 ± 0.01	7.39 ± 0.01	7.40 ± 0.01	7.39 ± 0.01	7.39 ± 0.01
	vc	7.39 ± 0.01	7.39 ± 0.01	7.39 ± 0.01	7.40 ± 0.01	7.40 ± 0.01	7.40 ± 0.01	7.40 ± 0.01
Glucose ($mmol \cdot l^{-1}$)	a	6.0 ± 0.2	5.8 ± 0.2	5.8 ± 0.3	6.0 ± 0.2	6.1 ± 0.3	6.3 ± 0.3	6.3 ± 0.4
	vh	5.3 ± 0.4	5.4 ± 0.3	5.6 ± 0.2	5.7 ± 0.2	5.8 ± 0.2	5.9 ± 0.3	6.1 ± 0.3
	vc	5.1 ± 0.4	5.3 ± 0.3	5.5 ± 0.3	5.5 ± 0.2	5.7 ± 0.2	5.7 ± 0.2	5.9 ± 0.2
Lactate ($mmol \cdot l^{-1}$)	a	1.2 ± 0.3	1.2 ± 0.3	1.1 ± 0.2	1.1 ± 0.2	1.1 ± 0.2	1.3 ± 0.2	1.5 ± 0.3
	vh	1.1 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.4 ± 0.2
	vc	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.4 ± 0.2
Noradrenaline ($nmol \cdot l^{-1}$)	a	2.4 ± 0.9	-	-	3.4 ± 1.3	-	-	2.6 ± 0.6
	vh	3.8 ± 0.5	-	-	3.2 ± 0.6	-	-	3.0 ± 0.7
	vc	3.2 ± 0.7	-	-	4.2 ± 1.8	-	-	3.6 ± 0.7
Adrenaline ($nmol \cdot l^{-1}$)	a	1.7 ± 0.4	-	-	1.4 ± 0.9	-	-	0.8 ± 0.2*
	vh	0.5 ± 0.2	-	-	0.7 ± 0.3	-	-	0.8 ± 0.4
	vc	0.4 ± 0.1	-	-	0.9 ± 0.6	-	-	0.5 ± 0.1

Values are mean ± SEM for 8 participants (venous samples) and 7 participants (arterial samples). Catecholamines were measured at time-points 0, 30, and 60 only (n = 5). a, arterial; vh, femoral venous heated leg; vc, femoral venous cooled leg. PO_2, PCO_2, and pH were corrected for changes in blood temperature. * Significantly different from baseline; $P < 0.05$.

Exercise responses

No significant differences in power output were observed at any stage of incremental exercise between the heated and cooled legs (max. 52 ± 2 vs. 50 ± 3 W; $P = 0.48$), whilst T_c and $\overline{T}_{sk}$ were also comparable throughout (~ 38 °C for both; $P > 0.05$). As expected, however, leg $\overline{T}_{sk}$ and T_b were significantly higher throughout the exercise bouts with the heated compared to the cold leg (mean ~ 36 vs 24 °C for leg $\overline{T}_{sk}$ and 37.7 vs 36.3 °C for T_b, respectively; $P < 0.01$; Table 6.1 and Fig. 6.5D). Blood flow through the common femoral artery of the heated leg (i.e. leg blood flow; LBF) was consistently ~ 0.6 $l \cdot min^{-1}$ higher than that of the cooled leg at each stage of incremental exercise (final stage values 3.7 ± 0.1 vs. 3.1 ± 0.2 $l \cdot min^{-1}$; $P < 0.01$; Fig. 6.5A), coinciding with an elevated leg vascular conductance of ~ 6 $ml \cdot min^{-1} \cdot mmHg^{-1}$ during heated limb exercise in comparison to cooled. The differences in blood flow and vascular conductance between legs were once again closely associated with differences in femoral venous blood temperature draining each of the legs ($R^2 = 0.77$ and 0.63 for heated and cooled legs, respectively; $P < 0.05$; Fig. 6.7B). Blood haemoglobin, osmolality, and electrolytes showed no difference over both exercise bouts (Table 6.3). No significant difference was observed in a-vO_2 difference between the two legs, although results suggested that this tended to be lower over the duration of the heated leg exercise protocol ($P = 0.059$; Fig. 6.5B). The higher blood flow at each incremental stage, coupled with an unchanged arterial O_2 content, resulted in increased O_2 delivery in the heated compared to the cooled limb throughout the duration of exercise ($P < 0.05$). $\dot{V}O_2$ was significantly higher over the entire duration of the incremental exercise bout with the heated leg (Fig. 6.5C), coinciding with a decreased leg net lactate release (19.4 vs. 36.5 $mmol \cdot min^{-1}$; $P < 0.05$) and attenuated drop in venous blood pH (absolute decrease of 0.11 vs. 0.19; $P < 0.05$; Table 6.3). At the systemic level, heart rate (~ 90 to 150 $beat \cdot min^{-1}$), stroke volume (~ 85 ml throughout), and $\dot{Q}$ (~ 8 to 13 $l \cdot min^{-1}$) were similar during the 4 stages of incremental exercise with the heated and the cooled leg ($P > 0.05$; Table 1 and Fig. 6.5E). MAP and FVP both significantly increased over the duration of the exercise tests, with smaller increases in both being observed during exercise with the heated compared to the cooled leg (final values for MAP and FVP 124 ± 4 vs. 141 ± 5 mmHg and 21 ± 2 vs. 30 ± 4 mmHg, respectively; $P < 0.05$; Fig. 6.5F).

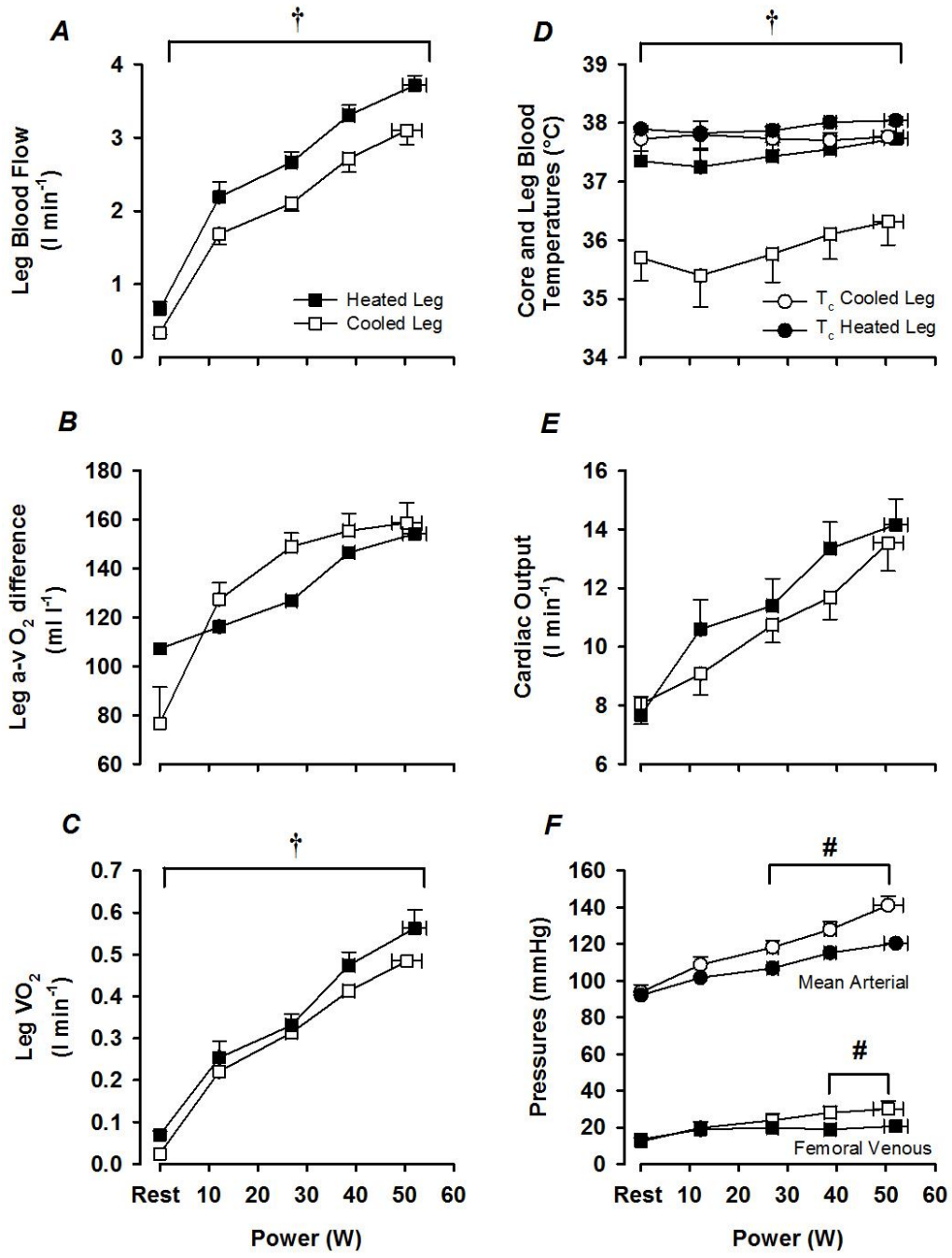

Figure 6.5. Leg and systemic haemodynamic and temperature responses during incremental single-legged knee extensor exercise at 20, 40, 60, and 80% peak power output. Participants were previously exposed to 1 h of full-body heat stress with simultaneous isolated single leg cooling before carrying out an incremental exercise test in both the heated and cooled limb. Values are mean ± SEM for seven participants except for cardiac output (n=6) and core temperature (n=5). Missing values are due to equipment failures or catheterisation issues prior to the experiment. † Mean effect for temperature (heated vs. cooled leg). # Significantly different from cooled leg; *P* < 0.05.

Table 6.3. Blood variable responses to exercise in the heated and cooled leg during systemic and leg heat stress combined with leg cooling

		Exercise with Cooled Leg (W)					Exercise with Heated Leg (W)				
		Rest	12 ± 1	27 ± 1	39 ± 1	50 ± 3	Rest	12 ± 1	27 ± 1	39 ± 1	52 ± 2
Hb ($g \cdot l^{-1}$)	a	156 ± 8	152 ± 4	151 ± 4	154 ± 4	152 ± 6	152 ± 4	153 ± 4	150 ± 6	152 ± 4	157 ± 4
	vh	152 ± 4	152 ± 4	153 ± 4	152 ± 5	161 ± 3	150 ± 4	152 ± 5	149 ± 9	152 ± 6	152 ± 4
	vc	152 ± 4	150 ± 4	153 ± 3	152 ± 4	161 ± 4*	153 ± 4	150 ± 5*	153 ± 10	155 ± 4	156 ± 3
O_2 Sat. (%)	a	98 ± 0.2	98 ± 0.3	98 ± 0.2	98 ± 0.2	98 ± 0.2	98 ± 0.4	98 ± 0.2	98 ± 0.4	98 ± 0.2	98 ± 0.2
	vh	71 ± 4	61 ± 3	52 ± 4*	52 ± 4*	53 ± 5*	48 ± 6	43 ± 4	36 ± 4	28 ± 3*	27 ± 4*
	vc	59 ± 7	37 ± 4*	26 ± 2*	25 ± 3*	21 ± 3*	66 ± 6	59 ± 5	56 ± 3*	52 ± 4*	48 ± 4*
PO_2 (mmHg)	a	105 ± 6	103 ± 4	101 ± 2	106 ± 4	109 ± 3	111 ± 6	100 ± 3	110 ± 10	102 ± 2	105 ± 2
	vh	34 ± 1	34 ± 1	31 ± 1	32 ± 2	32 ± 2	30 ± 2	28 ± 2	26 ± 1	23 ± 1	23 ± 2
	vc	32 ± 4	23 ± 1*	20 ± 1*	20 ± 1*	20 ± 1*	38 ± 4	32 ± 2	31 ± 2	30 ± 2	29 ± 2
CtO_2 ($ml \cdot l^{-1}$)	a	200 ± 4	205 ± 4	204 ± 5	208 ± 4	207 ± 7	206 ± 5	206 ± 5	202 ± 7	205 ± 4	212 ± 5
	vh	138 ± 1	127 ± 1	110 ± 1*	108 ± 1*	116 ± 1*	99 ± 1	90 ± 1	75 ± 1*	59 ± 1*	58 ± 1*
	vc	127 ± 2	78 ± 1*	55 ± 0.3*	53 ± 0.4*	48 ± 1*	135 ± 2	111 ± 2*	112 ± 1*	110 ± 1*	100 ±1*
PCO_2 (mmHg)	a	41 ± 1	40 ± 1	42 ± 1	39 ± 2	38 ± 2	36 ± 3	39 ± 2	36 ± 2	39 ± 2	38 ± 2
	vh	47 ± 1	49 ± 1	52 ± 1*	53 ± 1*	53 ± 2*	50 ± 2	55 ± 2*	59 ± 2*	64 ± 2*	71 ± 4*#
	vc	47 ± 3	56 ± 3*	65 ± 3*	73 ± 4*	80 ± 3*	43 ± 2	44 ± 2	46 ± 2*	48 ± 1*	50 ± 1*
pH	a	7.42 ± 0.01	7.41 ± 0.01	7.39 ± 0.01	7.39 ± 0.01	7.40 ± 0.01	7.41 ± 0.03	7.40 ± 0.01	7.41 ± 0.02	7.39 ± 0.01	7.39 ± 0.01
	vh	7.39 ± 0.01	7.37 ± 0.01	7.36 ± 0.01*	7.35 ± 0.01*	7.34 ± 0.01*	7.36 ± 0.02	7.34 ± 0.01	7.31 ± 0.01*	7.29 ± 0.01*	7.25 ± 0.02*#
	vc	7.40 ± 0.01	7.33 ± 0.01*	7.29 ± 0.01*	7.25 ± 0.02*	7.21 ± 0.02*	7.36 ± 0.02	7.37 ± 0.02	7.36 ± 0.01	7.36 ± 0.01	7.35 ± 0.01
Glucose ($mmol \cdot l^{-1}$)	a	6.5 ± 0.4	6.6 ± 0.3	6.6 ± 0.2	6.4 ± 0.2	6.3 ± 0.2	6.6 ± 0.2	6.7 ± 0.3	6.8 ± 0.3	6.8 ± 0.4	6.8 ± 0.4
	vh	6.1 ± 0.3	6.1 ± 0.3	6.1 ± 0.2	6.1 ± 0.2	6.0 ± 0.3	6.1 ± 0.3	6.4 ± 0.5	6.3 ± 0.5	6.2 ± 0.6	6.1 ± 0.6
	vc	5.9 ± 0.2	6.2 ± 0.3	6.3 ± 0.2	6.1 ± 0.2	6.3 ± 0.3	5.9 ± 0.4	6.2 ± 0.3	6.4 ± 0.4	6.6 ± 0.4	6.6 ± 0.4
Lactate ($mmol \cdot l^{-1}$)	a	1.5 ± 0.3	1.7 ± 0.3	2.2 ± 0.3*	3.0 ± 0.5*	4.1 ± 0.7*	3.6 ± 0.9	3.4 ± 0.7	3.4 ± 0.7	4.0 ± 0.4	4.7 ± 0.5*
	vh	1.4 ± 0.2	1.4 ± 0.2	1.7 ± 0.2	2.2 ± 0.3*	3.3 ± 0.5*	2.9 ± 0.5#	3.4 ± 0.5#	3.5 ± 0.5*	4.0± 0.6*	5.3 ± 0.9*
	vc	1.4 ± 0.2	2.3 ± 0.3*	3.0 ± 0.4*	4.3 ± 0.6*	6.0 ± 0.8*	4.4 ± 0.9	3.5 ± 0.7	3.4 ± 0.5	3.3 ± 0.5	3.8 ± 0.5

Values are mean ± SEM for seven participants (venous samples) and six participants (arterial samples). a, arterial; vh, femoral venous heated leg; vc, femoral venous cooled leg. PO_2, PCO_2, and pH were corrected for changes in blood temperature. * Significantly different from baseline. # Significantly different from cooled femoral venous blood during cooled leg exercise; $P < 0.05$.

6.3.2 – Haemodynamic responses to isolated changes in leg temperature

Resting responses

T_c and $\overline{T}_{sk}$ were maintained at 37 and 32 °C throughout both isolated heating and cooling protocols, whereas hot and cold leg T_{sk} were comparable to that obtained during Study 1 (final values 38.4 ± 0.9 and 19.5 ± 1.6 °C, respectively). LBF responses to isolated heating were similar to that observed in the heated leg during systemic heat stress, with increases observed in the common (0.25 ± 0.02 to 0.76 ± 0.08 l·min^{-1}), superficial (0.13 ± 0.01 to 0.46 ± 0.07 l·min^{-1}) and profunda femoral arteries (0.08 ± 0.01 to 0.22 ± 0.05 l·min^{-1}; $P < 0.01$ for all). As in Study 1, these changes in LBF were once again associated with increasing local tissue temperatures ($R^2 = 0.55$; $P < 0.01$). Isolated cooling of the limb led to a small but significant decrease in flow to the common, superficial, and profunda femoral arteries (0.19 ± 0.01 to 0.16 ± 0.01 l·min^{-1}, 0.09 ± 0.02 to 0.08 ± 0.01 l·min^{-1}, and 0.07 ± 0.02 to 0.04 ± 0.01 l·min^{-1}; $P < 0.01$). The difference in flow between the heated and cooled legs at the end of the 1 h intervention was similar to that observed during Study 1 (~ 0.6 l·min^{-1}; $P < 0.01$). The increases in LBF with isolated heating were paralleled by increases in leg vascular conductance, which in turn was significantly correlated with T_m ($R^2 = 0.55$; $P < 0.01$), but not T_c or T_{sk} ($R^2 = 0.07$ and 0.37, respectively; $P > 0.05$).

Exercise responses

There were no differences in power output, T_c, or $\overline{T}_{sk}$ during each incremental exercise test with either isolated leg heating or cooling ($P > 0.05$ for all). However, leg $\overline{T}_{sk}$ was as expected significantly elevated throughout incremental exercise in the heated compared to the cooled leg (~ 37 and 15 °C, respectively; $P < 0.01$). LBF of the heated leg was consistently ~ 0.6 l·min^{-1} higher than that of its cooled counterpart at each stage of incremental exercise and was not different between Study 1 and 2 (final stage values ~ 3.7 vs. 3.1 l·min^{-1}; $P < 0.01$; Fig. 4B). Increases during

incremental exercise with the heated and cooled legs were associated with progressive increases in T_m.

6.3.3 – Haemodynamic responses to altered leg temperature with and without systemic heat stress: a comparison of both studies

At rest, the effect of heating or cooling the leg with and without systemic heat stress resulted in similar LBF responses over the 1 h intervention, despite differences in systemic temperatures and haemodynamic responses. LBF in the heated leg at the end of Study 1 was within ~ 0.1 l·min^{-1} of that recorded in Study 2 (0.94 ± 0.1 vs. 0.80 ± 0.11 l·min^{-1}; $P > 0.05$), with similar responses being observed in the cooled leg also (0.19 ± 0.02 vs. 0.33 ± 0.03 l·min^{-1}; $P > 0.05$; Figure 6.6A). These similar flows occurred despite significant differences in both T_c (37.7 vs 37.1 °C; $P < 0.01$) and $\dot{Q}$ (8.8 ± 0.6 vs. 6.4 ± 0.5 l·min^{-1}; $P < 0.05$) between the two studies.

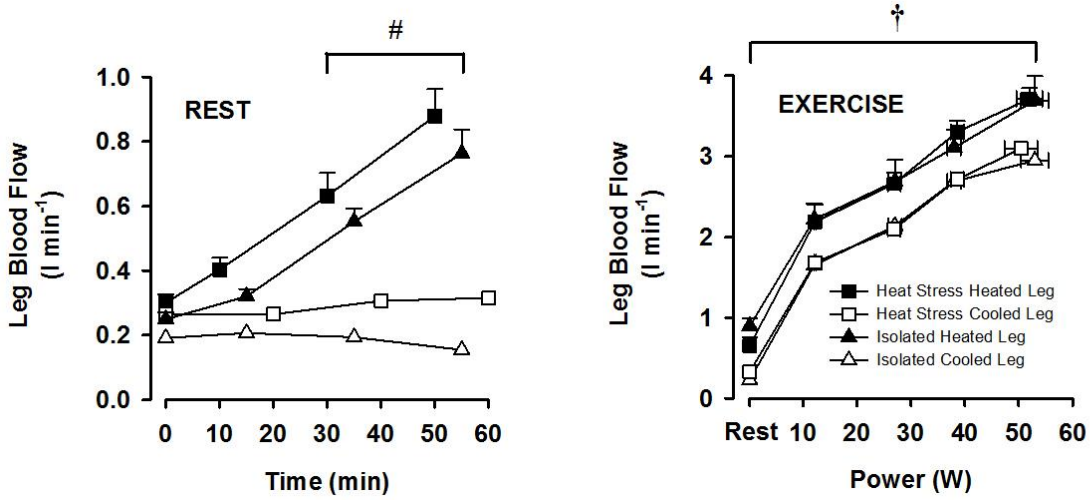

Figure 6.6. Leg blood flow responses to leg heating and cooling with and without systemic heat stress. Values are mean ± SEM for eight participants during whole-body hyperthermia (exercise data n=7 due to equipment failure) and seven participants during normothermia. † Mean effect for temperature (both heated vs. both cooled legs) # Significantly different from both cooled legs; $P < 0.05$.

During incremental exercise, LBF was determined by a combination of exercise intensity and local blood and/or tissue temperatures; with heated and cooled leg blood flows in the systemic heat stress incremental exercise tests showing no difference to their isolated heated and cooled leg conditions (Fig. 6.6B), despite differences in T_c of ~ 1 °C (final heated and cooled leg blood flows ~ 3.7 vs. 3.1 $l \cdot min^{-1}$ during both heat stress and control conditions).

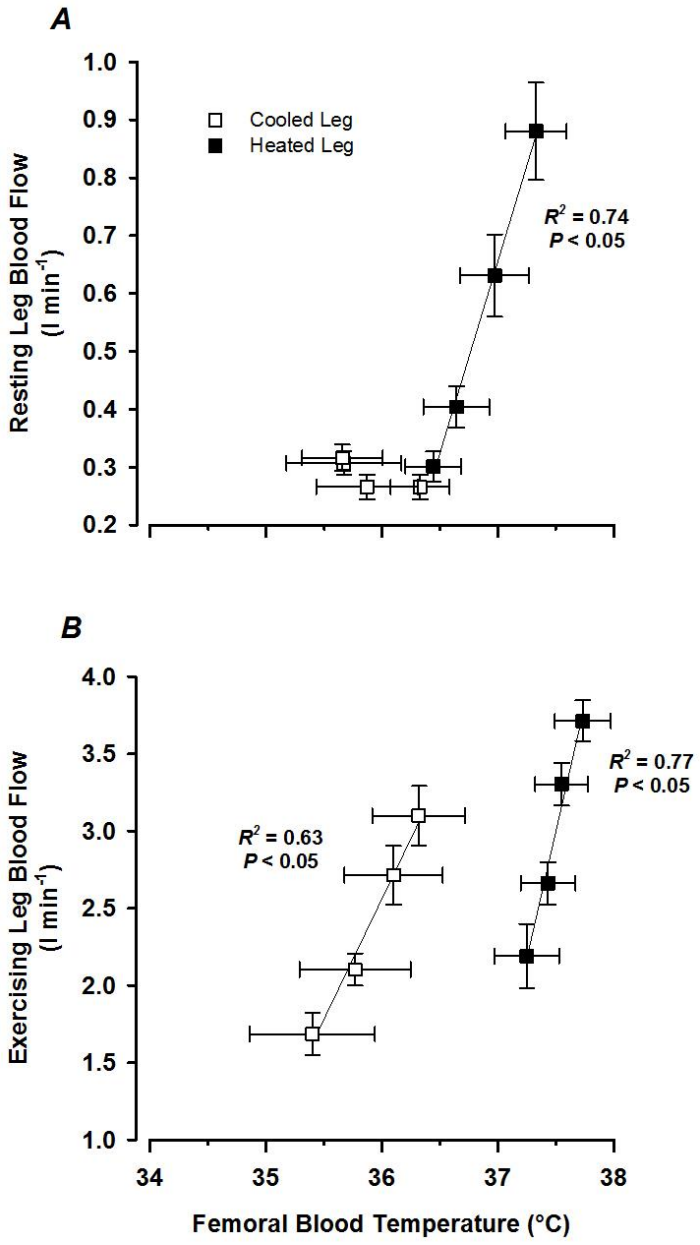

Figure 6.7. Relationship between leg blood flow and femoral venous blood temperature in cooled and heated legs during both rest (top) and exercise (bottom). Values are mean ± SEM for 8 participants in Study 1; P < 0.05 for all indicated relationships.

6.4 – Discussion

This investigation sought to elucidate the contribution of local vs. systemic thermosensitive mechanisms on global leg perfusion and its distribution in a variety of thermal and exercise conditions in healthy humans. A major finding was that increases in resting blood flow in the leg's largest conduit arteries in response to both isolated limb and systemic heat stress were strongly correlated to increases in local tissue and/or blood temperatures, but were unrelated to the distinct core temperature and systemic haemodynamic responses. Consistent with this notion, we also found that blood flow in the cooled leg's common, superficial and profunda femoral arteries remained essentially unchanged, despite physiologically significant increases in systemic temperature and blood flow with heat stress. The presence of an increased profunda femoral artery blood flow (main conduit vessel supplying blood to deep thigh tissues) is indicative of a contribution of thigh skeletal muscle hyperaemia to the overall increase in limb tissue perfusion with heat stress, a response which may be partly attributable to increases in metabolic mediated vasodilatation. These increases in blood flow to leg tissues at rest are maintained during incremental single-legged knee-extensor exercise to near maximal power output. This suggests an additive effect of local thermoregulatory and metabolic stimuli on the regulation of leg perfusion during exercise activities engaging a small muscle mass.

6.4.1 – Local and systemic influences on leg tissue perfusion during heat stress

We systematically altered leg tissue temperatures under conditions of systemic and isolated limb thermal stress, with a major finding being the tight coupling between elevations in resting blood flow in the leg's major conduit arteries and the increases in local tissue and/or blood temperatures, mechanistically dissociated from core temperature and systemic haemodynamic stimuli. Several observations underpinned this novel finding. Firstly, and in agreement with a recent study from our laboratory

(Pearson *et al*, 2011), we found significant increases in blood flow in the passively heated leg (0.6 $l \cdot min^{-1}$) during systemic heat stress, accompanied by increases in T_c (0.5 °C), heart rate (30 $beats \cdot min^{-1}$), $\dot{Q}$ (2 $l \cdot min^{-1}$), and decreased MAP and leg perfusion pressure (~ 15%). The increased leg hyperaemia in the face of a decreased perfusion pressure gradient was strongly associated with a corresponding elevation in leg vascular conductance, a response which was closely related to the rise in femoral venous blood temperature (R^2 = 0.74; P < 0.01), with weaker relationships observed with T_c (R^2 = 0.67; P < 0.05) and $\overline{T}_{sk}$ (clamped at ~ 38 °C throughout the experiment; R^2 = 0.45; P < 0.05). Secondly, an involvement of local temperature in the regulation of LBF was revealed by the prevention of the hyperaemic response during the simultaneous cooling of the contralateral limb, despite the same increases in central haemodynamics and core temperature. Lastly, data during isolated heating of a single leg showed comparable LBF responses to that seen during systemic heat stress with a significant correlation observed between leg vascular conductance and T_m (R^2 = 0.55 P < 0.01), but not T_c or T_{sk} (R^2 = 0.07 and 0.37, respectively). Together, these observations indicate that local temperature, independent of central temperature and haemodynamic reflexes, distinctly and noticeably influences limb blood flow during moderate passive heat stress.

Conflicting evidence over the past decades has rendered the role of temperature on functional hyperaemia as highly contentious. Several previous studies have reported no change in exercise hyperaemia during one-legged knee extensions (Savard *et al*, 1988; Ferguson *et al*, 2006), two-legged cycling (Savard *et al*, 1988), or walking uphill to exhaustion in the heat (Nielsen *et al*, 1990), whereas our recent study showed significant increases in LBF during submaximal knee-extensor exercise across different levels of heat stress (up to 0.7 $l \cdot min^{-1}$; Pearson *et al.* 2011). These elevations are of a similar magnitude to the ~ 0.5 $l \cdot min^{-1}$ higher LBF observed by Ferguson *et al* (2006) when thigh muscle temperature was heated by ~ 3 °C prior to knee-extensor exercise, although the difference in flow was deemed not statistically significant in this earlier study. The small changes in temperature between heated and cooled conditions (~ 0.3 °C in Savard *et al*, 1988) and the use of an exercise protocol that engages a large muscle mass (Savard *et al*, 1988; Nielsen *et al*, 1990)

and thus markedly increases sympathetic vasoconstrictor drive (Pawelczyk *et al*, 1992; Saito *et al*, 1993; Rosenmeier *et al*, 2004; Calbet *et al*, 2004; Mortensen *et al*, 2005) might explain the discrepant results. Here we hypothesised that if local temperature is important for functional hyperaemia, an additive effect of both thermoregulatory and metabolic stimuli should result in further increases in blood flow during combined heat stress and exercise. In support of recent findings, the 0.6 $l \cdot min^{-1}$ increase in heated leg blood flow seen with passive heat stress was maintained at near identical levels in all participants throughout incremental exercise to near maximal power output, with increases in leg vascular conductance once again being tightly coupled to femoral blood temperatures as exercise intensity gradually increased (R^2 = 0.63 and 0.77 for cooled and heated legs respectively; $P < 0.05$ for both). Strikingly, LBF responses following isolated leg heating and cooling were virtually identical to those observed following systemic heat stress, despite differences in T_c and cardiac output of up to 1 °C and 2 $l \cdot min^{-1}$ prior to the commencement of exercise. Hence, these observations lend support to an independent and additive effect of thermoregulatory factors on LBF during heat stress and small muscle mass exercise.

6.4.2 – Blood flow distribution to vascular beds of the leg during heat stress

A second aim of the present study was to examine the distribution of blood flow through the main conduit arteries of the leg in response to passive heat stress. A salient finding is the 2 - 4.5 fold increase in blood flow in profunda and superficial femoral arteries in tandem with elevations in local tissue temperature, with a preferential distribution of flow to the superficial femoral artery (major supplier of skin and lower leg) compared to the profunda femoral artery (major supplier to muscle tissue of the thigh). This increase equates to approximately 75 vs. 25% of the 0.6 $l \cdot min^{-1}$ global leg hyperaemia induced by both systemic and isolated leg heat stress, respectively. Although not a direct measurement of skeletal muscle perfusion, the anatomical layout of the profunda femoral artery indicates that the vast majority of flow through this vessel supplies thigh tissue and may be used to quantify muscle

perfusion (Mamatha *et al*, 2012). In agreement with studies carried out in recent years (Keller *et al*, 2010; Pearson *et al*, 2011; Heinonen *et al*, 2011), we found that the ~ 130 ml·min^{-1} increase in profunda femoral artery blood flow was accompanied by a 40% decrease in oxygen extraction from blood draining the deep portion of the femoral vein, a normal physiological response to augmented blood and oxygen supply. The measure of oxygen extraction therefore further supports a contribution of muscle to the global hyperaemic response to heat stress.

Comparison of the haemodynamic effects of heat stress in different experimental conditions including exercise requires detailed knowledge of blood flow distribution in different tissues. A recent study by Heinonen *et al*. (2011) quantified blood flow to different tissues of the calf and reported a modest increase in muscle blood flow of ~ 1 ml· (dl of muscle)$^{-1}$·min^{-1} following local heating, compared to skin blood flow of ~ 5 ml· (dl of skin)$^{-1}$·min^{-1}. These findings would translate into a larger distribution of blood flow in absolute terms to the underlying skeletal muscle when taking into account typical relative volumes of calf muscle and skin tissue (i.e., roughly 75% and 10% of total calf volume; (Wang *et al*, 1999). If we assume baseline thigh muscle blood flow values of ~ 2.9 ml·(dl of muscle)$^{-1}$·min^{-1} (Heinonen *et al*, 2013) and a similar relative increase during heating as in the calf (~ 80% increase from baseline; Heinonen *et al*, 2011), thigh muscle perfusion would be expected to increase to ~ 5.2 ml· (dl of muscle)$^{-1}$·min^{-1} following exposure to heat stress. This would equate to a total thigh muscle blood flow increase of ~ 150 ml·min^{-1}, equivalent to results reported in this study for the profunda femoral artery. Methodological limitations prevented the measurement of profunda femoral artery blood flow during the incremental exercise test, and it was therefore not possible to quantify skeletal muscle blood flow in the same manner as at rest. However, the persistent 0.6 l·min^{-1} elevation in global leg perfusion mirrored by a decreased a-vO_2 difference suggests that the enhanced skeletal muscle hyperaemia seen with passive heat stress was maintained during incremental exercise in the heated leg. In this setting, global perfusion and femoral venous blood temperature increased gradually in both legs up to ~2.8 l·min^{-1} and 0.3-0.6°C above baseline values, respectively, whilst core and skin temperatures remained stable at ~38°C. The leg blood flow temperature sensitivity value of 0.9 l·min^{-1}·°C^{-1} established in the current study

during passive heat stress indicates that increases in blood temperature during exercise can only account for a fraction of functional hyperaemia.

6.4.3 – Mechanisms of blood flow control in the heat-stressed human leg

Severe heat stress induces a hyperadrenergic state, characterised by augmented circulating catecholamines (Kim *et al*, 1979), enhanced muscle and skin sympathetic nerve activity (Niimi *et al*, 1997; Crandall *et al*, 1999), and hyperthermia and hyperkinetic haemodynamics at limb and systemic levels (Rowell, 1990). A key integrative physiology question in this study was whether central neural and humoral reflexes drive local tissue blood flow responses to moderate heat stress or whether local thermosensitive mechanisms are more important. The virtual abolition of a hyperaemic effect in the cooled leg in the face of significant increases in both systemic drive and opposing contralateral heated leg blood flow suggests local temperature-sensitive mechanisms, a finding supported by a similar magnitude increase in heated leg blood flow in the isolated limb thermal protocol.

The increased perfusion to different leg tissues with heat stress was associated with net vasodilation, as indicated by the increases in conduit artery vascular conductance, irrespective of the perfusion pressure gradient response. Skin blood flow has been shown to be regulated by afferent-activated axon reflexes (Stephens *et al*, 2001), NO (Kellogg *et al*, 1999; Kellogg *et al*, 2008) and sympathetically mediated component (Charkoudian, 2010). The presence of increased skeletal muscle perfusion suggests that these mechanisms do not entirely account for current whole-leg findings, however, as temperature stimuli also appear to affect skeletal muscle. In this construct, temperature might be acting either directly or indirectly at the microcirculation level via potentiation of thermosensitive pathways. In respect to skeletal muscle, *in vitro* studies provide no evidence of direct vasoactive effects of temperature elevations on arterial and venous microvessel preparations from humans and canines (Vanhoutte and Shepherd; 1970; Vanhoutte and Shepherd; 1970; Ives *et al*, 2011). It therefore seems that temperature exerts its vascular effects through

thermosensitive signal transduction pathways. Evidence from *in vivo* and *in vitro* studies suggest the involvement of ATP release from human erythrocytes in the regulation of tissue hyperaemia in hyperthermic conditions (Pearson *et al*, 2011; Kalsi and González-Alonso; 2012). The direct relationship between increases in erythrocyte ATP release and temperature (Kalsi and González-Alonso, 2012) and the potent vasodilatory and sympatholytic properties of ATP in the human leg and arm circulations (González-Alonso *et al*, 2002; Rosenmeier *et al*, 2004; Kirby *et al*, 2008) make temperature-dependent erythrocyte ATP release an attractive mechanism. Another possibility based on the presently observed 64% increase deep tissue $\dot{V}O_2$ in the heated leg is a metabolic contribution to hyperthermia-mediated hyperaemia. Although the contributions of extracellular and intracellular themosensitive mechanisms remain to be fully established, the parallel decreased in deep tissue O_2 extraction and unaltered glucose uptake would point to potential intracellular signalling pathways playing a part in the metabolic stimulated vasodilatation.

6.4.4 – Limitations

Methodological considerations prevented the counterbalancing of the heated and cooled legs during exercise in Study 1, with the cooled leg always being exercised first to maintain the highest possible local temperature difference between legs. Previous research has shown elevations in muscle blood flow and aerobic metabolism following repeated, maximal knee-extensor exercise bouts in humans, separated by a short recovery (Bangsbo *et al*, 2001; Krustrup *et al*, 2001). The current experimental design, however, employed submaximal exercise to a level no greater than 80% of peak power output, with exercise bouts separated by 20 min and performed with different legs. Leg blood flows in Study 2, which used a counterbalanced experimental design, were identical to those observed in Study 1. This indicates that the additive increases in flow documented here during incremental exercise were associated with elevations in local tissue and blood temperatures, rather than the potentiating effects of previous exercise.

6.4.5 – Conclusion

This study provides compelling evidence that increases in limb tissue perfusion during passive heat stress and small-muscle mass exercise are mediated by local temperature-sensitive mechanisms, independent of systemic temperature and haemodynamic responses. In addition, strong evidence is provided that skeletal muscle blood flow contributes to the observed hyperaemic response, potentially accounting for up to ~25% of the increase in limb blood flow during both localised and whole-body hyperthermia and in part due to increases in metabolic vasodilatation. These findings emphasise the importance of local temperature-sensitive mechanisms in the regulation of peripheral blood flow and suggest a potential therapeutic use of local heating to improve oxygen and substrate delivery to specific tissues without the additional cardiac strain of whole-body hyperthermia.

CHAPTER 7

Study 4: Blood flow responses, distribution, and regulation in the human leg during severe passive heat stress

7.0 – Abstract

Elevations in leg tissue perfusion during isolated leg and whole-body passive heat stress occur in tandem with reductions in oscillatory shear index in vessels perfusing both skin and skeletal muscle, with these changes regulated through local temperature-sensitive mechanisms during heat stress to moderate levels. Whether this close association between local tissue temperatures, perfusion, and flow profiles is maintained during more severe levels of heating remains unclear. To address this question, eight healthy males (age 25 ± 3 yr) had core temperatures elevated $\geq$ 2 °C (mean heating time 94 ± 5 min) using a whole-body water-perfused suit, followed by rapid cooling of a single leg via 10 min of crushed ice application. Core (T_c), deep muscle, subcutaneous, and skin temperatures were measured throughout; as were systemic and local haemodynamic responses in the common (CFA), superficial (SFA), and profunda femoral arteries (PFA) and great saphenous vein (GSV). Blood flow through all vessels increased in line with increasing core and local limb temperatures from rest to intense heat stress (T_c + 1.5 °C). Subsequently; CFA, SFA, and GSV effectively plateaued until the termination of heating (T_c + 2 °C), while PFA continued to increase in line with mean leg and core temperatures. Leg blood flow remained elevated during rapid single-leg cooling compared to equivalent mean leg temperatures during progressive heat stress (~ 1.0 *vs.* 0.6 l·min^{-1}). This elevation was most likely due to the maintenance of high skin blood flow through underlying central-mediated reflexes, with no change observed in the GSV ($P > 0.05$) despite significant reductions in skin and subcutaneous temperatures (39.7 ± 0.2 to 25.9 ± 2 °C and 39.0 ± 0.4 to 30.3 ± 1.3 °C, respectively; $P < 0.05$ for both). In contrast, skeletal muscle blood flow decreased in line with local muscle temperatures at a rate similar to that seen during heating ($P < 0.05$). Oscillatory shear index decreased in all three arteries throughout heating, and was virtually abolished upon reaching severe heat stress (0.01 ± 0.01, 0.01 ± 0.01, and 0.06 ± 0.02 for CFA, SFA, and PFA, respectively; $P < 0.05$). However, flow profile responses up to levels of moderate heat stress were generally not different to those obtained during isolated single leg heating in a previous study ($P < 0.05$). We conclude that while both skin and skeletal muscle are controlled predominantly by local tissue temperatures during direct whole-body heat stress, underlying central reflexes maintain vasodilation in the skin

(but not muscle) upon rapid cooling. In addition, evidence is provided that isolated local heating may provide the same beneficial conditions for endothelial health as moderate whole-body heat stress.

7.1 – Introduction

Severe passive heat stress places a significant circulatory strain on the human body that can reach levels similar to that seen during moderate intensity exercise in normal ambient conditions. The systemic responses to this thermal stress are well-characterised, with an increased demand for peripheral blood flow for heat dissipation leading to significant increases in cardiac output and heart rate (up to 12 $l \cdot min^{-1}$ and 120 $beats \cdot min^{-1}$, respectively), occurring alongside decreases in central blood volume, total peripheral resistance and mean arterial and central venous pressure (Rowell *et al*, 1969; Rowell *et al*, 1970; Minson *et al*, 1998; Crandall *et al*, 2008; Pearson *et al*, 2011). In contrast to the wealth of knowledge gathered on alterations at a systemic level, the haemodynamic responses and overall control of blood flow to peripheral tissues in the lower limbs during exposure to severe heat stress remain poorly understood. Recent work investigating leg blood flow responses during mild to moderate passive heat stress have characterised a linear increase in leg blood flow that appears closely related to increases in both local tissue and systemic temperatures (Pearson *et al*, 2011). The previous chapter of this thesis (Chapter 6) has expanded upon these findings by showing that these increases in whole-leg perfusion during moderate heat stress are controlled solely through local temperature-sensitive mechanisms, with blood flow in the limb prevented from rising alongside systemic temperatures and haemodynamics if local temperatures are kept low. However, whether leg blood flow continues this close association with increasing local temperatures at more severe levels of thermal stress remains unclear. Also unknown is whether the same local control mechanisms continue to dominate haemodynamic responses in all tissues of the leg. Previous studies in both animals and humans have shown skin blood flow to be controlled by a combination of both central and local mechanisms under exposure to high levels of heat stress (Wyss *et al*, 1974; Wyss *et al*, 1975; Wenger *et al*, 1975; Proppe *et al*, 1976; Proppe, 1981; Wenger *et al*, 1985). Skeletal muscle blood flow, however, has recently been shown to be under direct local control alone, at least at levels approaching moderate heat stress (Heinonen *et al*, 2011). Whether this control remains at a local level only, or is influenced by over-riding central mechanisms with more severe levels of heat stress (i.e., with increases in $Tc \geq 2$ °C), has yet to be investigated.

A number of studies have previously suggested both whole-body and indirect heating as a potential non-pharmacological means of improving vascular function in humans, partly based on improvements in flow-mediated dilation in the brachial artery following repeat exposures to whole-body heating protocols (Imamura *et al*, 2001; Kihara *et al*, 2002; Carter *et al*, 2014). Although clearly effective at increasing brachial conduit artery flow, the presence of increased core temperatures, cardiovascular strain and feelings of thermal discomfort associated with systemic heat stress may make it an unsuitable intervention in certain elderly and clinical populations. Results obtained Chapter 5 of this thesis provided novel evidence of increased antegrade and decreased retrograde flows and shear rates in all three major vessels supplying the leg; a number of which are highly susceptible to vascular dysfunction and potential peripheral arterial disease (Kroger *et al*, 1999). These haemodynamic changes resulted in increases of flow of up to 200% combined with a 66% decrease in oscillatory blood flow within the vessels, potentially providing suitable conditions for improving local vascular function in the leg similar to that observed in the arm during whole-body heat stress (Thijssen *et al*, 2009; Padilla *et al*, 2011; Simmons *et al*, 2011), despite an unchanged core temperature, thermal comfort, and systemic haemodynamics. No study to date has assessed flow profiles and shear rates within the lower limb during exposure to whole-body heat stress, and as such the relative benefits of this intervention in comparison to local tissue heating also remains unclear.

The aims of this study were therefore to test the following three hypotheses: 1) whole-leg blood flow would continue to increase in a linear fashion from ambient conditions to levels of severe passive heat stress, 2) these increases would be accompanied by further improvements in flow and shear rate profiles compared to isolated heating alone, and 3) control of blood flow would differ between the deep and superficial tissues of the leg during severe heat stress.

7.2 – Methods

7.2.1 – Participants

Following completion of a medical questionnaire and the provision of informed written consent, eight healthy males (age 25 ± 3 yr; height 182 ± 5 cm; weight 79 ± 6 kg) were recruited to participate in the study. Participants abstained from alcohol, caffeine, and strenuous exercise in the 24 h leading up to the day of testing. All procedures in this experiment conformed to the Declaration of Helsinki and were approved by the Brunel University London Research Ethics Committee.

7.2.2 – Experimental protocol

Each participant visited the laboratory on two occasions, separated by at least one week. These consisted of a shortened familiarisation trial in the initial visit in order to estimate whole-body sweat rate followed by the main experimental trial at least one week later. In the familiarisation trial, participants attended the laboratory at 9 am following ingestion of their usual breakfast, and were weighed and measured as described previously. Following instrumentation, participants were fitted with a custom-built water-perfused suit designed to cover the entire body, before being wrapped in survival blankets to prevent heat loss to the surrounding environment and resting in the supine position for the remainder of the study (Fig. 7.1). The suit was connected to a thermostatically-controlled water circulator (Julabo F-34, Seelbach, Germany) to allow the constant perfusion of 50 °C water and subsequent manipulation of body temperature, and participants were passively heated until T_c increased by 1 °C. At 20 min intervals throughout the heating protocol, complete arterial occlusion at the level of the knee was performed in order to assess blood flow distribution to the upper and lower leg. Occlusions were carried out for a maximum of 5 min via the inflation of a pneumatic cuff to 240 mmHg (Hokanson E20 Rapid Cuff Inflator and CC17 Cuff, Bellevue, WA, USA). Following the end of the intervention, participants were weighed once again and whole-body sweat rate was

estimated in $l \cdot min^{-1}$ as (pre-heating mass in kg – post-heating mass in kg) / duration of heating in min.

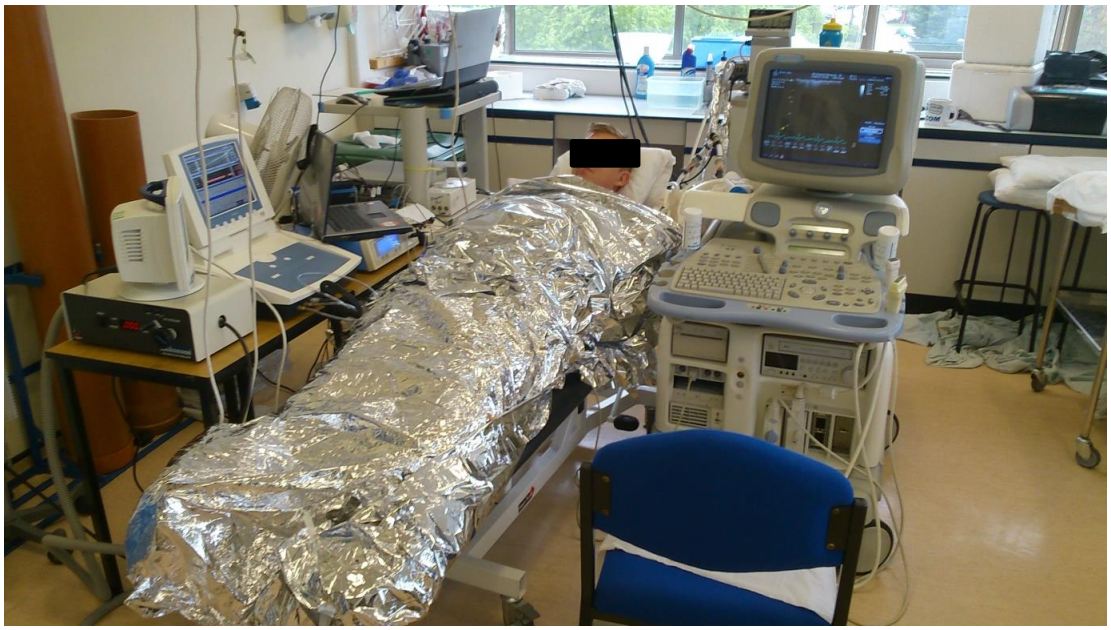

Figure 7.1: Experimental set-up showing subject resting in supine position while core and skin temperatures are elevated through the use of a water-perfused suit.

The experimental trial consisted of an identical protocol to the familiarisation trial, but with participants exposed to a more severe level of heat stress than their initial visit (Fig. 7.2). Full-body passive heating was continued until participants reached their level of thermal tolerance or achieved a T_c of at least 2 °C above baseline. Thermal tolerance was defined by participants displaying symptoms of pre-syncope, reporting symptoms of nausea, or indicating they were no longer willing to continue. Participants were provided with water every 20 min at a rate equal to their calculated sweat rate in the familiarisation trial and at a temperature approximately equal to that of T_c. Following the attainment of the target T_c, one leg of the water-perfused suit was removed and the exposed limb was completely surrounded in bags of crushed ice for 10 min in order to facilitate rapid skin cooling. Following measurements of whole-leg blood flow after 5 min of local cooling, an arterial occlusion was performed at the level of the knee using the pneumatic cuff and temperature and

haemodynamic responses were measured at various depths within the cooled thigh in order to assess the contribution of local and systemic mechanisms to blood flow distribution within the deep and superficial tissues.

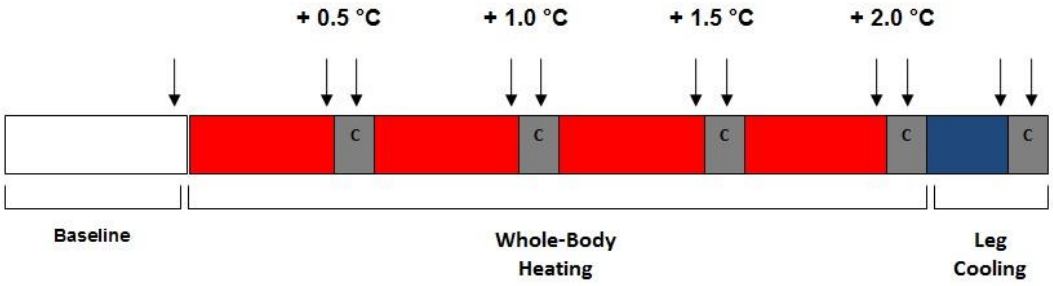

Figure 7.2: Sequence of the experimental protocol for main experimental visit. Following baseline measurements, participants were heated to T_c + 2 °C with measurements (denoted by arrows) taken during mild (+ 0.5 °C), moderate (+ 1 °C), intense (+ 1.5 °C), and severe (+ 2 °C) heat stress, both with and without arterial cuff occlusions (denoted by C) at the level of the knee. Following the heating protocol, an isolated leg was rapidly cooled and further measurements taken as before.

7.2.3 – Temperature measurements

Core temperature was measured using an oesophageal thermocouple (PhysiTemp, Clifton, NJ, USA). Following application of local anaesthetic gel (1% lidocaine) to the dominant nostril, the probe was fed into the nasal cavity and advanced to a level ¼ that of standing height to sit behind the left atrium (Fujii *et al*, 2013). Skin temperature was measured using wireless data loggers (iButtons, Maxim, USA) fitted to the arm, leg, thigh, and calf; and mean skin temperature ($\overline{T}_{sk}$) was calculated using the formula devised by Ramanathan (1964). Mean leg skin temperature was calculated as the average of thigh and calf measurements. Muscle and subcutaneous temperatures were measured using sterile implantable thermocouples inserted into the mid-portion of the vastus lateralis muscle using a 22-gauge catheter (BD Venflon, Oxford, UK; Fig. 7.3). Local anaesthetic gel (1% lidocaine) was applied to the site of measurement prior to insertion to minimise any discomfort. Muscle

temperature was measured at a depth of approximately 2-3 cm, while subcutaneous temperature was measured directly under the skin.

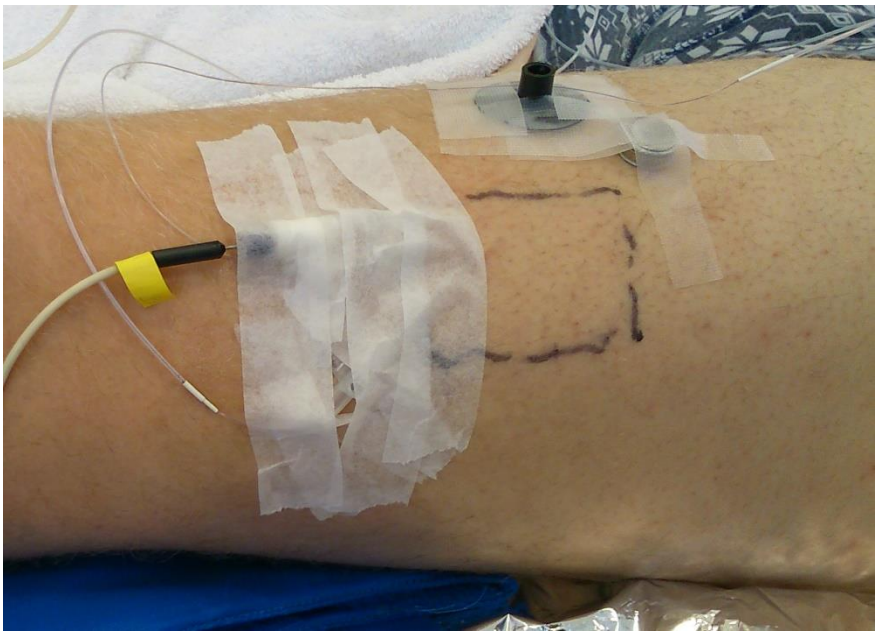

Figure 7.3: Instrumentation of the thigh for measurement of skin blood flow (laser Doppler flowmetry; black disk at top); skin temperature (wireless data logger; top right); muscle blood flow (laser Doppler flowmetry; yellow label); and muscle and subcutaneous temperatures (fine-wire thermocouples).

7.2.4 – Haemodynamic measurements

Arterial blood pressure was measured non-invasively by infrared photoplethysmography (Finometer, FMS, Netherlands) via a cuff on the middle finger of the control hand. Heart rate was determined using a 3-lead ECG. Stroke volume was estimated using the ModelFlow method included with the Beatscope computer software package (Beatscope, FMS, Netherlands), with cardiac output calculated as stroke volume x heart rate following corrections for age, height, and

weight (Wesseling *et al*, 1993). Blood flow was repeatedly measured during the different stages of heat stress and subsequent isolated leg cooling and occlusion in the common, superficial, and profunda femoral arteries (CFA, SFA, PFA, respectively) and the great saphenous vein (GSV) using a duplex Doppler ultrasound system (Vivid 7 Dimension, GE Medical, Horton, Norway) with a 10 MHz linear array transducer probe (GE Medical Systems, UK). All arterial measurements were taken at a distance of at least 2 cm from the bifurcation into the superficial and profunda femoral arteries in order to minimise disruption to measurements due to turbulent flow, whereas flow through the GSV was measured immediately distal to the pre-terminal valve separating the vein from the saphenofemoral junction. Blood flow through each vessel was calculated as the product of the average vessel cross-sectional area obtained from three 2D B-mode images and the mean velocity averaged over two 16 s Doppler scans (32 s total). Arterial diameter was consistently measured at peak systole (Rådegran, 1999), identified by an overlaid ECG trace, while venous diameter was measured every 2 s over the duration of one velocity trace (16 s total) to account for diameter changes due to both cardiac and respiratory cycles. Arterial and venous blood flows (BF) were calculated in $ml \cdot min^{-1}$ using the equation $BF = V_{mean} \times \pi \times (D/2)^2 \times 60$ where V_{mean} is the time-averaged mean velocity of the blood expressed as $cm \cdot s^{-1}$, π is a mathematical constant, D is the diameter of the vessel in cm, and 60 is a constant employed to convert the units to $ml \cdot min^{-1}$. Skin (SkBF) and muscle blood flow (MBF) were measured via laser-Doppler flowmetry (Periflux Flowmetry System, Jarfalla, Sweden), with skin probes positioned on the surface of the vastus lateralis and intramuscular probes placed at a depth of 2 cm within the muscle using the same technique as previously described for temperature thermocouples. Arterial shear rates and oscillatory flow index were calculated as described in previous chapters, while leg and systemic vascular conductance were calculated as CFA / MAP and $\dot{Q}$ / MAP, respectively.

7.2.5 – Data analysis

Blood flow and vessel diameters were recorded using the techniques described previously, burned to DVD, and analysed on specialist software installed on a stand-

alone laptop (EchoPac, GE Medical, UK). All other data were collected via a data acquisition system (Powerlab 16S, AD Instruments, UK) and fed to a desktop computer data acquisition software package (LabChart 5, AD Instruments, UK). All analysed data were stored in a PC-based computer spreadsheet software programme (Excel, Microsoft, US).

7.2.6 – Statistical analysis

A one-way repeated measures ANOVA was used to test for differences over time, with Holm-Bonferroni *post-hoc* testing employed to identify the time-points at which changes occurred once a significant effect was found. All statistical analyses were carried out using SPSS (Version 20, IBM, Armonk, US) with results expressed as mean ± SEM. Significance is set at $P < 0.05$.

7.3 – Results

7.3.1 – Temperature responses to progressive passive heat stress followed by occlusion and isolated leg cooling

Full temperature responses can be seen in Table 7.1. Whole-body passive heat-stress increased T_c by 2.0 ± 0.1 °C (mean heating duration 94 ± 5 min). T_m increased in a linear fashion from 34.9 ± 0.3 to 38.3 ± 0.1 °C, while $\overline{T}_{sk}$ and T_{sc} increased rapidly upon commencement of heating before levelling off and remaining significantly elevated for the remainder of the experiment (final values ~ 6 °C higher than baseline for both; $P < 0.05$ for all; Fig. 7.4). Isolated leg cooling following heat stress resulted in decreases in all temperature variables, with the largest responses occurring in skin and subcutaneous tissues.

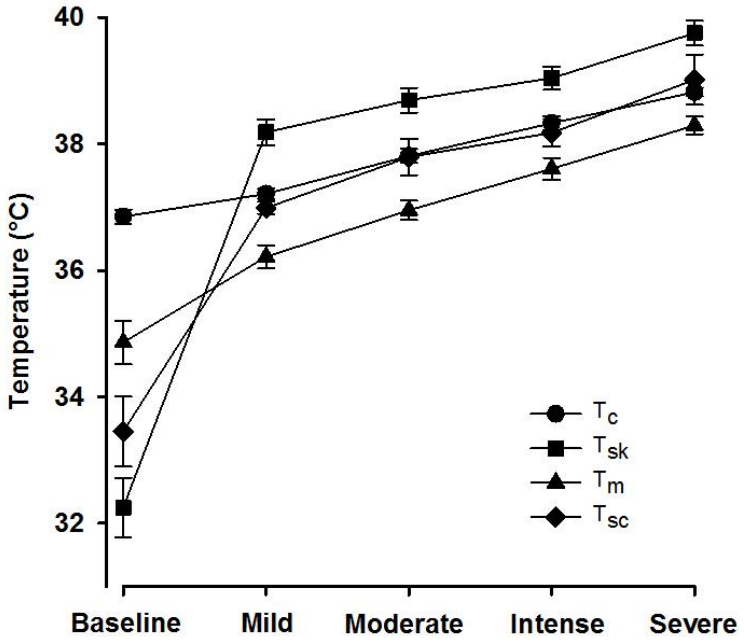

Figure 7.4: Temperature responses to progressive whole-body heat stress. T_c, core temperature; T_{sk}, skin temperature; T_m, muscle temperature; T_{sc}, subcutaneous temperature.

7.3.2 – Leg and systemic haemodynamic responses to progressive passive heat stress and subsequent occlusion and isolated leg cooling

Whole-leg blood flow (LBF) through the common femoral artery (CFA) increased in a linear fashion from 0.30 ± 0.03 l·min^{-1} at baseline to 1.24 ± 0.10 l·min^{-1} during intense heat stress (T_c + 1.5 °C), but showed no further increases upon progression to severe heat stress (final values 1.22 ± 0.11 l·min^{-1}; Table 7.1). Superficial femoral artery (SFA) blood flow showed a similar response, with an apparent plateau during the final heating stage, while profunda femoral artery (PFA) flow displayed a modest yet steady increase over the entire duration of passive heating (0.10 ± 0.01 to 0.27 ± 0.04 l·min^{-1}).

Table 7.1 – Temperature and haemodynamic responses to whole-body heat stress followed by isolated leg cooling

	Baseline	Whole-Body Heat Stress				
		Mild	Moderate	Intense	Severe	Leg Cooling
T_c (°C)	36.8 ± 0.1	37.2 ± 0.1*	37.8 ± 0.1*	38.3 ± 0.1*	38.8 ± 0.1*	38.4 ± 0.1#
T_m (°C)	34.9 ± 0.3	36.2 ± 0.2*	37.0 ± 0.2*	37.6 ± 0.2*	38.3 ± 0.1*	37.8 ± 0.2#
T_{sc} (°C)	33.5 ± 0.6	37.0 ± 0.1*	37.8 ± 0.3*	38.2 ± 0.2*	39.0 ± 0.4*	31.5 ± 1.2#
$\overline{T}_{sk}$ (°C)	33.1 ± 0.4	37.5 ± 0.3*	38.4 ± 0.2*	39.0 ± 0.2*	39.4 ± 0.2*	28.0 ± 1.6#
CFA ($l \cdot min^{-1}$)						
Whole-Leg	0.31 ± 0.03	0.53 ± 0.07*	0.86 ± 0.07*	1.24 ± 0.10*	1.22 ± 0.11	0.98 ± 0.04#
Occluded	0.22 ± 0.02	0.44 ± 0.02*	0.64 ± 0.05*	0.65 ± 0.05	0.71 ± 0.04	0.55 ± 0.05#
SFA ($l \cdot min^{-1}$)						
Whole-Leg	0.17 ± 0.01	0.41 ± 0.05*	0.73 ± 0.08*	0.91 ± 0.08*	0.95 ± 0.09	0.76 ±0.06#
Occluded	0.11 ± 0.02	0.28 ± 0.03*	0.39 ± 0.04*	0.42 ± 0.04	0.43 ± 0.04	0.29 ± 0.05#
PFA ($l \cdot min^{-1}$)						
Whole-Leg	0.10 ± 0.02	0.12 ± 0.02*	0.18 ± 0.02*	0.22 ± 0.04*	0.26 ± 0.04*	0.19 ± 0.02#
Occluded	0.08 ± 0.02	0.16 ± 0.02*	0.20 ± 0.03*	0.24 ± 0.03*	0.27 ± 0.03*	0.17 ± 0.02#
GSV ($l \cdot min^{-1}$)						
Whole-Leg	0.02 ± 0.01	0.13 ± 0.02*	0.21 ± 0.02*	0.25 ± 0.02*	0.25 ± 0.03	0.22 ± 0.05
Occluded	0.01 ± 0.01	0.10 ± 0.01*	0.15 ± 0.01*	0.16 ± 0.02	0.17 ± 0.02	0.15 ± 0.03
SkBF (LDU)						
Whole-Leg	9 ± 2	49 ± 8*	67 ± 14*	87 ± 20*	92 ± 21	-
Occluded	10 ± 2	60 ± 12*	76 ± 17*	83 ± 18	85 ± 13	-
$\dot{Q}$ ($l \cdot min^{-1}$)	6.4 ± 0.5	7.3 ± 0.3*	8.5 ± 0.6*	9.3 ± 0.6*	9.7 ± 0.6*	8.4 ± 0.5#
HR ($beats \cdot min^{-1}$)	60 ± 3	72 ± 3*	85 ± 4*	96 ± 4*	106 ± 3*	93 ± 3#
SV (ml)	107 ± 8	102 ± 8	100 ± 8*	98 ± 9	91 ± 7*	92 ± 7
MAP (mmHg)	91 ± 3	78 ± 2*	80 ± 2	81 ± 2	82 ± 3	80 ± 2

Values are mean ± SEM for eight participants. T_c, core temperature; T_m, muscle temperature; T_{sc}, subcutaneous temperature; T_{sk}, skin temperature; CFA, common femoral artery; SFA, superficial femoral artery; PFA, profunda femoral artery; GSV, great saphenous vein; SkBF, skin blood flow; $\dot{Q}$, cardiac output; HR, heart rate; SV, stroke volume; MAP, mean arterial pressure. * Significantly different from previous measurement # Significantly different from end of heating; $P < 0.05$.

Thigh blood flow (as measured in the CFA following arterial occlusions at the level of the knee) increased rapidly from baseline to moderate heat stress (0.22 ± 0.02 to 0.64 ± 0.05 l·min^{-1}; $P < 0.05$), with only minor increases from this point until the end of the protocol (final values 0.71 ± 0.04 l·min^{-1}). These changes were predominantly due to a plateau in SFA blood flow during moderate heat stress, as PFA continued to increase throughout. Blood flow to the lower leg was supplied exclusively through the SFA and showed a relatively greater increase (8-fold) over the duration of heating than thigh blood flow alone (3.5-fold), although absolute increases in flow were similar between the upper and lower leg (~ 0.5 l·min^{-1} increase to both).

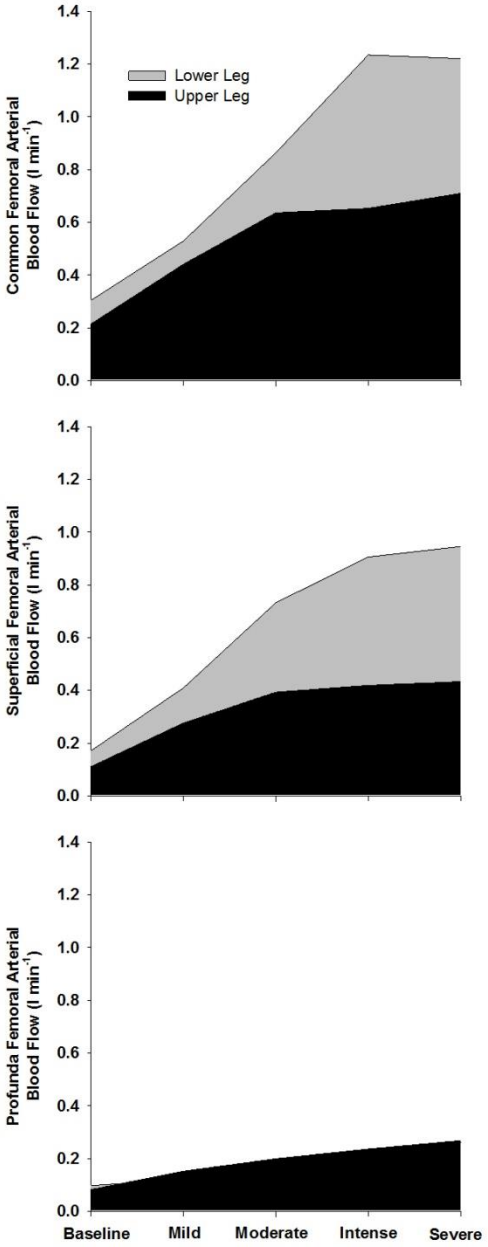

Figure 7.5: Blood flow distribution to the upper leg (black shaded area) and lower leg (grey shaded area) through each of the major conduit arteries during severe passive heat stress. Total area of top graph represents whole-leg blood flow. Data are expressed as mean values for eight subjects.

Skin blood flow (SkBF) values measured by laser-Doppler correlated well with Doppler ultrasound measurements of blood draining the superficial tissues of the leg through the great saphenous vein (GSV; R^2 = 0.74; P < 0.05), suggesting that changes in GSV flow can be used as an effective measure to investigate relative changes in skin blood flow. SkBF increased from 9 ± 2 to 87 ± 20 LDU from baseline to intense heat stress, but showed no further increase during severe heat stress (final value 92 ± 21 LDU). GSV blood flow draining both the whole-leg and thigh alone showed similar responses, with increases from 0.02 ± 0.01 to 0.25 ± 0.03 and 0.01 ± 0.01 to 0.16 ± 0.02 l·min^{-1} between baseline and intense heat stress for whole-leg and thigh, respectively, followed by a plateau during severe heat stress (final values 0.25 ± 0.03 and 0.17 ± 0.02 l·min^{-1}; NS compared to intense heat stress). Muscle blood flow (MBF) was also measured in five participants using intramuscular laser Doppler flowmetry. Results were variable between subjects, with two participants showing substantial increases, one showing a small steady increase, and two showing little to no change (Fig. 7.5).

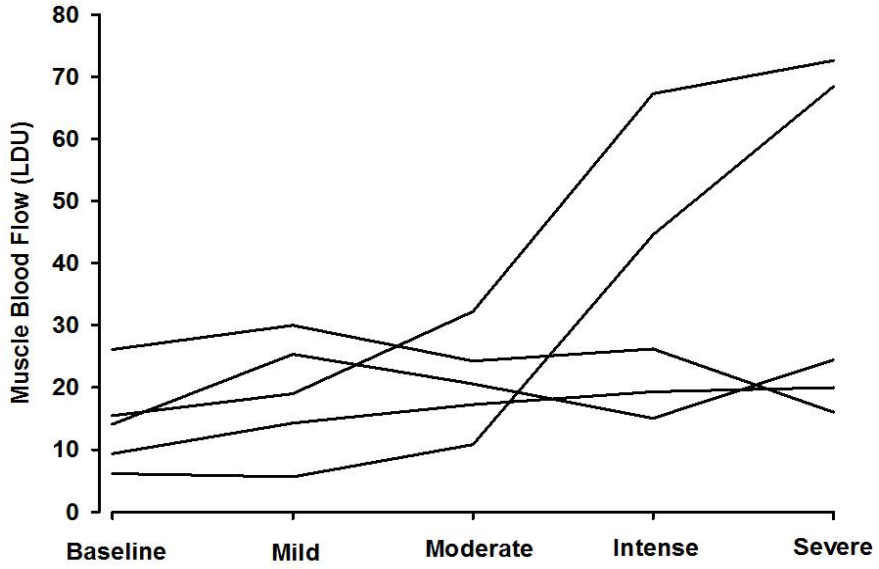

Figure 7.6: Muscle blood flow responses to severe passive heat stress as measured by intramuscular laser Doppler flowmetry. Due to problems with catheter placement and subsequent signal quality in three individuals, data shown are individual responses for five participants.

Full systemic haemodynamic responses can be seen in Table 7.1. Cardiac output ($\dot{Q}$) increased from 6.4 ± 0.5 l·min^{-1} at baseline to 9.7 ± 0.7 l·min^{-1} during severe heat stress. These increase in $\dot{Q}$ were solely due to increases in heart rate (60 ± 2 to 106 ± 3 beats·min^{-1}), as stroke volume significantly decreased throughout the duration of heating (107 ± 8 to 91 ± 6 ml; $P < 0.05$). Mean arterial pressure (MAP) decreased from baseline to moderate heat stress (90 ± 3 to 80 ± 2 mmHg; $P < 0.05$) before plateauing for the remainder of heating (final value 82 ± 3 mmHg).

7.3.3 – Flow profile and shear rate responses to progressive whole-body heat stress

Mean shear rate significantly increased in all three arteries over the duration of passive heat stress (Table 7.2; $P < 0.05$). Increased shear in the CFA and SFA occurred as a result of an increased antegrade flow and shear rate component in conjunction with a significant decrease in opposing retrograde flow and shear. Mean PFA shear increased due to increases in antegrade shear rates only, as retrograde flow and shear remained unchanged throughout. The combination of an increased antegrade and decreased retrograde shear component in both the CFA and SFA led to significant decreases in oscillatory shear index (0.25 ± 0.02 to 0.05 ± 0.04 and 0.27 ± 0.03 to 0.04 ± 0.03 for CFA and SFA, respectively), signifying almost uniform forward flow in each of these vessels during heat stress. PFA oscillatory shear index was significantly lower than CFA and SFA at baseline, and showed a downwards trend throughout heating ($P = 0.06$), finishing at levels similar to that of the other two vessels (final value 0.08 ± 0.03).

Table 7.2 – Arterial diameters and flow profiles during progressive whole-body heating

		Whole-Body Heating				
		Baseline	Mild	Moderate	Intense	Severe
Diameter (cm)	CFA	0.96 ± 0.04	0.95 ± 0.04	0.96 ± 0.04	0.96 ± 0.04	0.96 ± 0.04
	SFA	0.75 ± 0.03	0.73 ± 0.03	0.76 ± 0.03	0.76 ± 0.03	0.76 ± 0.03
	PFA	0.56 ± 0.03	0.57 ± 0.02	0.57 ± 0.02	0.58 ± 0.03	0.58 ± 0.03
V_{mean} ($cm \cdot s^{-1}$)	CFA	6.6 ± 0.6	13.3 ± 2.1*	20.8 ± 1.7*	28.4 ± 2.1*	28.5 ± 2.4*
	SFA	6.1 ± 0.3	16.6 ± 2.1*	26.8 ± 1.9*	33.9 ± 2.7*	34.4 ± 2.8*
	PFA	6.2 ± 0.6	7.7 ± 1.0*	11.7 ± 0.8*	14.4 ± 1.2*	16.8 ± 1.8*
V_{ant} ($cm \cdot s^{-1}$)	CFA	10.5 ± 1.0	16.8 ± 1.9*	23.5 ± 0.9*	30.3 ± 1.2*	30.2 ± 1.3*
	SFA	10.1 ± 0.8	19.8 ± 1.8*	28.9 ± 0.8*	35.1 ± 1.4*	36.1 ± 1.6*
	PFA	7.3 ± 0.7	9.0 ± 1.1*	12.6 ± 0.9*	15.5 ± 1.5*	18.7 ± 1.7*
V_{ret} ($cm \cdot s^{-1}$)	CFA	3.6 ± 0.5	3.1 ± 0.6	2.7 ± 0.6	0.5 ± 0.7*	0.5 ± 0.4*
	SFA	3.9 ± 0.7	2.3 ± 0.3*	1.4 ± 0.2*	0.1 ± 0.6*	0.3 ± 0.3*
	PFA	1.1 ± 0.2	1.0 ± 0.3	1.3 ± 0.2	1.1 ± 0.3	1.1 ± 0.4
SR_{mean} (s^{-1})	CFA	28.9 ± 3.7	59.8 ± 12.2*	88.5 ± 6.6*	126.0 ± 11.9*	126.3 ± 11.0*
	SFA	33.1 ± 2.4	96.4 ± 12.2*	143.4 ± 5.0*	185.5 ± 16.0*	187.4 ± 14.6*
	PFA	44.0 ± 5.6	56.9 ± 10.4*	81.6 ± 7.9*	101.3 ± 11.7*	124.1 ± 15.2*
SR_{ant} (s^{-1})	CFA	44.0 ± 5.8	72.4 ± 10.7*	99.6 ± 5.7*	128.1 ± 10.4*	128.2 ± 11.0*
	SFA	53.7 ± 5.6	108.5 ± 12.9*	150.6 ± 5.0*	185.9 ± 13.9*	188.9 ± 14.7*
	PFA	52.0 ± 6.2	64.1 ± 9.3*	91.1 ± 8.2*	109.2 ± 12.2*	132.7 ± 16.3*
SR_{ret} (s^{-1})	CFA	15.1 ± 2.6	12.7 ± 2.1	11.2 ± 2.3*	2.1 ± 2.9*	1.9 ± 1.6*
	SFA	20.6 ± 4.1	12.1 ± 1.7*	7.2 ± 0.8*	0.5 ± 3.1*	1.5 ± 1.5*
	PFA	8.0 ± 1.7	7.1 ± 1.9	9.5 ± 1.9	7.9 ± 2.6	8.6 ± 2.8
OSI	CFA	0.25 ± 0.02	0.16 ± 0.04*	0.10 ± 0.02*	0.02 ± 0.02*	0.01 ± 0.01
	SFA	0.27 ± 0.02	0.10 ± 0.02*	0.05 ± 0.01*	0.01 ± 0.02*	0.01 ± 0.01
	PFA	0.13 ± 0.03	0.12 ± 0.04	0.09 ± 0.02	0.07 ± 0.02*	0.06 ± 0.02*

Values are mean ± SEM for eight participants. V_{mean}, time-averaged mean velocity; V_{ant}, time-averaged antegrade velocity; V_{ret}, time-average retrograde velocity; SR_{mean}, mean shear rate; SR_{ant}, antegrade shear rate; SR_{ret}, retrograde shear rate; OSI, oscillatory shear index. * Significantly different from previous measurements; $P < 0.05$.

7.3.4 – Comparison of flow profile and shear rate responses during localised vs. whole-body heating

Flow profiles and shear rate responses were compared between localised leg heating (data from Chapter 5; T_m + 2.3 °C), moderate whole-body heat stress (current study T_c + 1 °C; T_m + 2 °C), and severe whole-body heat stress (current study T_c + 2 °C; T_m + 3.7 °C). Exposure to moderate whole-body heating resulted in similar blood flows, shear rates, and oscillatory shear indices in the CFA and PFA compared to local heating alone (Chapter 5), although SFA blood flow and OSI were significantly higher and lower, respectively ($P < 0.05$; Figs. 7.6 and 7.7). Further heating to severe heat stress (T_c + 2 °C) resulted in additional increases in flow to all vessels alongside an almost complete abolition of oscillatory flow (maximum OSI = 0.05).

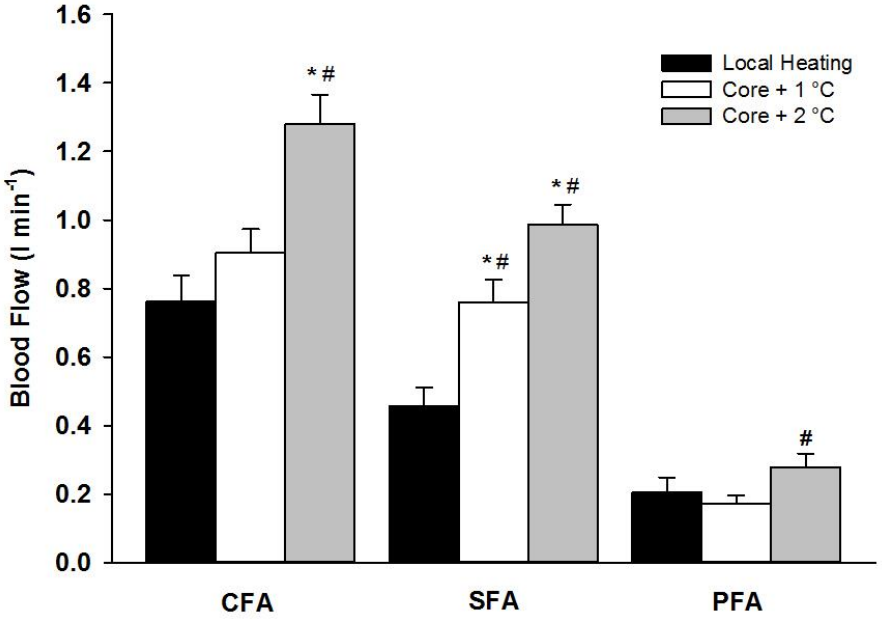

Figure 7.7 (above): Common, superficial, and profunda arterial blood flows following isolated leg heating and whole-body heating to a core temperature of + 1 and + 2 °C. Data are mean ± SEM for seven subjects for isolated heating (data from Chapter 5) and eight subjects for whole-body heating (except PFA n =7; data from current study). * Significantly different from isolated heating. # Significantly different from Core + 1 °C; *P* < 0.05.

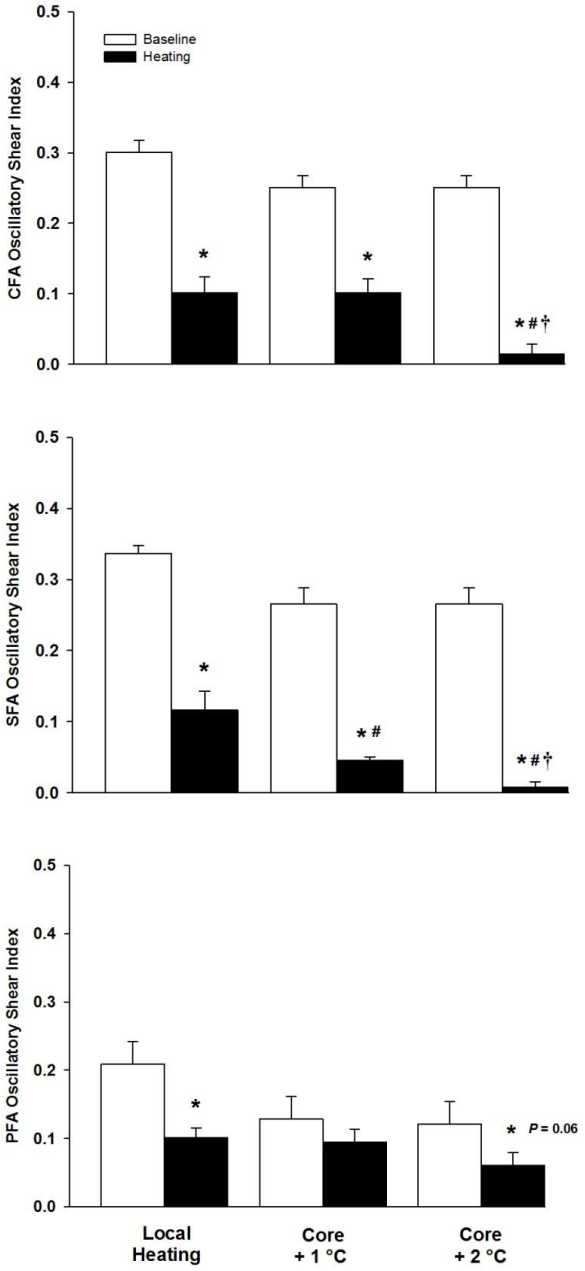

Figure 7.8 (right): Changes in oscillatory shear index in common (top), superficial (middle), and profunda (bottom) femoral arteries following exposure to isolated local heat stress and whole-body heating to a core temperature of + 1 and + 2 °C. * Significantly different from baseline. # Significantly different from local heating. †Significantly different from Core + 1 °C; *P* < 0.05.

7.3.5 – Relationship between haemodynamic responses and temperatures during severe heat stress

CFA (+ 0.93 l·min^{-1}), SFA (+ 0.73 l·min^{-1}), GSV (+ 0.23 l·min^{-1}), and SkBF (+ 78 LDU) all increased with increasing whole-body temperatures from baseline to conditions of intense heat stress (T_c + 1.5 °C; Fig. 7.8). Thereafter, no significant increases in flow were seen in any of these vessels, despite further increases in core temperature of 0.5 °C. This phenomenon was not observed in the PFA, however, with a modest yet steady increase being observed throughout the entire heating protocol (+ 0.16 l·min^{-1}). Isolated leg cooling maintained intense whole-body heat stress (+ 1.6 °C) whilst simultaneously lowering leg skin and subcutaneous temperatures to values similar to that seen at baseline (28.0 ± 1.6 *vs.* 32.4 ± 0.6 °C and 31.5 ± 1.2 *vs.* 33.5 ± 0.7 °C for $\overline{T}_{sk}$ and $T_{sc,}$ respectively; $P > 0.05$). Modest decreases in flow were observed in the CFA (- 0.24 l·min^{-1}), SFA (- 0.19 l·min^{-1}), and PFA (- 0.08 l·min^{-1}; $P < 0.05$ for all), but not in the GSV ($P > 0.05$).

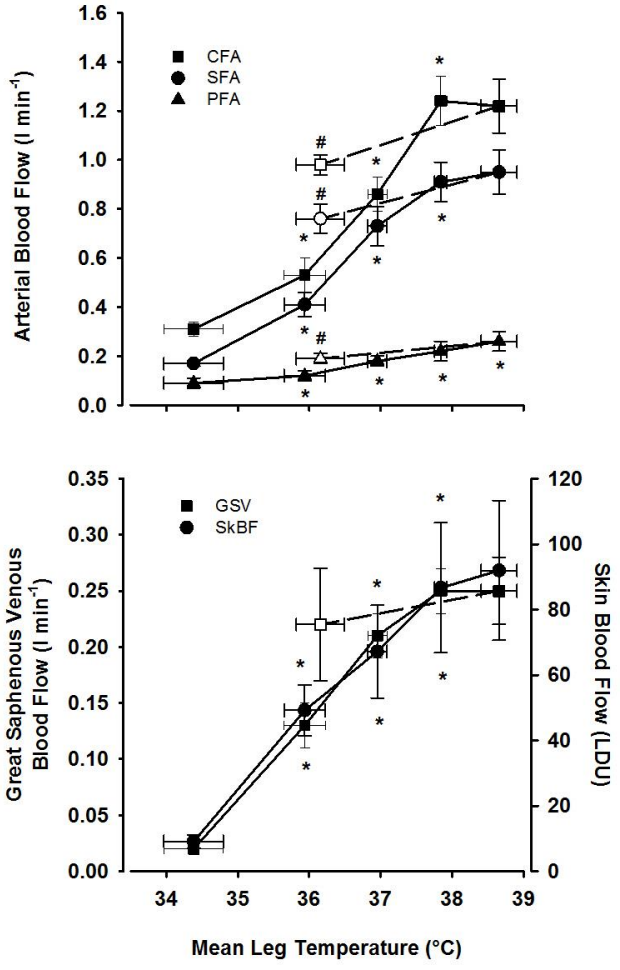

Figure 7.9: Relationship between mean leg temperature vs. common, superficial, and profunda femoral artery blood flows (top) and great saphenous vein and skin blood flow (bottom) during progressive heat stress (closed symbols) and rapid single leg cooling (open symbols). Data are mean ± SEM for eight subjects. Methodological issues prevented the measurement of SkBF during isolated leg cooling. * Significantly higher than previous measurement; # Significantly lower than end of heating; $P < 0.05$.

In order to investigate the relationship between different haemodynamic responses within the limb and their respective tissue temperatures, additional blood flow measurements were made in the great saphenous vein and profunda femoral artery during leg cooling following an arterial occlusion at the level of the knee. Systemic haemodynamic responses can be seen in Table 7.1. Blood flow through the great saphenous vein (major drainage vessel of the skin and subcutaneous tissues of the thigh) was closely related to increases in surrounding subcutaneous temperatures throughout passive heating, with rapid increases in both responses being observed at the start of the protocol before a subsequent plateau in both when approaching severe heat stress. This relationship was lost when T_{sk} and T_{sc} were rapidly cooled under conditions of high T_c, with GSV flow remaining virtually unaffected despite decreases in T_{sk} and T_{sc} to levels lower than that recorded at baseline (25.9 ± 2.0 and 30.3 ± 1.3 °C, respectively; $P < 0.05$). In contrast, blood flow through the PFA (main arterial supply to the deep tissues of the thigh) was closely related to both increases in local muscle temperature during passive heating and the subsequent decreases in T_m observed during isolated leg cooling.

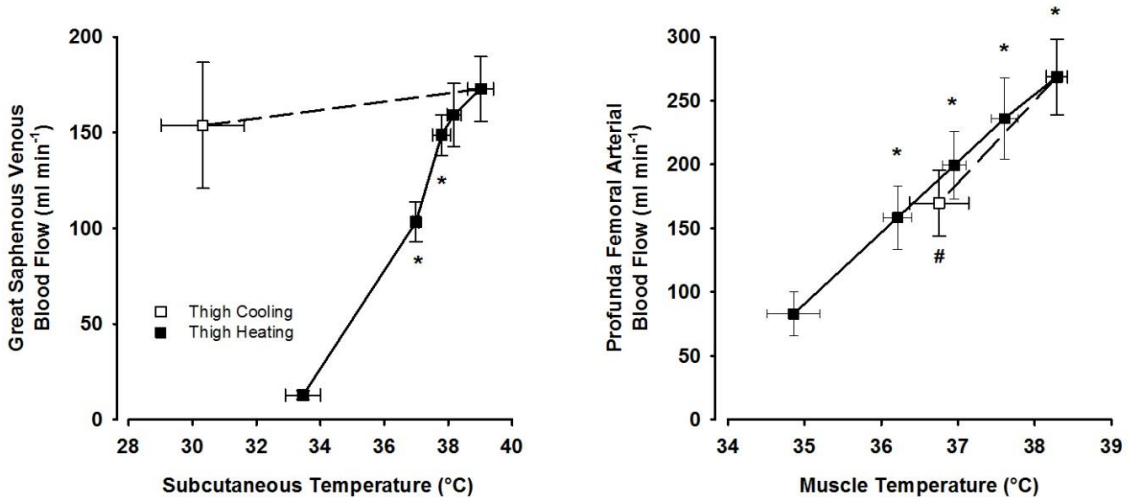

Figure 7.10: Left graph – Relationship between local subcutaneous temperatures and great saphenous venous blood flow (main drainage vessel of superficial tissues of the thigh) during both severe whole-body passive heat stress and subsequent local thigh cooling with arterial cuff occlusion. Right graph – Relationship between local deep tissue temperatures and profunda femoral arterial flow (main supply vessel to the deep tissues of the thigh). Data are mean ± SEM for eight subjects. * Significantly higher than previous measurement. # Significantly lower than end of heating; *P* < 0.05.

7.4 – Discussion

Previous chapters in this thesis reported the findings from experiments investigating blood flow responses within the human leg to both isolated heating (Chapter 5) and moderate whole-body heat stress (Chapter 6). This study aimed to expand upon these findings by investigating haemodynamic responses and their potential control mechanisms to severe passive heat stress to levels approaching that of an individual's thermal tolerance. Novel findings of this study were that 1) contrary to our initial hypothesis, the tight coupling between local temperatures and whole-leg blood flow observed in previous studies was not maintained during severe passive heat stress or subsequent rapid cooling, with leg blood flow essentially plateauing prior to the limit of thermal tolerance despite continued increases in both local and core temperatures, 2) in contrast to the rapid increase and subsequent relative plateau in whole-leg blood flow, deep tissue blood flow in the thigh (predominantly perfusing skeletal muscle) showed a steady increase throughout severe heat stress in line with increasing muscle temperatures, although absolute increases in flow were relatively modest, 3) a whole-body T_c of + 1 °C resulted in flow profiles and shear rates similar to that observed during isolated local heating alone, although further increases in heat stress to T_c + 2 °C led to the virtual abolition of oscillatory shear profiles in all three arteries, and 4) different mechanisms appear to be responsible for blood flow control in the superficial and deep tissues of the leg during severe heat stress, with skin blood flow controlled by a combination of central and local mechanisms while deep tissues are mediated at the local level alone.

7.4.1 – Leg haemodynamic responses to severe heat stress

Whole-leg blood flow increased from ~ 0.3 $l \cdot min^{-1}$ in thermoneutral ambient conditions to 1.2 $l \cdot min^{-1}$ when core and local tissue temperatures were raised by + 1.5 °C and 2.9 °C, respectively (intense heat stress). Despite further increases in whole-body temperature, leg blood flow plateaued for the remainder of heating, remaining essentially unchanged as core temperature and local tissue temperatures continued to increase to + 2 °C and + 3.6 °C (severe heat stress). These findings

expand upon previous research (previous chapter; Pearson *et al*, 2011) and show that the tight coupling between increasing local temperatures and leg blood flow is lost at severe levels of passive heat stress due to a plateau in blood supply to the leg. There are a number of potential explanations for this apparent restraint of leg blood flow at these high levels of thermal stress. Firstly, a combination of baroreceptor unloading and increased levels of circulating catecholamines during severe heat stress (Kim *et al*, 1979; Niimi *et al*, 1997; Crandall *et al*, 1999) could potentially result in decreases in leg vascular conductance in an attempt to minimise the drop in mean arterial pressure. Although previous studies have reported an attenuation in α-adrenoreceptor responsiveness in the skin (Wilson *et al*, 2001), skeletal muscle α-adrenoreceptor function has been shown to be preserved (Keller *et al*, 2010). While catecholamines were not measured in this study, the presence of an attenuated increase in deep tissue blood flow compared to that observed in Chapter 5 (55 $ml \cdot min^{-1} \cdot °C^{-1}$ muscle temperature *vs.* 44 $ml \cdot min^{-1} \cdot °C^{-1}$ muscle temperature) suggests a potential restraint of muscle blood flow during severe levels of heat stress. The relatively modest contribution of deep tissue flow to the overall hyperaemic response (~ 30-35%) suggests that this may not be the over-riding factor, however. A second, and more likely, explanation may be that a maximal vasodilation, and therefore capacitance, of cutaneous vessels during exposure to high core and skin temperatures may prevent any further significant increases in flow despite an increased drive from temperature-sensitive mechanisms. An interesting observation in the current study was an early plateau in thigh blood flow of 0.7 $l \cdot min^{-1}$ upon reaching moderate heat stress, with further increases in core temperature having no effect on thigh skin temperature (~ 39 °C), superficial femoral arterial flow (main supplier of thigh superficial tissues) and great saphenous venous return (major drainage vessel of thigh cutaneous superficial tissues). These findings point to a maximal perfusion of the cutaneous tissues in this portion of the leg, as lower leg blood flow continued to increase in line with a slower warming of the distal portion of the limb.

7.4.2 – Leg blood flow distribution during severe heat stress

Early attempts to estimate maximal whole-skin blood flow in humans by the extrapolation of forearm blood flow and various other systemic measurements have suggested a cutaneous capacity of ~ 7 – 8 $l \cdot min^{-1}$ (Rowell *et al*, 1969). If all of the observed 0.5 $l \cdot min^{-1}$ increase in thigh blood flow observed here was directed solely to the skin, extrapolation of this figure would result in a whole-body skin blood flow of > 11 $l \cdot min^{-1}$ (assuming a thigh skin volume of 0.55 litres and a whole-body skin volume in a 75 kg human of 12.5 litres; Wang *et al* 1999); a value much higher than that reported in classical studies. In contrast to the plateau in blood flow through superficial vessels, flow in the profunda femoral artery supplying the deep thigh tissues displayed a modest yet continual increase throughout heating in line with increases in local tissue temperatures (~ 0.8 $ml \cdot dl^{-1} \cdot min^{-1} \cdot °C^{-1}$). Taking this deep tissue blood flow increase into account, the remaining 0.35 $l \cdot min^{-1}$ increase in flow to the thigh superficial tissues would extrapolate to a whole-skin blood flow of 7.5 – 8 $l \cdot min^{-1}$, similar to that reported in previous studies (Rowell *et al*, 1969) and once again suggesting a contribution of skeletal muscle blood flow to the heat-stress hyperaemic response. In order to try to confirm the presence of this increased muscle blood flow, we employed an intramuscular laser Doppler flowmetry technique previously unreported in humans in an attempt to detect changes in blood flow in the muscle microcirculation itself. Of the five participants tested; two showed significant increases in muscle blood flow throughout the heating protocol, one showed a gradual yet steady increase, and two showed no change (Fig. 7.5). Given the sampling area covered by the laser Doppler technique (~ 1 mm from the tip of the probe) and the extensive heterogeneity present in skeletal muscle perfusion during heat stress (Akyürekli *et al*, 1997; Heinonen *et al*, 2011), the variation in results is perhaps not surprising. The presence of significant blood flow increases in a number of participants, however, would appear to lend further evidence to the ability of skeletal muscle fibres to increase perfusion in response to increasing local temperatures.

7.4.3 – Flow profiles and shear rate responses

Previous authors have suggested whole-body or indirect heating as a potential non-pharmacological means for improving cardiovascular health in certain clinical populations (Imamura *et al*, 2001; Kihara *et al*, 2002; Carter *et al*, 2014). Part of these improvements have been attributed to improvements in vascular endothelial health resulting from increased antegrade and decreased retrograde shear rates; the former of which has been shown to be beneficial to endothelial function (Tinken *et al*, 2009; Tinken *et al*, 2010) while the latter appears detrimental (Thijssen *et al*, 2009; Schreuder *et al*, 2014). The majority of studies to date looking at repeated heat exposures as a method of 'thermoregulatory conditioning' have employed whole-body heating to moderate levels of heat stress (Imamura *et al*, 2001; Kihara *et al*, 2002; Carter *et al*, 2014). Whilst severe whole-body heating in the current study (T_c + 2 °C) led to the complete abolition of oscillatory shear profiles and apparent idealised flow profiles within the major arterial vessels of the leg, the significant cardiac strain and thermal discomfort associated with heat stress at these levels would make it unfeasible as a potential therapeutic intervention. Findings from this study provide evidence that moderate whole-body heating to T_c + 1 °C results in similar blood flow increases and shear rate patterns as that observed during localised heating of the limb alone (Chapter 5), with no significant difference observed in any variable in the CFA and PFA, and only modest improvements in blood flow and OSI values recorded in the SFA (~ 300 ml·min^{-1} and 0.05, respectively for flow and OSI; $P < 0.05$). These findings show for the first time that localised heating of the leg has the potential to increase blood flow and anti-atherogenic shear rates in the major arterial vessels of the leg to levels equivalent to that seen during moderate whole-body heating, although without the added cardiac strain (increase in heart rate of up to 20-30 beats·min^{-1} and cardiac output of up to 2 l·min^{-1}) and thermal discomfort associated with whole-body heating protocols.

7.4.4 – Relationship between local tissue temperatures and haemodynamic responses during severe heat stress

In order to assess the importance of core vs. local temperature mechanisms to haemodynamic responses within the leg, we employed a rapid leg cooling protocol following severe heat stress in order to quickly lower local temperatures whilst core temperatures remained high. In an attempt to separate the individual effects of local temperature changes between skin and muscle tissues, we occluded blood flow at the level of the knee and assessed the relationship between 1) subcutaneous tissue temperature and great saphenous venous flow (major drainage vessel of the superficial tissues of the thigh and taken to predominantly represent relative changes in skin tissue perfusion alone), and 2) deep muscle temperature and profunda femoral arterial flow (major supplier to deep tissues of the thigh and taken to predominantly represent relative changes to skeletal muscle tissues alone). Rapid cooling of the leg following heat stress had no noticeable effect on great saphenous venous blood flow (Δ -19 ml·min^{-1}; $P > 0.05$), despite significant decreases in local subcutaneous temperatures to levels below that recorded at baseline (final value ~ 30 °C compared to 33 °C at baseline; Figure 7.8). These findings are in contrast to our previous results displaying exclusively local control of leg blood flow during moderate heat stress (Chapter 6), but agree with previous heat stress studies where an over-riding cutaneous vasodilation was found to be maintained during cooling from prior heat stress (Brengelmann *et al*, 1973; Wyss *et al*, 1975; Vuksanović *et al*, 2008). With the relative contribution of central vs. local mechanisms to skin blood flow control reported to be in the region of 10:1 (i.e. a 1 °C change in core temperature exerting 9x the effect of the same increase in the skin temperature) (Wyss *et al*, 1974; Wyss *et al*, 1975; Wenger *et al*, 1975; Proppe *et al*, 1976), the rapid elevation in thigh skin temperature to ~ 39 °C would most likely have resulted in maximal dilation of cutaneous vessels through local-temperature sensitive mechanisms (Black *et al*, 2008) before core temperature increased sufficiently to exert reflex central effects. When cooling the limb following severe heat stress however, a decrease in skin and subcutaneous temperatures by 11 °C and 7.5 °C, respectively, would be insufficient to counteract the central drive exerted by the ongoing high core temperature (+ 1.6 °C). These findings suggest a differential control of limb blood flow during heating

and subsequent cooling, with local temperature-sensitive mechanisms being responsible for tissue hyperaemia during heating, but underlying central reflexes mediating haemodynamic responses when skin and subcutaneous tissues are rapidly reduced.

A novel finding of this study was that in contrast to the control of skin blood flow, deep tissue blood flow appeared to continue to be mediated solely at a local level during all stages of heat stress, with profunda femoral arterial blood flow not only increasing in a linear fashion with muscle temperature to the limits of thermal tolerance, but also decreasing at the same rate as local muscle tissues were rapidly cooled (Figure 7.8). This finding expands upon previous research showing the local control of skeletal muscle blood flow during moderate heat stress (Chapters 4 and 5, Heinonen *et al*, 2011) by showing that this response is maintained throughout severe levels of heat stress (despite an accompanying plateau in skin blood flow) and can be reversed with local cooling even when core temperatures are significantly elevated.

7.4.5 – Limitations

Whilst an argument has been made for a potential therapeutic effect of heating on vascular function, future studies are required to test this hypothesis further. Results from this study have provided evidence of alterations in flow rates and shear rate responses in the leg that have previously been shown to have positive anti-atherogenic effects within the brachial artery. Whether the magnitude of changes in flow profiles observed is sufficient to cause endothelial adaptation in the leg, however, and whether the same benefits are observed in elderly/clinical populations in comparison to the healthy study group reported here remains to be investigated

7.4.6 – Conclusions

Whole-leg blood flow effectively plateaus during severe heat stress, most likely due to the maximal vasodilation of cutaneous blood vessels. The control of this skin blood flow during severe levels of heat stress is mediated to an extent by elevated core temperatures, as evidenced by a relatively unchanged skin blood flow response when local temperatures were rapidly cooled. Muscle blood flow on the other hand increases modestly throughout all levels of heating at a linear rate calculated to be ~ 0.8 $ml \cdot dl^{-1} \cdot min^{-1} \cdot °C$. This control appears to be exerted solely by local temperature-sensitive mechanisms, as rapid thigh cooling leads to decreases in deep tissue blood flow in line with decreasing deep tissue temperatures, despite continued elevated core temperatures. The observation of comparable improvements in flow rates and shear profiles during both localised and moderate whole-body heating suggests that local tissue heating in the lower limbs may provide the same potential therapeutic benefits in certain clinical populations (e.g. lower limb peripheral arterial disease) without the confounding factors of cardiac strain and thermal discomfort associated with whole-body heat stress.

CHAPTER 8

General Discussion and Conclusions

8.1 – Introduction

The primary aims of this thesis were to 1) investigate the global haemodynamic responses of the resting human arm and leg and the blood flow distribution among the leg's main conduit arteries to direct thermal challenges both at a local level and during progressive elevations in systemic heat stress, 2) to ascertain the contribution of local vs. systemic thermosensitive mechanisms towards this regulation, and 3) to investigate the same responses during single-legged small-muscle mass exercise to near maximal levels. In Chapters 4 and 5, haemodynamic responses within the human arm and leg were investigated in order to systematically characterise blood flow responses and regulation whilst undergoing changes in local tissue temperatures alone. Chapter 6 looked to not only characterise these responses during moderate whole-body heat stress, but to identify the relative contribution of local *vs.* central mechanisms in their regulation through the use of both a contralateral leg cooling and heating protocol and the inclusion of isolated leg heating data from the previous chapter. In addition to resting measurements, incremental small muscle mass exercise to levels approaching peak power output were also assessed in this chapter in an attempt to clarify discrepancies in the literature as to the effects of heat stress on exercising leg blood flow. Finally, Chapter 7 extended the severity of heat stress to levels approaching that of an individual's thermal tolerance to establish whether the similar regulatory mechanisms operated in these conditions, and also performed a rapid limb cooling protocol whilst heat-stressed in order to offer further insight into this mechanistic control.

The following chapter will review the main findings of the thesis and explain the novel contribution made by these results to the existing literature in the area. In addition, any limitations inherent to the studies carried out will be discussed and recommendations for future research will be made.

8.2 – Main findings

8.2.1 – Limb haemodynamic responses during passive local and whole-body thermal challenges

Results from Chapters 4 and 5 revealed a similar haemodynamic response in both the arm and leg during local cooling and heating, with small reductions in flow below baseline blood/tissue temperatures of ~ 35 °C and a substantially greater magnitude of response at temperatures above this level, indicative of distinct temperature sensitivities of the limb tissues to cold and hot temperatures. Arm blood flow showed a relatively greater decrease than that of the leg during 1 h of localised cooling (50% *vs* 20%) as well as a greater increase during the same duration of both localised and moderate whole-body heating (~ 400% *vs.* 200%; Chapters 4,5,6). While part of this response may have occurred due to the larger surface-to-volume ratio within the arm – and therefore higher rate of heat transfer to/from the tissues over the same period of time – the normalisation of flow to units of tissue per °C change in local temperature displayed a clear heterogeneous response between the limbs, with isolated heat stress resulting in increases of 5.5 and 2.3 $ml \cdot dl^{-1} \cdot min^{-1} \cdot °C^{-1}$ for the arm and leg, respectively (Chapters 4 and 5). Although methodological differences between isolated arm and leg studies resulted in different sites of temperature measurements (blood and muscle, respectively), the small tissue mass present in the arm combined with records of muscle temperatures from similar previous interventions suggests that the magnitude of change in forearm blood and muscle temperatures are similar (Abramson *et al*, 1958). The reason for the differences in haemodynamic responses could not be directly elucidated from these studies, but could potentially be due to the presence of an increased vasodilator (Newcomer *et al*, 2004) and decreased vasoconstrictor (Pawelczyk and Levine; 2002) responsiveness in the arm in comparison to the leg, or due to the higher skin-to-muscle ratio in the arm in comparison to the leg.

During whole-body heat stress, the association between increasing local tissue temperatures and leg blood flow was similar to that observed during isolated leg heating alone (2.6 *vs.* 2.3 $ml \cdot dl^{-1} \cdot min^{-1} \cdot °C^{-1}$). Blood flow in the leg essentially plateaued at ~ 1.2 $l \cdot min^{-1}$ prior to severe heat stress, most likely due to a combination of maximal cutaneous vasodilation (as evidenced by plateaus in SFA and GSV) and only modest increases in underlying muscle blood flow with increasing temperatures (as evidenced by a continual linear increase in PFA flow until the end of heating). Although arm blood flow was not measured during severe heat stress, if a similar increase to that of isolated arm heating (5.5 $ml \cdot dl^{-1} \cdot min^{-1} \cdot °C^{-1}$) was observed during whole-body heat stress, a maximal arm blood flow of ~ 0.6 $l \cdot min^{-1}$ could be predicted, matching previously reported maximal levels during forearm warm water immersion (Naylor *et al*, 2011). Taken together, these figures indicate that up to 33% of the total cardiac output is directed to the limbs during severe whole-body heat stress in comparison to ~ 15% in resting thermoneutral conditions.

Attempts to quantify changes in skeletal muscle perfusion during heat stress in the forearm (Chapter 4) and leg (Chapters 6 and 7) provide evidence of ~ 30% of the observed hyperaemic response being directed to this tissue bed. Reductions in the a-vO_2 difference of venous blood predominantly draining the deep tissues of both of these limbs suggests the presence of a reciprocal increase in blood flow despite only modest changes in limb $\dot{V}O_2$. The use of the Fick Equation in the forearm and the measurement of profunda femoral arterial flow in the leg provided an estimation of blood flow delivery to underlying skeletal muscle tissues, with 0.08 $l \cdot min^{-1}$ out of a total hyperaemic response of 0.34 $l \cdot min^{-1}$ occurring in the forearm and 0.18 $l \cdot min^{-1}$ out of 0.51 $l \cdot min^{-1}$ occurring in the thigh (25% and 35% of total response, respectively). The estimated 80% increase in forearm muscle blood flow broadly agrees with previous PET measurements of flow in the human calf during equivalent levels of direct local heat stress (Heinonen *et al*, 2011). Previous studies have shown the presence of significant heterogeneity in muscle perfusion during heating (Akyürekli *et al*, 1997; Heinonen *et al*, 2011), which may explain the mixed results observed when intramuscular laser Doppler flowmetry was performed in Chapter 7 to confirm increases in thigh tissue perfusion. Together, these findings reveal the control of limb blood flow through thermosensitive mechanisms is significantly

increased at temperatures above resting baseline values of ~ 35 °C. This effect is more pronounced in the arm per unit volume of tissue, but the smaller muscle mass results in absolute increases only ~ ½ that of the leg. Furthermore, increases in skeletal muscle perfusion may account for up to 30% of this observed hyperaemic response.

8.2.2 – Role of local vs. central mechanisms of limb blood flow control during passive whole-body heat stress

The collective findings from Chapters 5 – 7 of this thesis suggest that local temperature-sensitive mechanisms are the major driving force for increases in limb blood flow during progressive increases in direct heat stress ranging from isolated leg heating through to severe whole-body hyperthermia.

Leg blood flow during isolated leg heat stress in Chapter 5 increased ~ 3-fold from 0.25 to 0.75 l·min^{-1}. These effects were exerted almost entirely through local thermosensitive mechanisms (> 90 %), as contralateral control leg blood flow increased only 10% of that in the experimental limb. With no increase in core temperature, however, and previous research using brachial plexus nerve blockade showing no central contribution to flow during localised forearm heating (Wenger *et al*, 1986; Minson *et al*, 2001), the small elevation in control leg blood flow was most likely due to heat transfer between the limbs and/or the activation of local thermosensitive mechanisms as opposed to a central reflex response. Further evidence for this local control came from comparisons with local tissue temperatures within the leg, with a strong association found between the two ($R^2 = 0.55$; $P < 0.01$). To assess the relative contribution of these local mechanisms during whole-body heating, a within-subjects contralateral limb model was employed in Chapter 6 to allow the manipulation of local temperatures within opposing legs whilst simultaneously exposing participants to moderate levels of whole-body heat stress, and showed that leg blood flow during 1 h of moderate whole-body heat stress remained essentially unchanged if local temperatures were prevented from rising in

line with the rest of the body and opposing leg (0.27 to 0.32 l·min^{-1} *vs.* 0.30 to 0.88 l·min^{-1} for cooled and heated legs, respectively). Furthermore, when comparing heated leg blood flow responses following isolated heating in Chapter 5 to whole-body heating in Chapter 6, the magnitude of change was almost identical (within 0.1 l·min^{-1}), despite differences in core temperature and cardiac output of 0.6 °C and 2.4 l·min^{-1}, respectively. Collectively, these studies suggest that during heat stress to levels of moderate hyperthermia, leg blood flow is controlled through a predominantly locally-mediated vasodilation in both skin and skeletal muscle in line with increasing local blood and tissue temperatures and independent of systemic responses.

These findings contrast with classical studies investigating limb vasomotor control, in which the majority of influence was attributed to central reflex drive (Grant and Holling; 1938; Doupe *et al*, 1943; Edholm *et al*, 1957; Roddie *et al*, 1957; Blair *et al*, 1960). This fundamental difference in findings can most likely be explained, however, by the different methodological approaches used to raise body temperature. The use of indirect passive heating (Grant and Holling; 1938; Edholm *et al*, 1957; Roddie *et al*, 1957) or lower body exercise (Blair *et al*, 1960) to raise body temperatures in previous studies would have resulted in increased core temperatures but relatively little change in skin temperature at the site of measurement in the forearm, thereby providing predominantly reflex mechanisms of action. In contrast, the direct whole-body heating approach in the current study resulted in rapid elevations of skin temperature to ~ 39 °C, a response which has been shown to cause locally-mediated near-maximal skin vasodilation within 20-30 min (Minson, 2010). With skeletal muscle blood flow controlled through local mechanisms alone (Heinonen *et al*, 2011) and only accounting for a maximum of ~ 30% of total increases in limb blood flow, the current form of direct heat stress would likely increase leg blood flow to near maximal-levels before increases in core temperature could rise sufficiently to exert any significant effects through reflex cutaneous drive.

Further evidence supporting this theory was provided in Chapter 7, in which heat stress was continued to levels approaching each participant's thermal tolerance ($T_c \geq$

2 °C). This study once again showed an elevated leg blood flow in line with increasing local tissue temperatures, with both the threshold and sensitivity of the hyperaemic response comparable to results obtained during isolated heating alone (Fig. 8.1).

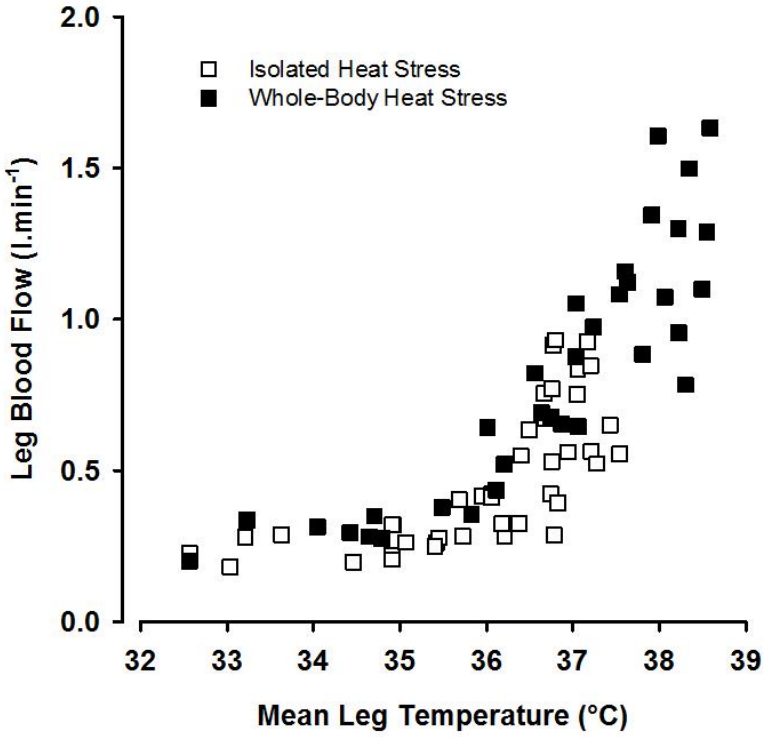

Fig. 8.1 – Relationship between leg blood flow and mean leg temperature during isolated leg heating (Chapter 5) and severe whole-body heat stress (Chapter 7). Individual data points from both studies (n = 7 for both).

This response plateaued in the SFA and GSV prior to the attainment of maximal heat stress, suggesting a maximal vasodilatory capacity in the cutaneous circulation. In contrast, blood flow through the major vessel supplying the skeletal muscle of the thigh (PFA) showed a modest linear increase until the termination of heat stress (0.10 to 0.26 l·min^{-1}). Subsequent rapid cooling of an occluded thigh reduced local tissue temperatures back towards baseline values whilst maintaining a significantly

elevated core temperature. PFA flow decreased in line with reductions in muscle tissue temperature, providing further evidence that changes in the skeletal muscle vascular bed are unaffected by any form of central drive. Blood flow through the GSV, however (the major drainage vessel of the cutaneous circulation of the thigh), showed very little change (0.17 to 0.15 $l \cdot min^{-1}$), despite skin and subcutaneous temperatures decreasing to sub-baseline levels. Whilst local mechanisms drive the increase in leg blood flow during progressive elevations in heat stress, the removal of local heat-sensitive stimuli when already hyperthermic appears to exert a significant effect on leg skeletal muscle perfusion alone, with skin blood flow most likely maintained due to the presence of a previously masked underlying reflex active vasodilation (Fig. 8.2).

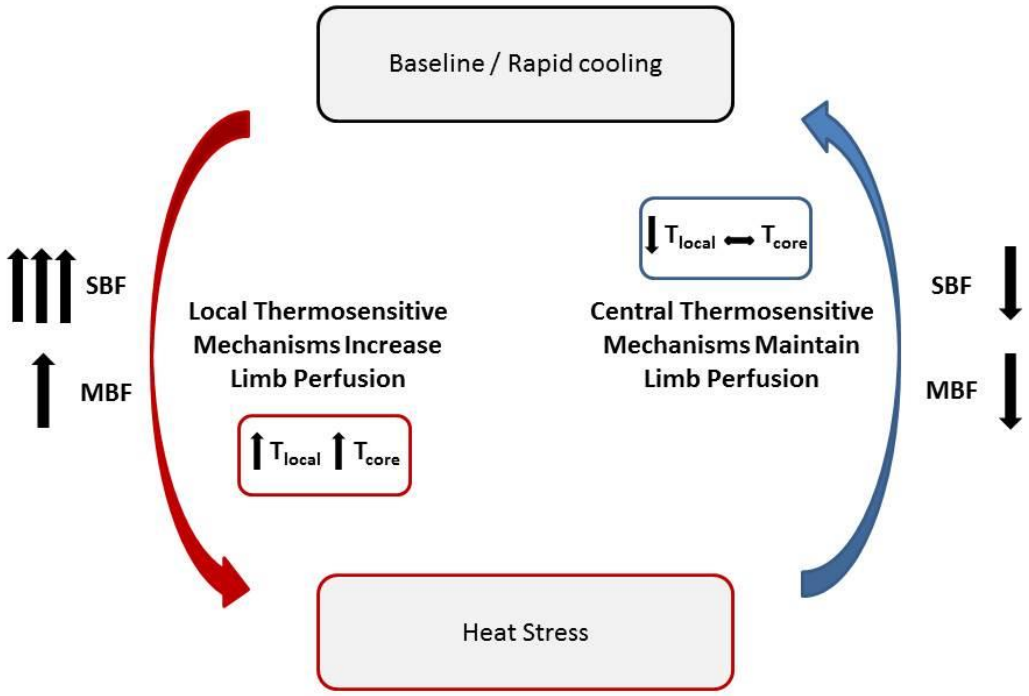

Fig. 8.2 – Proposed influence of local *vs.* central thermosensitive mechanisms on limb tissue perfusion in directly-heated humans. Rapid elevations in skin temperature during heating leads to the majority of elevations in skin blood flow through predominantly local mechanisms prior to increases in core temperature of a sufficient magnitude to activate reflex active vasodilation. Muscle blood flow increases to a lesser extent in line with local muscle temperatures. Upon rapid cooling, an unchanged high core temperature preserves the majority of skin blood through reflex mechanisms despite the removal of local stimuli, while muscle blood flow decreases in line with local muscle temperatures.

The finding of a persistently high skin blood flow agrees with early data in the forearm following the cooling of previously heated subjects (Detry *et al*, 1972). Interestingly, the same effect is also apparent during simulated haemorrhage (LBNP) in directly heated humans (Pearson *et al*, 2013); a condition in which it appears that the direct local effects of heating are acting to over-riding centrally-mediated signals to vasoconstrict. Together, these data demonstrate the ability of either local or reflex mechanisms to act as the dominant control mechanism for skin blood flow depending on the nature and timing of the challenge exerted on the body, and once again highlights the importance of the methodological technique used in investigations into the control mechanisms regulating limb blood flow.

While much work has gone into looking at the mechanisms underlying the control of vasodilation in the cutaneous circulation, the long-standing concept of an unchanged underlying skeletal muscle perfusion during exposure to heat stress has resulted in little investigation into potential mechanistic control within these tissues. With heat stress having no impact on inherent smooth muscle contractile function (Ives *et al*, 2011), control of muscle blood flow during heat stress would once again seem likely to result as a net effect of competing vasoconstrictor and vasodilator responses. The presence of increased sympathetic nerve activity (Niimi *et al*, 1997) and preserved α-adrenoreceptor responsiveness (Keller *et al*, 2010) in skeletal muscle arteries has recently been shown to be opposed by heat-stress induced vasodilatory metabolites (Keller *et al*, 2010), with nitric oxide and its sympatholytic properties identified as being at least partially responsible (Ives *et al*, 2012). Another sympatholytic agent shown to be released during heat-stress both *in vitro* (Kalsi and González-Alonso; 2012) and *in vivo* (Pearson *et al*, 2011, Kalsi *et al*, unpublished data) is ATP; a potent dilator of the skeletal muscle vasculature during exercise and a further potential candidate for the hyperaemic response observed in these studies. In Chapter 6 of this thesis, a locally-mediated increase in leg $\dot{V}O_2$ during whole-body heating may have provided a metabolically-driven stimulus for at least a portion of the observed hyperaemic response. The presence of this 65% increase in heated leg $\dot{V}O_2$ alongside a decrease in deep venous a-vO_2 difference and unaltered glucose uptake suggests that the extracellular mechanisms of hyperaemia often associated with increased

oxygen uptake during exercise are not the same as those observed during passive heat stress.

8.2.3 – Leg blood flow responses and regulation during incremental small muscle mass exercise

Incremental single-legged knee extensor exercise was carried out to 80% peak power output under four different experimental conditions: 1) isolated cooled leg 2) isolated heated leg 3) cooled leg during whole-body heat stress, and 4) heated leg during whole-body heat stress. The use of these four different protocols once again allowed the relative contributions of the local *vs*. central mechanisms controlling leg blood flow to be elucidated, although this time with the addition of the metabolic stress of exercise. Findings presented here indicate that leg blood flow during small muscle mass exercise in the heat is determined by a combination of both the metabolic workload of the exercise bout and the local tissue temperatures of the leg. This regulation also appears to occur independently of systemic responses, as cooled and heated leg blood flows during whole-body heat stress were virtually identical to their counterparts when systemic heat stress was absent, despite differences in core temperature of up to 1 °C between the conditions. These findings agree with previous work from this laboratory in which mild intensity single-legged exercise in the heat resulted in an elevated blood flow compared to the same exercise in control conditions (Pearson *et al*, 2011), but also extend upon them by 1) identifying local temperature-sensitive mechanisms as the primary factor regulating differences in leg blood flow, and 2) displaying that this response is maintained even at exercise intensities approaching peak power output.

The limited number of studies carried out prior to that of Pearson *et al.* (2010) looking at leg blood flow during exercise in the heat reported no change from exercise in control conditions, with muscle blood flow maintained and skin blood flow therefore presumably reduced (Savard *et al*, 1988; Nielsen *et al*, 1990). Although a reflex reduction in skin blood flow is commonly reported during exercise

in the heat (see Fig. 2.8 in Literature Review), a number of studies from Taylor *et al.* has shown that this effect is not apparent if absolute exercise intensity is less than ~ 125 W, and can be further attenuated in the presence of high local skin temperatures (Taylor *et al*, 1984; Taylor *et al*, 1988; Taylor *et al*, 1990). The use of a small muscle mass exercise modality in this thesis – combined with high skin and tissue temperatures due to direct whole-body heating – most likely negates any potential central reflex effects due to the low absolute exercise intensities achieved during single-legged knee extensions (~ 50 W even at 80% PPO) and the submaximal challenge presented to the cardiovascular system (~ 14 $l \cdot min^{-1}$); thereby allowing the local effects of increased temperatures alone to be identified.

Due to the inability to measure profunda femoral arterial flow during exercise in Chapter 6, the distribution of blood flow between skin and underlying muscle tissues could not be directly assessed. The presence in the heated leg of an increased $\dot{V}O_2$ combined with the tendency for a-vO_2 difference of blood draining the deep tissues to be lower, however, pointed to the presence of an elevated skeletal muscle perfusion throughout one-legged exercise. Increases in local tissue temperature have been postulated as one of the potential mechanisms contributing to the hyperaemic response during exercise, with skeletal muscle mechanical inefficiency rapidly generating vast amounts of heat within the exercising tissues (Buckwalter and Clifford; 2001). In line with this, recent research has shown that heat sensitises the endothelium within human arteries to shear stress, with elevations in temperature increasing both eNOS activation and nitric oxide production (Ives *et al*, 2012). As such, increases in muscle temperatures may help to facilitate the release of sympatholytic metabolites during both passive heating and exercise that aid in the attenuation of adrenergic drive and promote vasodilation. However, the leg blood flow sensitivity during passive heat stress of 0.9 $l \cdot min^{-1} \cdot °C^{-1}$, much of which likely supplies the cutaneous circulation, indicates that this effect only accounts for a fraction of the exercise hyperaemic response.

8.2.4 – Shear rates and oscillatory shear indices during heat stress

Data from this thesis has provided novel evidence of a similar response in each of the major arteries of the leg during both local and whole-body heating protocols compared to that previously reported in the arm. Localised heating of the leg (Chapter 5) led to a ~ 3-fold increase in mean shear rates over the course of 1 h and a reduction in oscillatory shear index from 0.3 to 0.1, broadly agreeing with responses observed in forearm models during direct heating (Naylor *et al*, 2011; Padilla *et al*, 2011), indirect heating (Carter *et al*, 2014) and lower-body exercise (Simmons *et al*, 2011; Padilla *et al*, 2011). Whole-body passive heat stress to a core temperature of + 2 °C in Chapter 7 virtually abolished the presence of any retrograde, and therefore oscillatory, flow profiles altogether. Interestingly, haemodynamic responses in the same study during moderate whole-body heating to T_c + 1 °C showed relatively little additional change from localised heat stress alone, with only the SFA showing any further reductions in oscillatory shear index.

The combined presence of low mean and high oscillatory shear patterns has repeatedly been shown to present a detrimental stimulus to the endothelium both *in vitro* in isolated vessels (Chiu *et al*, 1998; Chen *et al*, 2002; Hastings *et al*, 2007), and *in vivo* in the forearm (Thijssen *et al*, 2009) and the leg (Schreuder *et al*, 2014). The ability of increased tissue temperatures to create a high mean shear rate and/or low oscillatory shear index profile within the human forearm has been documented in a number of previous studies during both passive heating (Naylor *et al*, 2011; Carter *et al*, 2014) and lower-body dynamic exercise (Simmons *et al*, 2011; Padilla *et al*, 2011), and repeated exposure to these changes has been shown to improve vascular function (assessed via FMD) in the brachial artery (Naylor *et al*, 2011; Carter *et al*, 2014). No study to date, however, had assessed these same haemodynamic responses within the human leg, which could potentially differ due to the known heterogeneity between the limbs. Indeed, in the resting state, mean shear rates in the femoral arteries are only ~ 1/3 of that observed in the brachial artery (data from Chapters 4 and 5; Schreuder *et al*, 2014), while oscillatory shear index is almost 3x as high. With the forearm vasculature generally resistant to both atherosclerosis

and venous thrombosis, part of the vascular protective effect in the brachial artery could come through the presence of its significantly higher resting mean shear rates and lower oscillatory flow profiles when compared to that in the leg (mean shear rate ~ 100 s^{-1} *vs.* 30 s^{-1} and OSI ~ 0.1 *vs.* 0.3 in brachial and superficial femoral arteries, respectively) (Studies 1, 2 and Schreuder *et al.* 2014). Data from this thesis provides evidence that short durations of localised heating are sufficient to replicate these shear rate profiles in the leg, raising the possibility that repeated isolated leg heat stress may provide a feasible method for improving or preserving vascular function in the lower limbs of certain clinical or immobile populations.

8.3 – Significance of findings

The relative importance of central *vs.* local mechanisms towards the control of limb blood flow is commonly reported to be in the region of 10:1 – i.e. a 1 °C increase in core temperature will exert a 9-fold greater effect on limb hyperaemia than the equivalent rise in local skin temperature (Wyss *et al*, 1974; Wyss *et al*, 1975; Wenger *et al*, 1975; Proppe *et al*, 1976; Wenger *et al*, 1985). Whilst this may be true mechanistically, evidence presented in this thesis suggests that the significantly greater range of skin temperatures present during direct whole-body heating, along with the temporal differences in which peripheral and central body temperatures are altered, result in local temperature being the major regulatory stimulus controlling blood flow in the limb when subjects are exposed to rapid and direct whole-body heat stress. The presence of a significant contribution from skeletal muscle hyperaemia – itself controlled solely through local temperature-sensitive mechanisms – may also impact upon commonly reported estimates of whole-body skin blood flow that have previously been calculated using venous occlusion plethysmography and assuming a hyperaemic response confined to the skin alone (Rowell, 1974; Kenney *et al*, 2014). Taken together, these findings suggest that in conditions where skin temperatures are rapidly elevated, the detrimental cardiovascular strain experienced occurs almost solely due to locally-mediated haemodynamic changes, and as such can be prevented at distinct sites through localised cooling techniques. The presence of relatively slow elevations in core temperatures following prolonged

heating, however, result in an underlying central reflex vasodilation which may maintain skin blood flow at elevated levels unless steps are taken to lower core temperature.

Localised heating of the lower limbs leads to changes in vascular haemodynamics that are equivalent to those seen during moderate levels of whole-body heat stress and have previously been shown to benefit vascular health in forearm models (Naylor *et al*, 2011; Carter *et al*, 2014). The lower limbs are more susceptible to both chronic arterial insufficiency (Kroger *et al*, 1999) and an increased incidence of thrombus formation during acute periods of inactivity (Kelly *et al*, 2004), and novel evidence presented here suggests a potential use for local heat application as a non-pharmacological method of preserving endothelial health in these clinical and special populations. As well as the development of pharmacological interventions to minimise the aforementioned risks (Qaseem *et al*, 2011), non-pharmacological methods of mechanically increasing lower limb blood flow have also proved successful in reducing morbidity and mortality. Intermittent pneumatic cuff compressions (IPCC) are a commonly used technique in which inflatable sleeves are wrapped around the leg and periodically inflated, thereby compressing the leg tissues and physically pumping venous blood back towards the heart. This technique has been shown to improve both limb haemodynamics (Labropoulos *et al*, 2005) and claudication distance in subjects suffering from peripheral arterial disease(de Haro *et al*, 2010), as well as reducing thromboembolic deaths in hospitalised stroke patients by almost 4% (CLOTS - Clots in Legs Or sTockings after Stroke - Trials Collaboration *et al*, 2013). Despite animal studies suggesting a role for NO-mediated improvements in this response through increases in shear stress (Liu *et al*, 1999; Chen *et al*, 2002), acute exposure to IPCC in both the arm and leg has been shown to expose the vasculature to a pro-atherogenic high oscillatory/low net shear rate pattern during each compression cycle and results in an unchanged endothelial function as measured by FMD (Roseguini *et al*, 2011; Sheldon *et al*, 2012). Findings from Chapter 5 of this thesis provide the first data that localised limb heating of a similar duration to IPCC not only leads to larger elevations in vessel blood flow and mean shear rate (3-fold *vs.* 2-fold), but also a uniform decrease in oscillatory shear profiles (~ 0.3 to 0.1) in all three major arteries of the leg as opposed to the potentially

detrimental repeated high and low shear patterns experienced during intermittent compressions. Although more research is required, the ability to enhance peripheral haemodynamics in this manner without the added discomfort of whole-body heat stress or repeated mechanical compressions may offer a new and effective method of preserving vascular health in a number of special populations.

8.4 - Limitations

8.4.1 – Sample size

The sample size in each of the studies was relatively small (Chapter 4, n = 10; Chapter 5, n = 7; Chapter 6, n = 8 for invasive protocol; Chapter 7, n = 8) and therefore potentially risked the possibility of Type II errors affecting statistical analyses. However, there are a number of reasons why this was not thought to be problematic with regards to results reported here-in. Firstly, the primary outcome data analysed in all studies consisted of changes in haemodynamic parameters in relation to various temperature manipulations. The magnitude of change in all of these parameters, combined with the reproducibility of responses, resulted in the vast majority of measurements reaching statistical significance even when analysed using the relatively conservative Holm-Bonferroni post-hoc procedure, suggesting the presence of an adequately powered design. Whilst it is accepted that higher numbers may have been beneficial for the analysis of the various blood parameters collected during invasive procedures, the complex and intense procedures required to attain these samples meant that a) not all participants were able to complete the procedure and b) a small number of samples were lost due to technical difficulties.

8.4.2 – Measurement error

Sound scientific findings rely on the ability of the measurement techniques used to both accurately and precisely document changes in the variables being measured.

Although the accuracy of the Doppler ultrasound technique was not confirmed using a phantom device, the recent service of the equipment combined with flow measurements equivalent to that commonly reported in the literature indicated that this was not an issue. In order to check the precision of the technique, the co-efficient of variation was calculated for diameter and blood flow both at rest and during incremental exercise, with results well within commonly reported acceptable limits (0.8 – 3.5% and 3.4 – 12 % for diameter and flow, respectively) (Shoemaker *et al*, 1996; Rådegran, 1999). Although the value of 12% for brachial artery flow in Chapter 4 seems somewhat high, it is important to note that the magnitude of change during the intervention resulted in absolute flow changes of up to 380%, and as such the signal to noise ratio was minimal.

Although the Modelflow method of estimating cardiac output has been shown to correlate well with gold-standard measurements of thermodilution during normothermia and lower-body negative pressure, recent research has indicated that this technique underestimates cardiac output during heat stress conditions (Shibasaki *et al*, 2011), and as such may have influenced systemic haemodynamic measurements in Chapters 6 and 7. The use of intra-arterial pressure measurements have been shown to minimise this error in comparison to the use of infrared photoplethsymography (Finometer), and the use of the former technique in Chapter 6 is expected to have increased the accuracy of these readings. The relatively low values for cardiac output during severe heat stress in Chapter 7, however, are most likely a result of this measurement bias. As described previously in the literature review, cardiac output is generally accepted to increase at a rate of 3 $l \cdot min^{-1} \cdot °C$ $(T_c)^{-1}$, and therefore estimations of cardiac output in Chapters 6 and 7 would be in the region of ~ 9 and 12 $l.min^{-1}$, respectively. All other equipment used in each study was calibrated according to the manufacturers' instructions.

Muscle blood flow was directly measured in Chapter 7 using intramuscular laser Doppler flowmetry. Methodological difficulties (i.e. movement of the probe due to participants becoming agitated when heat stressed and failing to remain still) resulted in the sample size for this new technique being limited to n = 5. In addition, with the

field of measurement from the fibre-optic cable being limited to ~ 1 mm from the tip of the probe and the effect of heat stress on skeletal muscle tissues shown to be distinctly heterogeneous, increases in flow may easily have been missed due to probe placement.

8.5 – Directions for future research

Results from this thesis provide evidence that local thermosensitive mechanisms are responsible for the majority of increases in limb blood flow during direct whole-body heating in humans. Future studies using nerve blockades such as a brachial plexus block may extend these findings further by eliminating any central drive to one arm whilst comparing the haemodynamic responses to the opposite limb. Previous research performing this technique during rapid local heating of the forearm has reported no change in blood flow (Wenger *et al*, 1986; Minson *et al*, 2001), whereas the same procedure during indirect body heating has resulted in a significant attenuation in blood flow to the experimental arm, presumably due to the elimination of central neural drive (Edholm *et al*, 1957). No study to date has investigated the effect due to direct whole-body heating, however, in which it would be expected that the unaltered local effects would continue to elevate blood flow in the limb despite the lack of active reflex vasodilation.

The development of new technologies and refinement of existing ones provides more scope for the accurate separation of skeletal muscle and skin blood flow during cold and heat stress. The use of intramuscular laser Doppler flowmetry in Chapter 7 provided a novel method for measuring muscle blood flow changes during heat stress without interference from increases in the cutaneous circulation, although methodological considerations resulted in mixed findings from this technique. Future studies using the procedure should look to employ local heating of a smaller muscle mass in order to localise the response and minimise movement of the tissues in question. The use of multiple probes within a small sampling area may also help to identify if the failure to detect increases in flow in some participants occurred as a

result of heterogeneous perfusion within the tissues. Alternatively, the use of techniques such as contrast-enhanced ultrasound or advancements in NIRS technology may offer additional methods for assessing skeletal muscle perfusion independent to that of the skin.

Findings reported here provide multiple potential mechanisms through which local heat stress may be used as a non-pharmacological tool for improving the health of the peripheral vasculature and tissues. Evidence is provided for increases in skeletal muscle perfusion and potentially substrate delivery, as well as the generation of anti-atherogenic and anti-thrombotic blood flow profiles within the major vessels of the leg. Whilst repeated heating bouts have been shown to enhance endothelial function in human forearm models (Naylor *et al*, 2011; Carter *et al*, 2014), further research is required to examine if the magnitude of heating achieved in the present studies is sufficient to result in clinically significant adaptations in local vascular function. Studies assessing endothelial function using flow-mediated dilation or biopsy studies assessing the upregulation of factors such as VEGF or eNOS could address these questions. In addition, with the current study employing young healthy subjects as participants, research into appropriate clinical/elderly/immobile populations is required in order to assess whether responses differ between these groups.

8.6 – Summary of findings

Both arms and legs exhibit modest decreases and substantial increases in whole-limb blood flow during isolated limb cooling and heating, respectively; although with a higher temperature sensitivity observed in the arm. These alterations are apparent in both skin and skeletal muscle circulations and occur in response to local thermosensitive mechanisms alone. Muscle perfusion is estimated to contribute ~ 1/3 of the total hyperaemic responses observed during heating, and may be in part mediated through increases in local tissue $\dot{V}O_2$.

Local thermosensitive mechanisms also appear to be the primary stimuli for limb hyperaemia during direct rapid whole-body heating, most likely due to locally-induced elevations in tissue perfusion occurring before delayed elevations in core temperature act upon reflex skin vasodilation. Although not the major contributor to the hyperaemic response during heating, this underlying reflex response maintains elevated skin blood flow during rapid cooling of an isolated tissue mass following severe heat stress.

Finally, isolated heat stress is shown to provide similar beneficial alterations to arterial flow profiles in the leg compared to that previously reported in the arm. These alterations occur in all three major arteries of the leg and result in a high shear/low OSI environment that has been strongly suggested to preserve or improve vascular health. Isolated heating appears to be as effective as moderate whole-body heat stress for this effect, but without the additional cardiac and thermal strain often associated with whole-body heating.

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APPENDICES

I. Ethical Approval Letters

Head of School of Sport & Education
Professor Susan Capel

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Mr Scott Chiesa
4 Belsize Avenue
Palmers Green
London
N13 4TJ

15 November 2011

Dear Scott

RE04-11 - The role of local tissue temperature on limb muscle blood flow

I am writing to confirm the Research Ethics Committee of the School of Sport and Education received your application connected to the above mentioned research study. Your application has been independently reviewed to ensure it complies with the University/School Research Ethics requirements and guidelines.

The Chair, acting under delegated authority, is satisfied with the decision reached by the independent reviewers and is pleased to confirm there is no objection on ethical grounds to the proposed study.

Any changes to the protocol contained within your application and any unforeseen ethical issues which arise during the conduct of your study must be notified to the Research Ethics Committee.

On behalf of the Research Ethics Committee for the School of Sport and Education, I wish you every success with your study.

Yours sincerely

Dr Gary Armstrong
Chair of Research Ethics Committee
School Of Sport and Education

Head of School of Sport & Education
Professor Ian Rivers

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Scott Chiesa
PhD (Sport Sciences) Student
School of Sport and Education
Brunel University

10th February 2014

Dear Scott

RE27-13 Limb blood flow distribution during severe passive heat stress

I am writing to confirm the Research Ethics Committee of the School of Sport and Education received your application connected to the above mentioned research study. Your application has been independently reviewed to ensure it complies with the University/School Research Ethics requirements and guidelines.

The Chair, acting under delegated authority, is satisfied with the decision reached by the independent reviewers and is pleased to confirm there is no objection on ethical grounds to grant ethics approval to the proposed study.

Any changes to the protocol contained within your application and any unforeseen ethical issues which arise during the conduct of your study must be notified to the Research Ethics Committee for review.

On behalf of the Research Ethics Committee for the School of Sport and Education, I wish you every success with your study.

Yours sincerely

pp Dr Richard J Godfrey
Chair of Research Ethics Committee
School Of Sport and Education

II. Health questionnaire and consent form

PRE-PARTICIPATION HEALTH CHECK QUESTIONNAIRE

Health and safety within this investigation is of paramount importance. For this reason we need to be aware of your current health status before you begin any testing procedures. The questions below are designed to identify whether you are able to participate now or should obtain medical advice before undertaking this investigation, Whilst every care will be given to the best of the investigators ability, an individual must know his/her limitations.

Subject name: .....
Date of birth: .....
Doctors Surgery Address: .....
Emergency Contact Name and Phone No: .....

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

CONSENT FORM

THE ROLE OF LOCAL TISSUE TEMPERATURE ON MUSCLE BLOOD FLOW

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?	☐	☐
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:

III. Conference Abstracts

THE ROLE OF LOCAL TISSUE TEMPERATURE ON RESTING AND EXERCISING SKELETAL MUSCLE HAEMODYNAMICS IN THE HUMAN LEG

Poster presented at IUPS 2013, Birmingham, UK

S.T. Chiesa, S.J. Trangmar, K.K. Kalsi, and J González-Alonso

Centre for Sports Medicine and Human Performance, Brunel University London, UK

Introduction: The haemodynamic responses of skeletal muscle to localised changes in tissue temperature are still poorly understood, despite the widespread therapeutic use of heating and cooling. We aimed to systematically identify these responses both at rest and during one-legged exercise over a wide range of physiologically relevant temperatures. Methods: Leg tissue temperatures in 7 males (age 22 ± 1 years) were altered at rest over 1hr through the use of frozen gel packs (cooling) or a water perfused suit (heating). Core, skin and deep muscle (T_m) temperatures were measured throughout. Haemodynamic alterations in 3 major arteries of the leg (common, superficial, and profunda femoral arteries: CFA, SFA, and PFA) were assessed using duplex Doppler ultrasound, with the contralateral leg providing control measures. Systemic haemodynamic responses were measured non-invasively using infrared plethysmography. Following each intervention, CFA flow was measured during incremental single-legged knee extensor exercise in the experimental or control leg (10 ± 1, 16 ± 1, 23 ± 2, and 30 ± 2 W). All values are means ± SEM, with Tm and flows analysed using RM-ANOVA and conductance using linear regression. Results: At rest, 1hr of localised cooling (T_m 34.9 ± 0.3°C to 29.5 ± 0.6 °C; P <0.05) led to small but significant decreases in blood flow to all three vessels (40-60 ml.min^{-1} or 15-25%; P <0.05), with heating (T_m 34.5 ± 0.5 °C to 36.8 ± 0.1 °C; P <0.05) leading to significant increases (100-360 ml.min^{-1} or 63-99%; P <0.05). Blood flow through the PFA (the major supply artery of the thigh skeletal muscle and therefore representative of muscle blood flow) showed significant alterations following both interventions (25% decrease and 63% increase respectively; P <0.05). PFA vascular conductance showed a strong linear relationship with muscle temperature during both interventions (R^2 = 0.95 and 0.96; P <0.01), with the sensitivity of the response increasing considerably at ~35 °C (i.e. normal resting muscle temperature; 0.11 ± 0.03 to 0.70 ± 0.07ml.min^{-1}.mmHg^{-1}.°C^{-1}). Systemic and control leg values remained unchanged. During exercise, prior cooling of the leg had no effect on CFA flows. In contrast, exercise following heating resulted in significantly higher CFA flows throughout the duration of the protocol (~ 500 ml.min^{-1}; P <0.05). Conclusions: These findings suggest that local temperature exerts significant effects on skeletal muscle haemodynamics both at rest and during exercise over a wide range of temperatures, but with significantly increased sensitivity observed at temperatures ≥ 35 °C. The maintenance of an increased blood flow throughout exercise following heating suggests an independent role for temperature in the hyperaemic exercise response, potentially mediated through the rapid increases in local muscle and blood temperature experienced when undertaking dynamic muscular contractions.

LOCAL TEMPERATURE-SENSITIVE MECHANISMS, INDEPENDENT OF SYSTEMIC RESPONSES, MEDIATE INCREASES IN LIMB TISSUE PERFUSION IN THE RESTING AND EXERCISING HEAT-STRESSED HUMAN

Mini-Oral Presentation for Young Investigators Award, ECSS 2014, Amsterdam, Netherlands

Chiesa S.T. [1], Trangmar S.J. [1], Kalsi K.K. [1], Rakobowchuk M. [1], Banker D. [2], Lotlikar M.D. [2], Ali L. [2], González-Alonso J. [1]

[1] CSMHP, Brunel University, UK. [2] Dept. of Anaesthetics, Ealing Hospital, UK

Introduction: Limb and systemic blood flow increases with heat stress in the resting and exercising human, but the contribution of local vs. systemic mechanisms remains equivocal. This study tested the hypothesis that heat stress-mediated increases in limb tissue blood flow, including that to skeletal muscle, are regulated by local temperature-sensitive mechanisms. Methods: 15 male subjects (age 23 ± 4 years; mean ± SEM) were exposed to 1 h of either 1) full-body passive heat stress (water-perfused suit; n = 8) with simultaneous isolated single leg cooling (ice packs), or 2) isolated leg heating or cooling during core normothermia (n = 7). Core, skin, and deep femoral venous blood temperatures, limb (common and profunda femoral arteries) and systemic haemodynamics were measured at rest and during incremental single-legged knee extensor exercise. Results: Full-body passive heat stress led to significant increases in whole-body core, mean skin and heated leg blood temperatures (0.5 ± 0.1 ˚C, 6.6 ± 0.3 ˚C, and 1.1 ± 0.1 ˚C), heart rate (24 ± 3 bpm), and cardiac output (2.0 ± 0.3 $l.min^{-1}$; $P < 0.05$ for all) Heated leg blood flow (common femoral artery) increased 0.6 ± 0.1 $l.min^{-1}$, partly due to downstream increases in profunda femoral arterial flow (main arterial supply of thigh skeletal muscle; 0.20 ± 0.03 $l.min^{-1}$ increase; $P < 0.05$). In contrast, leg tissue blood flow in both arteries of the cooled leg remained unchanged throughout ($P > 0.05$). During incremental exercise (up to 50 W), heated leg blood flow was consistently maintained ~ 0.6 $l.min^{-1}$ higher than that in the cooled leg ($P < 0.01$), with vascular conductance and blood flow in both legs showing a strong correlation with their respective local venous blood temperature (R^2 = 0.98 and 0.96, $P < 0.05$). During isolated limb heating and cooling, leg blood flows were equivalent to those found during full-body heating ($P > 0.05$), despite unchanged systemic temperatures and haemodynamics. Similarly, during incremental exercise, leg blood flow responses were essentially identical to their heated and cooled counterparts during full-body heating, despite a difference in core temperature of almost 1 °C between studies. Discussion Local temperature-sensitive mechanisms, independent of systemic temperature and haemodynamic responses, directly influence limb tissue blood flow regulation both at rest and during small-muscle mass exercise. These findings support the use of local heating for the promotion of limb tissue blood flow and oxygen and substrate supply in both athletic and clinical populations, without the increased cardiovascular strain associated with full-body hyperthermia.