REGIONAL BLOOD FLOW IN THE HUMAN LEG WITH LOCAL HEATING AND LOW-INTENSITY EXERCISE IN YOUNG AND OLD HUMANS

A Thesis Submitted for the

Degree of Doctor of Philosophy

By

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23 February 2024

ABSTRACT

Passive heating has been used for millennia as a therapeutic method to improve health and wellbeing. Contemporary research indicates that heating may have the potential to instigate positive vascular adaptation due to large increases in blood flow and thus, shear stress. However, the precise vascular locus in which hyperthermia modulates perfusion remains elusive. Moreover, the literature exploring the acute haemodynamic responses to heating and exercise in elderly populations, who might benefit the most from heat and exercise interventions, is riddled with inconsistencies. Consequently, the present thesis aimed to (*1*) investigate the local temperature and haemodynamic responses to various levels of leg and leg-segmental heating, and (*2*) examine the leg haemodynamic responses among single-leg hyperthermia, knee-extensor exercise, and their combination in healthy, active aged adults. The results from *Chapter 4* and *4* demonstrate a strong relationship between local tissue temperature and perfusion, and between the volume of heated tissue and perfusion. The magnitude of hyperaemia during segmental-leg heating corresponded to the regional hyperaemia of that particular leg-segment during whole-leg hyperthermia. During these various levels of local heating, blood flow solely increased in the heated areas with no changes in temperature and perfusion in the unheated contralateral and adject limb segments, alongside a maintained core temperature and central haemodynamics. Furthermore, *Chapter 6* uncovered that single-leg heating, knee-extensor exercise, and their combination induced substantial increases in hyperaemia—from smallest to largest, respectively—with hyperthermia having an additive effect on functional hyperaemia. A similar magnitude of functional hyperaemia was observed between the elderly and young cohorts, despite the presence of age-associated structural vascular changes. Collectively, these novel data indicate that peripheral tissue perfusion during local hyperthermia is primarily regulated by local thermosensitive mechanisms and that local heating may be a suitable intervention for the improvement or maintenance of vascular health in elderly individuals.

ACKNOWLEDGEMENTS

At around the age of eight, I told my mum that I did not ever want to go to university. When she asked me why, I simply pointed out that my dad—who at the time was pursuing a PhD alongside a full-time lectureship—was constantly working, stressed and with very little free time. She laughed and assured me that, if I decided to go to university, it would not be as burdening and that I did not have to go so far as to undergo a PhD. And so, reassured, the possibility of attending university was back on the table. Fast forward to the present day, and I am on the verge of obtaining a PhD after four laborious years whilst juggling numerous jobs. Go figure. I guess the apple does not fall far from the tree. And thus, my first acknowledgement goes to my wonderful parents, without whose inspiration, support, and love, I do not think I would have achieved this milestone. *Obrigado*, *mama e papa*.

Next, to my supervisor, Prof. José González-Alonso, thank you for investing your time into supporting and developing me into the researcher that I am today. From my BSc to my MSc and now to my PhD, your mentorship (and patience) has been invaluable and a great inspiration. I am beyond grateful for all that you have done, truly, thank you. I would also like to extend my gratitude to my second supervisor, Prof. Ashraf Khir, despite not necessarily obtaining the wave intensity data that we had hoped for, your constant encouragement and optimism was highly cherished. Moreover, to Dr Kazuhito Watanabe, Dr Scott Chiesa, Dr Steven Trangmar, Dr Patrick Cazorla, Dr Oliver Gibson, Jeneil McDonald, Tom Howes and of course, Dylan Smith, thank you all for your tremendous and instrumental support during my PhD. This submission concludes my ten years at Brunel University London and as such, I would like to thank all my ‘Brunel friends’ and all the people that I have had the pleasure of working with or learning from, for making the last decade an enjoyable ride.

A special thanks to my dear partner, Francesca. In addition to your abundant love and encouragement, thank you for listening and putting up with all my craziness whilst I chattered on endlessly about random findings, ideas, and complaints. Furthermore, a big shout out to Ishan for being a relentless beacon of positivity and support.

Lastly, I would like to express my deepest gratitude to all the amazing individuals who participated in my studies—without you, this thesis would not have been possible.

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Glossary of Key Terms

Term	Definition
Arterial distensibility	An artery's ability to stretch and comply with changes in pressure or volume. It is the inverse of stiffness.
Blood velocity	The speed at which blood travels through the lumen of a blood vessel. Usually reported as centimetres per second.
Cardiac output	The volume of blood ejected by the left ventricle per unit of time. It is represented as litres per minute.
Central (systemic) haemodynamics	A general term referring to the movement of blood throughout the entire body. Parameters commonly referred to include heart rate, cardiac output, stroke volume, and arterial pressure.
Conduit artery	A large blood vessel that transports oxygenated blood away from the heart and feeds it into smaller blood vessels and organs.
Diathermy	A technique which utilises electromagnetic energy to heat a structure via radiation.
Haemodynamics	The study of blood flow, pressure, and the mechanical forces in the cardiovascular system, and the structures that it flows through.
Homeostasis	The body's ability to regulate and maintain stable internal conditions despite changes in the external environment for optimum functioning and survival.
Hyperaemia	The increase in blood flow/tissue perfusion to a specific region. Similar to hyperperfusion.
Hyperperfusion	A high amount of blood flow to a specific region. Similar to hyperaemia.
Hyperthermia	A state characterised by a significant elevation in tissue and/or body temperature. For hyperthermic conditions, it can refer to external, environmental conditions which, theoretically, threaten homeostasis.

Macrocirculation	The circulatory system that involves the circulation of blood through the larger, conduit arteries and veins.
Mean arterial pressure	The average pressure in the arterial circulatory system during a cardiac cycle. It is calculated as the sum of one-third systolic and two-thirds diastolic blood pressure—i.e., the integral of the pressure waves.
Microcirculation	The circulatory system that involves the circulation of blood through the small arteries and veins, arterioles, venules, and capillaries.
Normothermia	A state characterised by the maintenance of a stable, normal (basal) body temperature. For normothermic conditions, it can refer to external, environmental conditions which, theoretically, do not alter homeostasis.
Shear stress	A frictional force that occurs when blood cells come into contact with the walls of blood vessels. It is measured by shear rate.
Thermoregulation	A component of homeostasis which specifically refers to the body's ability to maintain a stable internal temperature which is optimal for functioning and survival.
Thermosensitive mechanisms	An array of biological processes and sensors that respond to changes in tissue temperature and act accordingly to help regulate body temperature.
Tissue oxygen saturation	A percentage measure of the oxygen saturated haemoglobin in the blood circulating a specific tissue region.
Vascular conductance	The ease at which blood can flow through a blood vessel. It is the inverse of vascular resistance.
Vasodilation	The widening of blood vessels, usually due to the relaxation of smooth muscle, which commonly result in an increased blood flow.

List of Abbreviations

Term	Definition
BCW	Backward compression waves (a parameter derived from wave-intensity analysis)
BF	Blood flow ($mL \cdot min^{-1}$ or $L \cdot min^{-1}$)
CFA	Common femoral artery
D_{mean}	Average internal arterial diameter, measured as the sum of one-third diameter at systole and two-thirds diameter at diastole (cm)
FCW	Forward compression waves (a parameter derived from wave-intensity analysis)
FEW	Forward expansion waves (a parameter derived from wave-intensity analysis)
HR	Heart rate (bpm)
LLH	Lower-leg heating
MAP	Mean arterial pressure (mmHg)
OSI	Oscillatory shear index
PFA	Profunda (deep) femoral artery
POA	Popliteal artery
PQH	Partial-quadriceps heating (equivalent to 16 % of total quadriceps area)
$\dot{Q}$	Cardiac output ($L \cdot min^{-1}$)
QH	Quadriceps heating
SFA	Superficial femoral artery
SR	Shear rate (s^{-1})
SV	Stroke volume (ml)
$\overline{T}_X$	Average temperature for a given area, denoted by X (º C)
T_c	Core temperature (º C)

T_m	Muscle temperature (º C)
T_{sk}	Skin temperature (º C)
T_{tym}	Tympanic temperature (º C)
ULH	Upper-leg heating (synonymous with WTH)
$\dot{V}O_{2\,max}$	Maximal oxygen consumption, ($L \cdot min^{-1}$)
VC	Vascular conductance ($mL \cdot min^{-1} \cdot mmHg^{-1}$)
V_{mean}	Average arterial blood velocity ($cm \cdot s^{-1}$)
WIA	Wave-intensity analysis
WLH	Whole-leg heating
WTH	Whole-thigh heating (synonymous with WTH)

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CHAPTER 1: Introduction

Background

Hyperthermia—whether whole-body or local limb—instigates numerous cardiovascular adjustments to dissipate heat and maintain core temperature within a slim range for optimal functioning (Chiesa *et al.*, 2019). Whilst the human body can withstand a vast array of environmental temperatures, with human settlements inhabiting environments as cold as -50 ºC in Yakutsk, Russia and as hot as 50 ºC in Death Valley, USA (Donaldson *et al.*, 1998; Roof & Callagan, 2003), a small increase in core temperature of 3 ºC can be fatal (Crandall & Gonzalez-Alonso, 2010). Consequently, when homeostasis is threatened, central and local thermoregulatory mechanisms engage to increase blood flow to the skin and peripheral limbs which, in doing so, allow the exchange of heat away from the body to the environment (Johnson & Proppe, 1996). Whilst the literature examining the effects of hyperthermia on tissue perfusion is extensive (Roddie *et al.*, 1956; Edholm *et al.*, 1957; Roddie *et al.*, 1957; Koroxenidis *et al.*, 1961; Detry *et al.*, 1972; Taylor *et al.*, 1984; Minson *et al.*, 1998; Davis *et al.*, 2006; Ooue *et al.*, 2007; Heinonen *et al.*, 2011; Naylor *et al.*, 2011; Pearson *et al.*, 2011; Chiesa *et al.*, 2015; Caldwell *et al.*, 2016; Chiesa *et al.*, 2016; Kuhlenhoelter *et al.*, 2016; Thomas *et al.*, 2016; van Mil *et al.*, 2016; Kalsi *et al.*, 2017; Romero *et al.*, 2017; Engelland *et al.*, 2020; Coombs *et al.*, 2021), there remains debate surrounding the contribution of central and local thermoregulatory mechanisms responsible for the observed hyperthermia-induced hyperaemia and the distribution of blood between the skin and muscle (Roddie *et al.*, 1956; Edholm *et al.*, 1957; Blair *et al.*, 1960; Detry *et al.*, 1972; Heinonen *et al.*, 2011; Pearson *et al.*, 2011; Chiesa *et al.*, 2015; Chiesa *et al.*, 2016).

As alluded to, tissue perfusion is controlled through the mediation of central and local mechanisms, with the majority of mechanisms ultimately increasing perfusion through vasodilation (Secomb, 2008). Numerous mechanisms are involved—commonly working in tandem—such as pressure changes, neural stimuli, circulating substances, shear-dependant responses, and metabolic demands (Secomb, 2008). Perfusion is not regulated homogenously between organs, with discrepancies existing between cutaneous and skeletal muscle perfusion. Though there is overlap between some mechanisms such as nitric oxide, which is known to play a key role in both cutaneous and muscle perfusion, it is how that vasodilator is released that can differ between the

two (Kellogg *et al.*, 1999; Mortensen *et al.*, 2007; Johnson & Kellogg, 2010; Hellsten *et al.*, 2012). In line with the present thesis' research domain, it is important to have a fundamental understanding of the primary mechanisms responsible for cutaneous and skeletal muscle perfusion.

Skeletal muscle perfusion is a dynamic process with increases in blood flow occurring in direct response to metabolic and thermoregulatory demands (Andersen & Saltin, 1985; González-Alonso *et al.*, 2000; Heinonen *et al.*, 2011; Gonzalez-Alonso, 2012). It is mediated through the interplay of sympathetic activity and vasoconstrictors versus vasodilators and compounds which regulate sympathetic activity (Hellsten *et al.*, 2012). In general, these mechanisms tend to act upon the arterioles and capillaries in the microcirculation rather than large conduit arteries in the macrocirculation. While vasodilatory signals in the microvasculature can be propagated along the proximal feed arteries of the muscle (Segal, 2000, 2005), studies that directly measured conduit artery diameter during exercise (Radegran, 1997; Hoelting *et al.*, 2001; Lutjemeier *et al.*, 2005) and hyperthermia (Pearson *et al.*, 2011; Chiesa *et al.*, 2016) have seldom found evidence of vasodilation at this level of the arterial tree. Since vascular conductance is well known to increase during exercise and hyperthermia (Pearson *et al.*, 2011), it appears that the vasodilation is occurring downstream in the microcirculation.

Put simply, microcirculatory vasodilation is achieved through two distinct pathways: functional sympatholysis and the presence of vasodilatory substances (Gliemann *et al.*, 2019). Firstly, functional sympatholysis is the process whereby vasoconstriction caused via the sympathetic stimulation of α-adrenoreceptors is blunted, likely due to the role of endothelial-derived hyperpolarisation factors (EDHFs), adenosine triphosphate (ATP), acetylcholine, and nitric oxide (Thomas & Victor, 1998; Rosenmeier *et al.*, 2004; Hearon Jr *et al.*, 2016; Gliemann *et al.*, 2019). Sympathetic activity is known to increase with intense exercise (Katayama & Saito, 2019) and severe whole-body hyperthermia (Powers *et al.*, 1982; Niimi *et al.*, 1997; Cui *et al.*, 2002; Cui *et al.*, 2004; Low *et al.*, 2011); however, this is not the case during local limb heating (Keller *et al.*, 2010; Takahashi *et al.*, 2011) and low-intensity small muscle mass exercise (Katayama & Saito, 2019). Thus, whilst functional sympatholysis has a role in the regulation of muscle perfusion during intense exercise and severe

hyperthermia, it appears unlikely to play a significant role during local limb hyperthermia and low-intensity small muscle mass exercise as sympathetic activity does not drastically increase.

Moving onto the role of vasodilatory substances, nitric oxide and prostaglandins alongside EDHFs appear to occupy a central function (Hellsten *et al.*, 2012). Nitric oxide and prostaglandins are predominately localised in the skeletal muscle cells and vascular endothelium, and are formed through the conversion of L-arginine to nitric oxide and arachidonic acid to prostaglandin E_2 via nitric oxide synthase and cyclo-oxygenase, respectively (Mortensen & Saltin, 2014). The vasodilatory effect of EDHFs remains largely unknown with its potential role being founded on the observation that specific substances—when in the presence of nitric oxide synthase and cyclo-oxygenase—stimulate hyperpolarisation and thus, relaxation of the arterial smooth muscle cells (Mortensen & Saltin, 2014). Furthermore, erythrocyte-released ATP has been postulated as a primary regulator of tissue perfusion (Ellsworth *et al.*, 1995). ATP is thought to be released from the erythrocytes through vascular shear stress (Burnstock, 1999; Gonzalez-Alonso *et al.*, 2002; Mortensen *et al.*, 2011), a metabolic signalling mechanism coupled to the offloading of oxygen (Ellsworth *et al.*, 1995), and increases in blood temperature (Kalsi & Gonzalez-Alonso, 2012). The intravascular ATP then binds to endothelial PY2 receptors which in turn stimulate the release of nitric oxide, prostaglandins and/or EDHFs and result in vasodilation (Ellsworth *et al.*, 1995; Sprague *et al.*, 1996; Mortensen *et al.*, 2009a; Kalsi & Gonzalez-Alonso, 2012). In addition to ATP inducing vasodilation through the aforementioned vasodilatory substances, evidence also suggests that ATP may have a direct endothelial-independent vasodilatory effect (van Ginneken *et al.*, 2004; Crecelius *et al.*, 2011). Moreover, interstitial ATP, alongside adenosine, in the skeletal muscle also appears to play a role in the regulation muscle perfusion, with both ATP and adenosine stimulating nitric oxide and prostacyclin (a derived vasodilator from prostaglandin H_2) formation (Smits *et al.*, 1995; Mortensen *et al.*, 2009a; Mortensen *et al.*, 2009b; Nyberg *et al.*, 2013; Mortensen & Saltin, 2014).

The cutaneous blood flow response to heat stress and exercise is well characterised in the literature and can occur via local mechanisms and central reflexes (Johnson *et al.*, 1986; Kellogg *et al.*, 1993; Johnson & Proppe, 1996; Johnson & Kellogg, 2010;

Ootsuka & Tanaka, 2015). During local heating of the skin, cutaneous vasodilation occurs in two predominant phases: (i) a local axon reflex that instigates a rapid but brief increase in vasodilation and thus, blood flow during the first 5 minutes, followed by (ii) a slower vasodilation that plateaus after 25–30 minutes but has the potential for maximum vasodilation (Charkoudian, 2003; Johnson & Kellogg, 2010). The sensory nerves responsible for the first transient phase of local vasodilation are thought to be C-fibre afferents that are activated by the stimulation of temperature-sensitive vanilloid receptors, which then stimulate vasodilation through the release of calcitonin gene-related peptide, neurokinin A and substance P (Smith *et al.*, 2002; Charkoudian, 2003). The second phase of the local cutaneous vasodilatory response is largely thought to be nitric oxide dependent, with nitric oxide likely being release through increased activation of endothelial nitric oxide synthase (Charkoudian, 2003; Kellogg *et al.*, 2008a, b; Johnson & Kellogg, 2010). Alternatively, during whole-body heating or exercise whereby core and/or blood temperature increase past the body's 'set-point' of 37 ºC, central mechanisms are engaged (Savage & Brengelmann, 1996; Sawka *et al.*, 2011; Ootsuka & Tanaka, 2015; Chou & Coyle, 2023). The central control of thermoregulation is in the preoptic/anterior hypothalamus: when internal temperatures are sensed to be above the 'set-point', the preoptic/anterior hypothalamus responds by instigating an efferent response to induce cutaneous vasodilation which involves the withdrawal of sympathetically-mediated vasoconstriction and/or the activation of sympathetically-driven active vasodilation (Johnson & Proppe, 1996; Charkoudian, 2003). On this note, when mediated by central reflexes, cutaneous vasodilation in glabrous (non-hairy) skin is solely achieved by the withdrawal of sympathetic vasoconstriction whilst in non-glabrous (hairy) skin, cutaneous vasodilation is mediated by a combination of a withdrawal of sympathetic vasoconstriction and active vasodilation—with the latter accounting for 80–90 % of cutaneous vasodilation during whole-body hyperthermia (Roddie *et al.*, 1957; Pergola *et al.*, 1994; Johnson & Proppe, 1996; Charkoudian, 2003). The mechanisms responsible for active cutaneous vasodilation are not fully understood; however, they likely include the release of a co-transmitter from cholinergic nerves alongside other vasodilatory pathways such as vasoactive intestinal peptide, substance P, histamine, H_1 receptors, acetylcholine, prostanoids, and neuronal nitric oxide synthase (Kellogg Jr *et al.*, 1995; Johnson & Proppe, 1996; Minson *et al.*, 2001; Wilkins *et al.*, 2004; Wong *et al.*, 2004; McCord *et al.*, 2006; Wong & Minson, 2006; Kellogg *et al.*, 2008b, a; Wong & Minson, 2011;

Francisco & Minson, 2018; Francisco *et al.*, 2023). It is important to note that this centrally-mediated cutaneous reflex to increase in body temperature, does not occur in isolation but rather alongside the locally-mediated mechanisms previously described (Charkoudian, 2003).

Irrespective of the primary mechanisms governing tissue perfusion, passive heating is commonly recommended for its potential therapeutic ability to maintain or improve cardiovascular health (Imamura *et al.*, 2001; Kihara *et al.*, 2002; Naylor *et al.*, 2011; Carter *et al.*, 2014; Brunt *et al.*, 2016; Romero *et al.*, 2017; Thomas *et al.*, 2017; Cullen *et al.*, 2020; Engelland *et al.*, 2020). With almost a fifth of the world's current population aged over 65 years old (Rudnicka *et al.*, 2020), cardiovascular morbidity and mortality pose a larger risk to the population (Heidenreich *et al.*, 2011; Costantino *et al.*, 2016). Whilst exercise has been shown to weaken the relationship between age and cardiovascular morbidity and mortality, over 50 % of aged adults do not, and nor do they want to, exercise (Allen & Morelli, 2011; Fiuza-Luces *et al.*, 2018). Heating may provide a simple, non-pharmaceutical means to improve cardiovascular health; however, whole-body heating methods such as saunas and hot baths can be physically and psychologically taxing due to the associated increase in core temperature (Campbell *et al.*, 2022). Thus, local limb heating may provide a suitable compromise whereby the stimulus is substantially more tolerable while remaining sufficient to evoke vascular adaptions. However, before such conclusions can be formed, further research is warranted to comprehensively examine the cardiovascular and temperature responses to local hyperthermia and its potential applicability to aid elderly populations.

Thesis Aims and Hypotheses

The exact aims and hypotheses of each individual study will be reported following the systematic scrutiny of the existing literature. Nevertheless, the main aim of the present thesis is to investigate the mechanisms that control hyperthermia-induced hyperaemia, combining data from both healthy, active young and aged participants to provide a comprehensive report on the acute haemodynamic adjustments to heat stress and exercise. In doing so, the thesis aimed to shed further light on the applicability and viability of passive heating as a therapeutic means to improve cardiovascular health in populations with a reduced exercise capacity. Overall, it is

hypothesised that hyperthermia will induce substantial increases in hyperaemia during rest and small muscle mass exercise in both young and elderly participants which are primarily governed by local thermosensitive mechanisms, with blood flow and tissue oxygenation directly increasing in relation to local temperature.

The succeeding chapter provides a comprehensive review of the current literature which is pertinent to addressing the aims of the present thesis. It will commence by exploring the acute cardiovascular responses and associated thermoregulatory mechanisms to passive whole-body and isolated-limb hyperthermia. The contribution of central vs local haemodynamic adjustments, alongside the difference in hyperaemia between different tissues, during passive hyperthermia will be critically appraised. Subsequently, attention will shift towards the impact of age on the cardiovascular system and how healthy ageing may affect one's cardiovascular—and in particular, hyperaemic—responses to local hyperthermia, exercise, and their combination. Lastly, the review of the literature will conclude with a precise outline of the individual aims and hypothesis for each individual study.

CHAPTER 2: Review of the Literature

Cardiovascular responses to passive hyperthermia and mechanisms governing hyperthermia-induced hyperaemia

Whole-body heating interventions have been used for millennia due to their believed beneficial health effects, ranging from Roman hot baths to Finnish saunas. Numerous studies have confirmed this, reporting the acute cardiovascular adjustments to passive whole-body and isolated-limb hyperthermia (Johnson & Proppe, 1996; Crandall & Gonzalez-Alonso, 2010; Crandall & Wilson, 2015; Chiesa *et al.*, 2019). Arguably, one of the most important adjustments—discovered by renowned French physiologist, Claude Bernard, in the late 19th century—is the hyperthermia-induced hyperperfusion as the body attempts to dissipate heat and maintain homeostasis (Benzinger, 1969; Holmes, 1986), which is linked to the improvement and maintenance of vascular health (Green *et al.*, 2017). Currently, there is debate surrounding the precise locus and mechanisms governing this increase in tissue perfusion during hyperthermia. Thus, the subsequent section will aim to comprehensively report the systemic and peripheral cardiovascular responses to passive whole-body and local limb hyperthermia, highlighting any discrepancies in the literature.

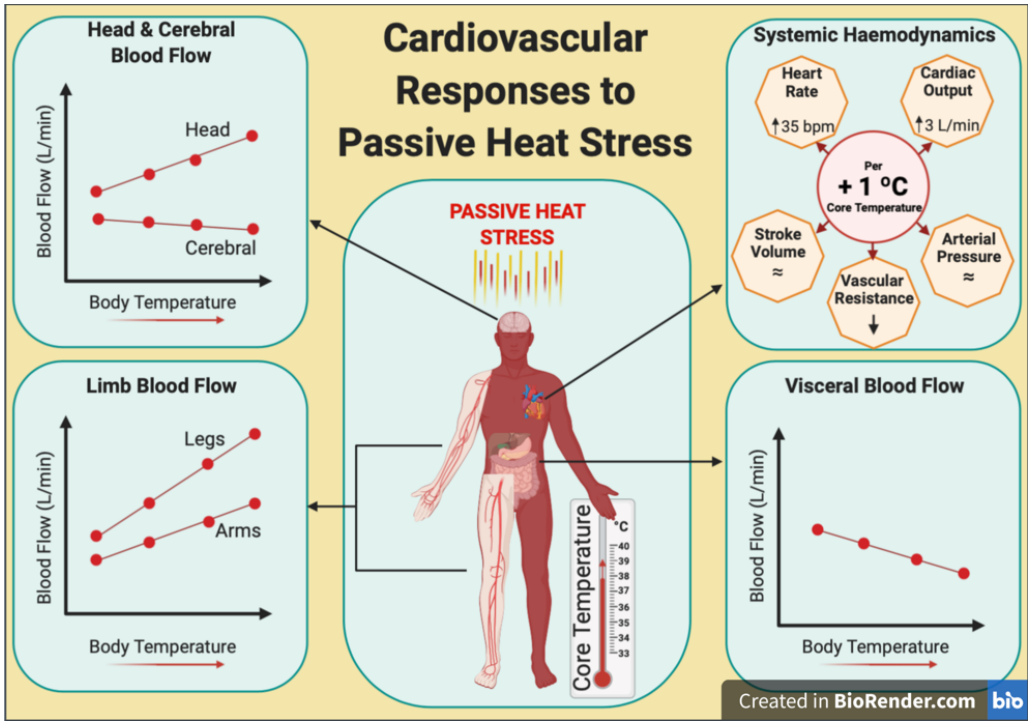

Figure 1. General summary of acute cardiovascular responses to passive whole-body heat stress. Data and trends obtained from (Chiesa *et al.*, 2019). Figure created in BioRender.com.

Acute cardiovascular responses to whole-body passive hyperthermia

Systemic cardiovascular responses. The human body has a remarkable ability to survive in a wide variety of environments, adapting through numerous physiological processes. In hot environments, thermoregulation and consequently, heat dissipation is paramount as an increase in core temperature of as little as 3 ºC can result in catastrophe (Crandall & Gonzalez-Alonso, 2010). When exposed to hot environments, systemic thermoregulatory mechanisms—such as increased sweat rate, cardiac output and increased cutaneous and peripheral circulation—engage to dissipate heat in attempt to maintain homeostasis and prevent significant increases in core temperature (Johnson & Proppe, 1996). However, during hyperthermic interventions whereby participants experience whole-body or limb heating via a temperature-regulated heated garment or hot water bath, homeostasis is seldomly maintained; thus, a significant increase in local tissue and/or core temperature is commonly observed. Alongside the substantial increase in hyperthermia-induced cutaneous and peripheral circulation, cardiac output—as well as potential reductions in splanchnic and renal blood flow—must also increase proportionally to cater to the demand in flow (Rowell *et al.*, 1969; Rowell *et al.*, 1970; Rowell *et al.*, 1971; Detry *et al.*, 1972; Minson *et al.*, 1998; Crandall & Gonzalez-Alonso, 2010). The relationship between severe whole-body heat stress and cardiac output is well documented, with numerous studies reporting increases of 27–100 % in cardiac output following whole-body heating where core temperature increased by 0.7–2.0 ºC (Koroxenidis *et al.*, 1961; Rowell *et al.*, 1969; Rowell *et al.*, 1970; Rowell *et al.*, 1971; Minson *et al.*, 1998; Fan *et al.*, 2008; Pearson *et al.*, 2011; Ganio *et al.*, 2012; Ogoh *et al.*, 2013; Chiesa *et al.*, 2016). However, whether cardiac output increases in hyperthermic conditions where skin temperature increases but core temperature remains relatively stable (i.e., increases of less than 0.5 ºC) remains unclear with some studies reporting modest increases of 14–30 % in cardiac output (Peters *et al.*, 2000; Pearson *et al.*, 2011; Ogoh *et al.*, 2013; Chiesa *et al.*, 2015; Chiesa *et al.*, 2016), whilst others reported no significant changes (Niimi *et al.*, 1997; Kenny *et al.*, 2017). Despite the variations reported in the literature, it remains clear that there is a positive correlation between increases in core temperature and an augmented cardiac output—with cardiac output increasing ~2.6 $L \cdot min^{-1} \cdot ºC^{-1}$ (Figure 2) (Koroxenidis *et al.*, 1961; Rowell *et al.*, 1969; Rowell *et al.*, 1970; Niimi *et al.*, 1997; Minson *et al.*, 1998; Peters *et al.*, 2000; Fan *et al.*, 2008;

Nelson *et al.*, 2011; Pearson *et al.*, 2011; Ganio *et al.*, 2012; Ogoh *et al.*, 2013; Chiesa *et al.*, 2015; Chiesa *et al.*, 2016; Kenny *et al.*, 2017).

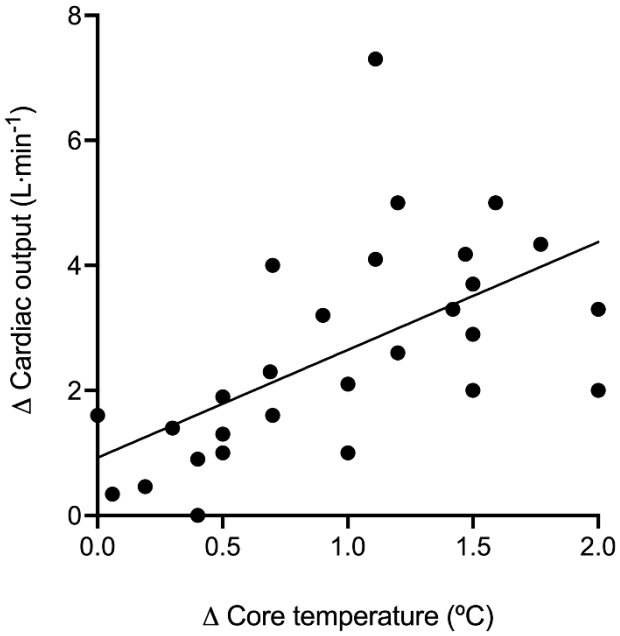

Figure 2. Analysis and plotting of delta change in cardiac output against delta change in core temperature. Data collated from 14 independent studies reporting the effects of whole-body hyperthermia. Where graphical data—as opposed to textual data—was solely reported in the studies, an online tool (WebPlotDigitizer) was used to extract accurate numerical data from the graphs (Rohatgi, 2021). The graph was adapted and updated from Chiesa (2014). Simple linear regression equation: $\boldsymbol{y = 1.73x + 0.92; R^2 = 0.35; p = 0.001}$.

Furthermore, since cardiac output is the product of heart rate and stroke volume, it appears that the increase in cardiac output is primarily the result of an increased heart rate as stroke volume remains largely unchanged during passive hyperthermia (Damato *et al.*, 1968; Minson *et al.*, 1998; Peters *et al.*, 2000; Fan *et al.*, 2008; Nelson *et al.*, 2011; Ogoh *et al.*, 2013; Chiesa *et al.*, 2015; Kenny *et al.*, 2017); although, on instances, increases (Koroxenidis *et al.*, 1961; Rowell *et al.*, 1969) and decreases (Rowell *et al.*, 1970; Chiesa *et al.*, 2016) in stroke volume have been reported. On the other hand, there is a well-established relationship between heart rate and core temperature—comparable to that between cardiac output and core temperature—with heart rate increasing ~31 beats·min^{-1}·ºC^{-1}, as shown in Figure 3 (Koroxenidis *et al.*, 1961; Rowell *et al.*, 1969; Rowell *et al.*, 1970; Niimi *et al.*, 1997; Minson *et al.*, 1998; Peters *et al.*, 2000; Fan *et al.*, 2008; Nelson *et al.*, 2011; Ganio *et al.*, 2012; Ogoh *et al.*, 2013; Chiesa *et al.*, 2015; Chiesa *et al.*, 2016; Kenny *et al.*, 2017; Coombs *et al.*, 2021). Thus, it seems that the increase in cardiac output during whole-body heat

stress is associated with an augmented heart rate as shown by the strong positive correlation ($R^2 = 0.79; p < 0.0001$), with cardiac output increasing ~0.13 L·min^{-1}·beat^{-1} during whole-body hyperthermia (Figure 4). The mechanisms contributing to the observed increase in heart rate during heat stress are a direct effect of temperature on the sinoatrial node, an increased sympathetic activity and reduced vagal tone, baroreceptor-mediated feedback, and a systemic global hyperadrenergic state (Badeer, 1951; Gorman & Proppe, 1984; Rowell, 1990; Wilson & Crandall, 2011). However, despite this close relationship between heart rate and cardiac output, Watanabe *et al.* (submitted) argue that the relationship between these two parameters may not be causal. Classic observations in the mammalian heart have demonstrated a linear response between blood temperature and heart rate—likely due to the aforementioned mechanisms; however, they found that this did not equate to an increased cardiac output due to a proportional decrease in stroke volume (Knowlton & Starling, 1912). Similarly, no changes in cardiac output have been observed in human *in vivo* studies whereby heart rate was systematically increased via cardiac pacing (Ross Jr *et al.*, 1965; Stein *et al.*, 1966; Parker *et al.*, 1971; Bada *et al.*, 2012; Munch *et al.*, 2014). Furthermore, studies demonstrating a proportional relationship between cardiac output and systemic vascular conductance during a maintained arterial pressure, reveal that cardiac output may be largely dependent on the regulation of the peripheral circulation (Pearson *et al.*, 2011; Stöhr *et al.*, 2011; Chiesa *et al.*, 2016). This challenges the traditional cardio-centric model whereby the heart is the chief regulator blood distribution—distributing blood away from the viscera towards the periphery during hyperthermia. Whilst this is undoubtably the most widely accepted and conventional model, it should be stated that an alternative model exists which focuses on the notion that blood is regulated locally within the tissues (Manteuffel-Szoege, 1960; Guyton, 1967; Furst, 2015; Alexander, 2017; Furst & Gonzalez-Alonso, 2023) and that regulation of the peripheral circulation may largely govern central haemodynamic parameters such as cardiac output.

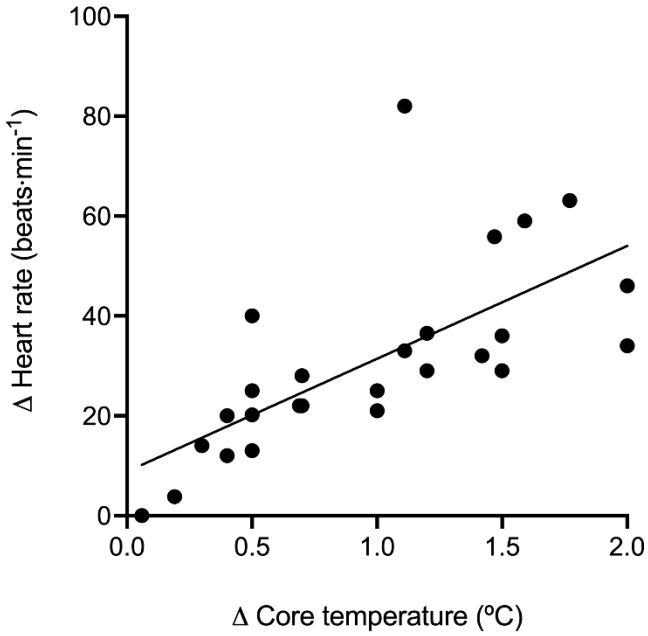

Figure 3. Analysis and plotting of delta change in heart rate against delta change in core temperature. Data collated from 14 independent studies reporting the effects of whole-body hyperthermia. Where graphical data—as opposed to textual data—was solely reported in the studies, an online tool (WebPlotDigitizer) was used to extract accurate numerical data from the graphs (Rohatgi, 2021). Simple linear regression equation: $\boldsymbol{y = 22.59x + 8.83; R^2 = 0.47; p < 0.0001}$.

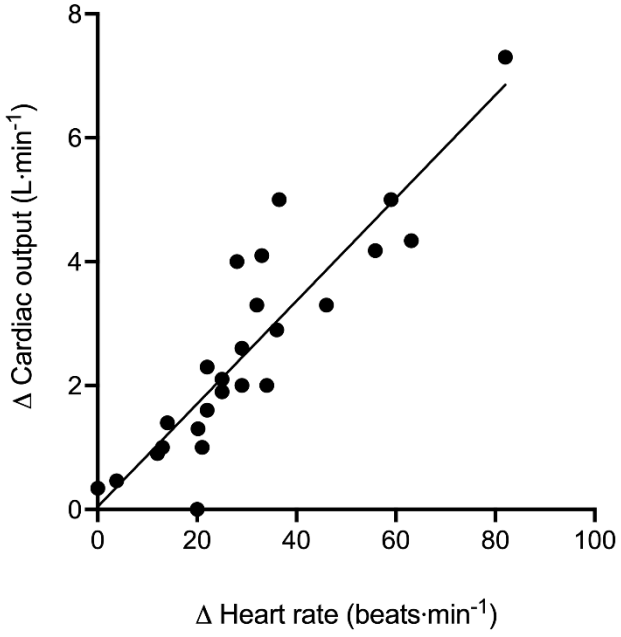

Figure 4. Analysis and plotting of delta change in cardiac output against delta change in heart rate during passive hyperthermia. Data collated from 13 independent studies reporting the effects of whole-body hyperthermia. Where graphical data—as opposed to textual data—was solely reported in the studies, an online tool (WebPlotDigitizer) was used to extract accurate numerical data from the graphs (Rohatgi, 2021). Simple linear regression equation: $\boldsymbol{y = 0.08x + 0.04; R^2 = 0.79; p < 0.0001}$.

As per the traditional cardio-centric model, it is widely believed that most of the increase in cardiac output during hyperthermia is directed to the cutaneous vascular beds, done so in attempt to dissipate heat to the external environment via the skin. This increase in cutaneous hyperaemia is likely achieved through (1) a reduction in sympathetic vasoconstrictor nerve activity, (2) an increase in sympathetic active cutaneous vasodilation, and/or (3) the activation of local thermosensitive mechanisms due to a heightened skin temperature (Johnson & Proppe, 1996). Classic studies report that skin blood flow can increase by ~6–8 $L \cdot min^{-1}$ following severe passive whole-body heating where core temperature (assessed via oesophageal or rectal temperature) increased by 1–2 ºC (Rowell *et al.*, 1969; Rowell, 1974; Minson *et al.*, 1998). However, the precise degree to which cutaneous circulation increases during hyperthermia is unknown as direct measures of whole-body skin blood flow are yet to be conducted. Current estimates are calculated as the difference between cardiac output and visceral blood flow (Koroxenidis *et al.*, 1961; Rowell *et al.*, 1969; Minson *et al.*, 1998), through direct laser Doppler flowmeter measurements (Pearson *et al.*, 2011; Ogoh *et al.*, 2013; Chiesa *et al.*, 2015; Kalsi *et al.*, 2017), or calculated as a whole-body extrapolation of a directly measured area of skin via positron-emission tomography or venous occlusion plethysmography (Taylor *et al.*, 1984; Johnson *et al.*, 1986; Heinonen *et al.*, 2011). Whilst these methods have advantages, they too hold flaws. Firstly, estimating whole-body skin blood flow by calculating the difference in cardiac output and visceral blood flow relies heavily on the assumption that muscle blood flow does not increase during hyperthermia; thus, concluding that the increase in change between cardiac output and visceral blood flow must be owed to hyperaemia in the cutaneous circulation. Similarly, Taylor *et al.* (1984) directly measured forearm blood flow pre- and post-heating and used this difference in blood flow as a direct index of cutaneous hyperaemia, which could then be extrapolated to estimate whole-body skin blood flow.

Numerous classic studies which manipulated vasomotor fibres to the cutaneous blood vessels via pharmacological interventions (Edholm *et al.*, 1957; Blair *et al.*, 1960; Detry *et al.*, 1972) or directly measured oxygen saturation in the superficial and deep veins during hyperthermia, concluded that muscle blood flow does not increase during hyperthermia (Roddie *et al.*, 1956). However, more contemporary research contradicts these findings, reporting that muscle blood flow can double during whole-body

hyperthermia (Wyper & McNiven, 1976; Heinonen *et al.*, 2011; Pearson *et al.*, 2011; Chiesa *et al.*, 2015; Chiesa *et al.*, 2016; Kalsi *et al.*, 2017). Interestingly, a past study demonstrated a ~16-fold increase in muscle blood during electromagnetic diathermic heating whilst the skin was simultaneously being cooled (Sekins *et al.*, 1980; Sekins *et al.*, 1982; Sekins *et al.*, 1984; Giombini *et al.*, 2007). Consequently, this method of estimating skin blood flow is likely to yield overestimations in the cutaneous hyperaemic response to hyperthermia. Irrespective of the differing research on whether muscle blood flow increases during hyperthermia, there is a consensus that the hyperaemia per unit of tissue ($ml \cdot 100g \cdot min^{-1}$) is higher in the skin than the muscle (Heinonen *et al.*, 2011; Pearson *et al.*, 2011; Chiesa *et al.*, 2015; Chiesa *et al.*, 2016).

On the other hand, calculating skin blood flow via laser Doppler has numerous advantages: it is a simple, direct measure which reports perfusion data directly under the probe and gives a reliable indication of cutaneous and/or microcirculatory blood flow (Holloway & Watkins, 1977; Nilsson *et al.*, 1980a; Nilsson *et al.*, 1980b; Leahy *et al.*, 1999). Yet, it is limited in the fact that (a) it does not have quantitative units, thus making it challenging to compare data, (b) it is extremely sensitive to movement artifacts, (c) lack of knowledge on the depth of measurement, and (d) discrepancies surrounding *instrument zero* and *biological zero* (Leahy *et al.*, 1999). Laser Doppler flowmetry and positron-emission tomography are generally accepted as being able to produce accurate skin blood flow data (Holloway & Watkins, 1977; Ruotsalainen *et al.*, 1997). However, these measurements are only accurate for the specific location where the measurements took place as cutaneous circulation is heterogenous (Braverman, 2000); thus, extreme caution is warranted when extrapolating these values to estimate whole-body skin blood flow as it introduces a considerable amount of error. Whilst the current methods of estimating whole-body skin blood flow are not perfect, they do have merit as they provide an estimate of the changes in skin blood flow during a time when it is not possible to obtain a direct and accurate measure due to current technological limitations.

Limb haemodynamic responses. Numerous publications have reported the blood flow response in the forearm (Roddie *et al.*, 1956; Edholm *et al.*, 1957; Roddie *et al.*, 1957; Koroxenidis *et al.*, 1961; Detry *et al.*, 1972; Minson *et al.*, 1998; Davis *et al.*, 2006; Ooue *et al.*, 2007; Coombs *et al.*, 2021) and the leg (Heinonen *et al.*, 2011; Pearson

et al., 2011; Chiesa *et al.*, 2015; Chiesa *et al.*, 2016) during whole-body hyperthermia using methods such as venous occlusion plethysmography, Doppler ultrasound and Positron-emission tomography. Much like the relationship between cardiac output and core temperature, a similar positive correlation exists between limb blood flow and core temperature. Studies reported increases in forearm blood flow as high as 10-fold (Roddie *et al.*, 1956; Edholm *et al.*, 1957; Roddie *et al.*, 1957; Koroxenidis *et al.*, 1961; Detry *et al.*, 1972; Minson *et al.*, 1998; Davis *et al.*, 2006; Ooue *et al.*, 2007; Coombs *et al.*, 2021), whereas 3–4-fold increases were observed in the leg (Pearson *et al.*, 2011; Chiesa *et al.*, 2015; Chiesa *et al.*, 2016). Whilst larger absolute increases in blood flow are observed in the leg due their larger mass, a higher relative increase is seen in the forearms which is likely due to the heterogeneity of the limbs, with the legs having a lower vasodilator and vasoconstrictor responsiveness in comparison to the forearm (Pawelczyk & Levine, 2002; Newcomer *et al.*, 2004).

Acute cardiovascular responses to local passive hyperthermia

The previous section explored the cardiovascular responses to whole-body passive hyperthermia. The current section will now focus on the cardiovascular responses to local passive hyperthermia. In this context, *local* hyperthermia refers to the heating of a single limb—i.e., the arm or leg—or the heating of two lateral limbs—i.e., the arms or legs.

Systemic cardiovascular responses. There is a great deal of literature that has explored the cardiovascular responses to local heating, whether that be single-arm heating (Barcroft & Edholm, 1943; Minson *et al.*, 2001; Davis *et al.*, 2006; Kalsi *et al.*, 2017), two-arm heating (Naylor *et al.*, 2011; van Mil *et al.*, 2016; Coombs *et al.*, 2021), single-leg heating (Pearson *et al.*, 2011; Chiesa *et al.*, 2015; Chiesa *et al.*, 2016), two-leg heating (Kuhlenhoelter *et al.*, 2016; Thomas *et al.*, 2016; Thomas *et al.*, 2017; Engelland *et al.*, 2020), or segmental limb heating—i.e., lower-leg heating of the two legs (Romero *et al.*, 2017; Cheng *et al.*, 2021), unilateral upper-leg heating (Kuhlenhoelter *et al.*, 2016) and unilateral lower-leg heating (Heinonen *et al.*, 2011; Takahashi *et al.*, 2011; Walsh *et al.*, 2019). Despite there being some discrepancies in the cardiovascular responses to local heating between the studies due to differences in methodology and the heated region, there is a general trend that central haemodynamics remain relatively stable, irrespective of a heightened hyperaemia to

the heated region, if core temperature is also unchanged. This is true for studies that conducted unilateral heating which reported minimal to no changes in core temperature and thus, systemic haemodynamics (Heinonen *et al.*, 2011; Pearson *et al.*, 2011; Takahashi *et al.*, 2011; Chiesa *et al.*, 2015; Chiesa *et al.*, 2016; Kuhlenhoelter *et al.*, 2016). However, studies that bilaterally heated the limbs reported increases in heart rate and cardiac output alongside an elevated core temperature (Kuhlenhoelter *et al.*, 2016; Thomas *et al.*, 2016; Romero *et al.*, 2017; Thomas *et al.*, 2017; Engelland *et al.*, 2020; Cheng *et al.*, 2021). Consequently, it appears that there is a thermal threshold for central haemodynamic forces or thermal reflexes, activated through elevations in core temperature, to be employed in the maintenance of homeostasis. Nonetheless, it should be noted that cardiac output may indeed increase during unilateral limb heating but is not observed due to the smaller magnitude of change (~0.3–0.5 $L \cdot min^{-1}$) alongside the measurement error associated with the ModelFlow method (Shibasaki *et al.*, 2011).

Limb haemodynamic responses. Irrespective of a stable core temperature and minimal to no increases in central haemodynamics during local unilateral hyperthermia, studies reported a substantial level of hyperaemia to the heated region. Studies have reported that forearm blood flow, via the brachial artery, increases by approximately ~0.4 $L \cdot min^{-1}$ during unilateral arm heating (Kalsi *et al.*, 2017), and by 0.1–0.4 $L \cdot min^{-1}$ during bilateral forearm heating (Naylor *et al.*, 2011; van Mil *et al.*, 2016; Coombs *et al.*, 2021). Conversely, regional leg blood flow has been shown to increase around ~0.5 $L \cdot min^{-1}$ at the common femoral artery during unilateral leg heating (Pearson *et al.*, 2011; Chiesa *et al.*, 2015; Chiesa *et al.*, 2016), ~0.3 $L \cdot min^{-1}$ and ~ 0.1 $L \cdot min^{-1}$ at the common femoral artery and popliteal artery, respectively, during bilateral leg heating (Thomas *et al.*, 2017; Engelland *et al.*, 2020), ~0.2 $L \cdot min^{-1}$ at the common femoral artery during unilateral upper-leg heating (Kuhlenhoelter *et al.*, 2016), 0.2 $L \cdot min^{-1}$ at the popliteal artery during unilateral lower-leg heating (Walsh *et al.*, 2019), and ~0.5 $L \cdot min^{-1}$ at the superficial femoral artery during bilateral lower-leg heating (Romero *et al.*, 2017). Whilst there is a considerable amount of variation in the level of hyperaemia observed during local hyperthermia due to differences in duration, intensity, mode of heating, as well as differences in the arterial sites used to measure the blood flow, local hyperthermia generally instigated 2–5-fold increases in tissue perfusion to heated region. As previously mentioned, the differences in blood flow

between limbs are likely due to the heterogeneity of the limbs' vasculature. The magnitude of hyperaemia observed in the locally heated limb is strikingly similar to that during whole-body heat stress despite large variances in core temperature and central haemodynamics. This is particularly well illustrated in Figure 5, where Chiesa *et al.* (2016) found that the hyperaemia observed in the leg was similar during single-leg and whole-body hyperthermia despite a 1 ºC difference in core temperature between the two conditions. Whilst it is apparent that regional hyperaemia is not dependent on increases in core temperature, there appears to be a direct, curvilinear relationship between local muscle temperature and local hyperaemia (Chiesa *et al.*, 2016). This challenges the classical dogma that central reflex mechanisms are responsible for the hyperthermia-induced hyperaemia (Edholm *et al.*, 1957; Roddie *et al.*, 1957; Blair *et al.*, 1960), and supports the contemporary notion that local thermosensitive mechanisms are the primary regulators of limb blood flow during local hyperthermia (Heinonen *et al.*, 2011; Pearson *et al.*, 2011; Chiesa *et al.*, 2015; Chiesa *et al.*, 2016; Kalsi *et al.*, 2017).

Specifically, these local thermosensitive mechanisms governing the reactive hyperaemic response to local limb hyperthermia may include heat-sensitive biochemical signals that (a) activate intravascular signalling-transduction mechanisms in the microvasculature such as shear-mediated nitric oxide release and cellular oxidative stress (Kellogg *et al.*, 1999; Minson *et al.*, 2001; Paniagua *et al.*, 2001; Laughlin *et al.*, 2008; Gifford *et al.*, 2014; Romero *et al.*, 2017) and/or (b) stimulate the release of vasoactive molecules from the circulating erythrocytes such as ATP (Pearson *et al.*, 2011; Kalsi & Gonzalez-Alonso, 2012; Kalsi *et al.*, 2017). However, it is difficult to definitively state which mechanisms are primarily responsible for local hyperthermia-induced hyperaemia as these mechanisms will work in tandem to meet the metabolic and/or thermal demands. Should one mechanism fail, the human body has redundancy mechanisms that will assume control (Hellsten *et al.*, 2012). For example, nitric oxide is a well-document potent vasodilator which increases blood flow during hyperthermia and exercise; however, during exercise when nitric oxide was blocked, functional hyperaemia was not significantly affected and it was only when multiple vasodilators were blocked that a reduction in blood flow was observed (Hellsten *et al.*, 2012). Furthermore, whilst these aforementioned mechanisms are vasodilators, which would certainly increase tissue perfusion, numerous studies

investigating the haemodynamic responses to hyperthermia, reported a maintained conduit artery diameter (Pearson *et al.*, 2011; Chiesa *et al.*, 2016; Teixeira *et al.*, 2017; Coombs *et al.*, 2019; Coombs *et al.*, 2021) and even a reduced diameter (Thomas *et al.*, 2017) despite vast increases in blood flow and vascular conductance. Consequently, the maintained conduit artery diameter, alongside the increases in vascular conductance, indicate that perhaps vasodilation may instead occur downstream in the microcirculation. Additionally, strong relationships exist between (a) increases in blood and tissue temperatures (Chiesa *et al.*, 2015; Kalsi *et al.*, 2017); (b) elevations in temperature and reductions in blood viscosity and frictional resistance (Snyder, 1971; Cinar *et al.*, 2001; Shin *et al.*, 2004; Lim *et al.*, 2010); and (c) increases in temperature and rises in red cell deformability and dispersion (Manteuffel-Szoege, 1960; Manteuffel-Szoege, 1969; Cinar *et al.*, 2001; Pinho *et al.*, 2016). Thus, in addition to the previously outlined mechanisms, it remains wholly conceivable that heat-modulated blood rheology also accounts for a portion of the hyperaemia observed during hyperthermia via reductions in blood viscosity and vascular resistance, and increases in erythrocyte dispersion, erythrocyte deformability, blood velocity, and blood kinetic energy.

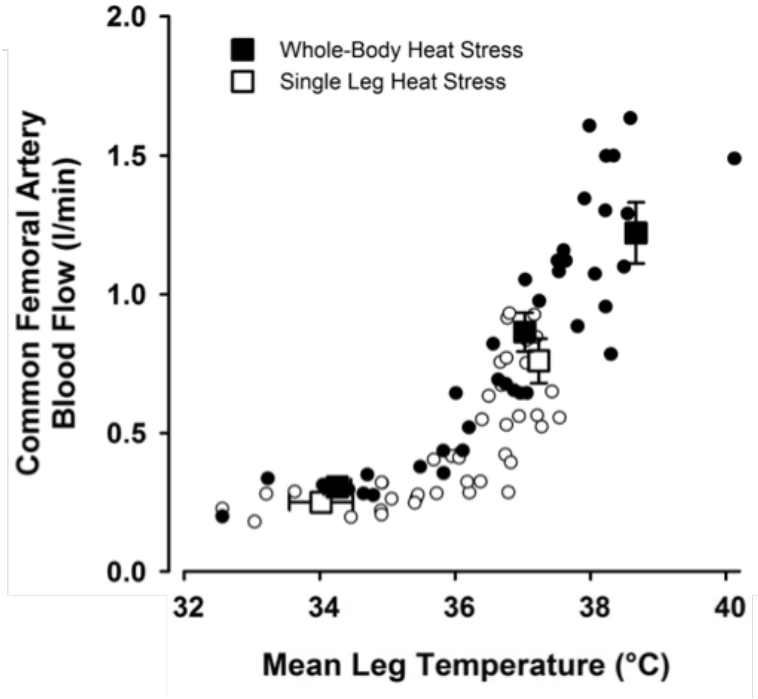

Figure 5. Comparison of leg blood flow vs. mean leg temperature during passive whole-body and single-leg heating. Data are represented as mean ± SE (large black squares) for baseline (thermoneutral), moderate (core temperature: ± 1 °C) and severe (core temperature: ± 2 °C) whole body heat stress. Single leg baseline and heat stress values are also displayed (large white squares)

(core temperature: ± 0 °C). Individual data points are also displayed (small circles). Figure reproduced from Chiesa *et al.* (2016).

Though several studies provide a strong argument for the role of local thermosensitive mechanisms (Heinonen *et al.*, 2011; Pearson *et al.*, 2011; Chiesa *et al.*, 2015; Chiesa *et al.*, 2016; Kalsi *et al.*, 2017), the effects of unilateral passive heating on the contralateral limb remain unclear. Increases in contralateral limb haemodynamics may be explained by increases in core temperature and consequent activation of central neural reflexes (Johnson *et al.*, 1976; Taylor *et al.*, 1984; Johnson & Proppe, 1996; Heinonen *et al.*, 2011; Caldwell *et al.*, 2016; Mallette *et al.*, 2016). In accordance, Chiesa *et al.* (2016) found no significant changes in core temperature and contralateral leg haemodynamics following isolated whole-leg heating. However, increases in contralateral limb haemodynamics have been reported following isolated arm heating and isolated thigh heating despite no changes in core temperature (Kuhlenhoelter *et al.*, 2016; Kalsi *et al.*, 2017). Nonetheless, these increases in contralateral limb blood flow were small and substantially lower than the magnitude of hyperaemia observed in the heated limb. These changes in contralateral limb blood flow could easily be attributed to a movement artefact or a cross-contamination effect of the heated cuff indirectly heating the contralateral limb, which may be in proximity. If one considers the assumption that unilateral heating indeed does not affect contralateral limb blood flow due to the primary instigator of the hyperthermia-induced hyperaemia being local thermosensitive mechanisms, then the question as to whether segmental limb heating causes changes in the blood flow to the adjacent limb remains unknown and of interest. For example, if one was to heat the upper leg, local temperature and thus, local blood flow would increase in the heated region; however, what would happen to the adjacent lower leg—would there be a *spill over* effect of an increased blood flow to the lower leg also, or would the hyperaemia be solely confined to the heated upper leg?

Summary

When exposed to hot environments, the human body engages systemic thermoregulatory mechanisms to dissipate heat, prevent significant increases in core temperature and thus, maintain dynamic homeostasis. During whole-body hyperthermia, it is traditionally believed that cardiac output is *redistributed* from the viscera to the cutaneous vascular beds to dissipate heat—likely achieved through a

reduction in sympathetic vasoconstrictor nerves, an increase in sympathetic active cutaneous vasodilation, and/or the activation of local thermosensitive mechanisms. However, contemporary studies demonstrate that the magnitude of limb hyperaemia observed during local isolated-limb heating is comparable to that during whole-body heating, despite no changes in core temperature and minimal increases in central haemodynamics. This strongly advocates that local thermosensitive mechanisms are the primary regulators of limb blood flow during hyperthermia, whether that be heat-sensitive biochemical signals that induce the release of vasoactive molecules or changes in heat-modulated blood rheology. Nonetheless, the functioning of these local thermosensitive mechanisms remains unclear within a limb.

Effect of ageing on the cardiovascular system: how age affects the hyperaemic response to exercise and hyperthermia

Currently, the ageing population is at its largest since the conception of humanity. In 1950, 11 % of the population were aged 65 years and over, which rose to 18 % by the start of the millennia (Rudnicka *et al.*, 2020). Astonishingly, by 2050 it is predicted that this will rise to 38 % which may very well be larger than the adolescent population (Rudnicka *et al.*, 2020). Traditionally, classical philosophers such as Plato, Cicero, Plutarch and Seneca postulated that ageing is to be revered as it is associated to greater wisdom, knowledge, life-experience, arguing for the benefits of physical decline as it allows one to exclusively exercise those virtues (Anton, 2016; Blasimme *et al.*, 2021). Conversely, more contemporary observations argue that ageing, at its very core, is potentially a pathological state due to its high prevalence of health issues, diseases, and economic burden (Martin & Sheaff, 2007; Gems, 2015; Gladyshev & Gladyshev, 2016). The prevalence of age-related diseases and health issues are further exacerbated by sedentary behaviour, with a systematic review concluding that over 60 % of individuals aged 60 years and over displayed sedentary behaviour (Harvey *et al.*, 2013). Therefore, a call for new methods of improving health and quality of life during old age is warranted. Passive heating, exercise and their combination have been suggested and postulated as potential therapeutic interventions to improve and maintain vascular health and function (Brunt *et al.*, 2016; Hellsten & Nyberg, 2016; Green *et al.*, 2017; Cullen *et al.*, 2020). The following sections of the literature review

will explore how age affects the cardiovascular system during rest, exercise, passive hyperthermia, and the combination of exercise and hyperthermia.

The effect of age on the cardiovascular system at rest

Ageing is associated with a vast number of cardiovascular changes. With ageing conduit arteries experience large structural changes which include an enlarged lumen (diameter) and a thickened wall due to increases in intima and media thickness, changes in the homogeneity of endothelial cells, increases in smooth muscle cells, and increases in collagen and calcium content and deposition (Rosenthal, 1987; Sonesson *et al.*, 1993; Sandgren *et al.*, 1999; Ferrari *et al.*, 2003; Greenwald, 2007; Heckman & McKelvie, 2008; Thijssen *et al.*, 2016). Moreover, the smooth muscle of conduit arteries is impaired with age as the artery is unable to alter tone as effectively and efficiently as it once could (Thijssen *et al.*, 2016). This is predominately due to an impairment in the vasodilator mechanisms, which could be due, but not limited, to deficiencies in the nitric oxide-dependent vasodilator response, reductions in endogenous bioavailability of vasoactive substance or an attenuation of specific β2-adrenoceptorreceptors antagonist receptors (Marin & Rodriguez-Martinez, 1999; Ferrari *et al.*, 2003; Heckman & McKelvie, 2008; Thijssen *et al.*, 2016). Consequently, when taken together, these changes result in reductions in vascular compliance and thus, cause increases in pulse pressure and arterial stiffness which impair the vessels' ability to cushion cardiac pulsations at the macrocirculatory level (Kawasaki *et al.*, 1987; Rosenthal, 1987; Sonesson *et al.*, 1993; Heckman & McKelvie, 2008; Hickson *et al.*, 2010; Li *et al.*, 2019). This impairment to cushion cardiac pulsations has been suggested to result in an increased ventricular afterload and left ventricular hypertrophy (Safar, 1990; O'Rourke & Hashimoto, 2007; Heckman & McKelvie, 2008). At the level of the heart—and in addition to the increases in left ventricular hypertrophy and ventricular afterload—left-ventricular stiffness, systolic blood pressure and end-diastolic filling increase while myocardial contractility, heart rate, responsiveness to sympathetic activity, diastolic blood pressure and early diastolic filling decrease (Rosenthal, 1987; Ferrari *et al.*, 2003; Heckman & McKelvie, 2008; Bolton & Rajkumar, 2011). Together, these modifications produce a diminished cardiac output which in turn is associated with a decrease in one's maximal exercise capacity with age, as well as having implications for one's tolerance and cardiovascular responses to acute

hyperthermia (Rosenthal, 1987; Rosen *et al.*, 1998; Kenney & Munce, 2003; Fleg *et al.*, 2005; Heckman & McKelvie, 2008; Roman *et al.*, 2016).

The effect of age on the cardiovascular system during exercise

Systemic cardiovascular responses. Figure 6 illustrates the effect of age on systemic and local cardiovascular adjustments during incremental cycling to exhaustion (Beere *et al.*, 1999). Following 20 years of maturity, albeit highly individualistic (Pollock *et al.*, 2015), humans commonly experience a reduction in $\dot{V}O_{2\,max}$ by approximately 10 % per decade in sedentary individuals (Betik & Hepple, 2008). Interestingly, and perhaps more insightful, Fleg *et al.* (2005) found that the decade decline in $\dot{V}O_{2\,max}$ during one's 20s and 30s was substantially lower than that following one's 70s: ~5 % vs ≥20 %. However, this decline in $\dot{V}O_{2\,max}$ can be minimised by regular engagement in physical activity and exercise, with active individuals reporting a 3-fold lower decline in $\dot{V}O_{2\,max}$ than their sedentary counterparts (Dehn & Bruce, 1972). Using the Fick equation, where $\dot{V}O_{2\,max}$ is the product of cardiac output and arteriovenous oxygen difference, it is plausible to assume that one of these factors must decline to explain the age-related reduction in $\dot{V}O_{2\,max}$. During submaximal to maximal large muscle mass, lower-limb exercise—i.e., one-legged and two-legged cycling, and treadmill running—it is commonly accepted that elderly participants display a diminished cardiac output, much like at rest (Brandfonbrener *et al.*, 1955; Hossack & Bruce, 1982; Hagberg *et al.*, 1985; Beere *et al.*, 1999; Proctor *et al.*, 2003; Proctor *et al.*, 2004; Roman *et al.*, 2016), particularly as heart rate declines ~5 % per decade (Fleg *et al.*, 2005). However, several studies challenge this dogma, reporting a maintained cardiac output during submaximal and maximal exercise (Rodeheffer *et al.*, 1984; Kenney & Ho, 1995; Proctor *et al.*, 1998a; Houghton *et al.*, 2016; Fuller *et al.*, 2021), which is related to an enhanced stroke volume (Rodeheffer *et al.*, 1984; Safar, 1990; Kenney & Ho, 1995; Houghton *et al.*, 2016; Fuller *et al.*, 2021). A comprehensive study by Beere *et al.* (1999) reported that elderly individuals had a similar oxygen consumption, cardiac output and systemic arteriovenous oxygen difference to their younger counterparts for the same workload but that their maximum oxygen consumption, cardiac output and workload was significantly attenuated (Figure 6). This raises important methodological concerns when selecting relative over absolute workloads. Utilising relative workloads—as the majority of the aforementioned studies have done—does have

merit as it is possible that when using absolute workloads, the workload may be a higher proportion of one's maximum work rate and therefore, would require one to recruit and perfuse a higher amount of muscle mass to perform the same absolute workload (Ray & Dudley, 1998; Donato *et al.*, 2006). However, it is well established that strong relationships exist between cardiac output, oxygen update and workload (Astrand *et al.*, 1964). Thus, caution is required when interpreting central and peripheral haemodynamic results and attributing them to an age-related effect, it could also be due to the use of a lower absolute workload as elderly cohorts typically have a reduced $\dot{V}O_{2\,max}$.

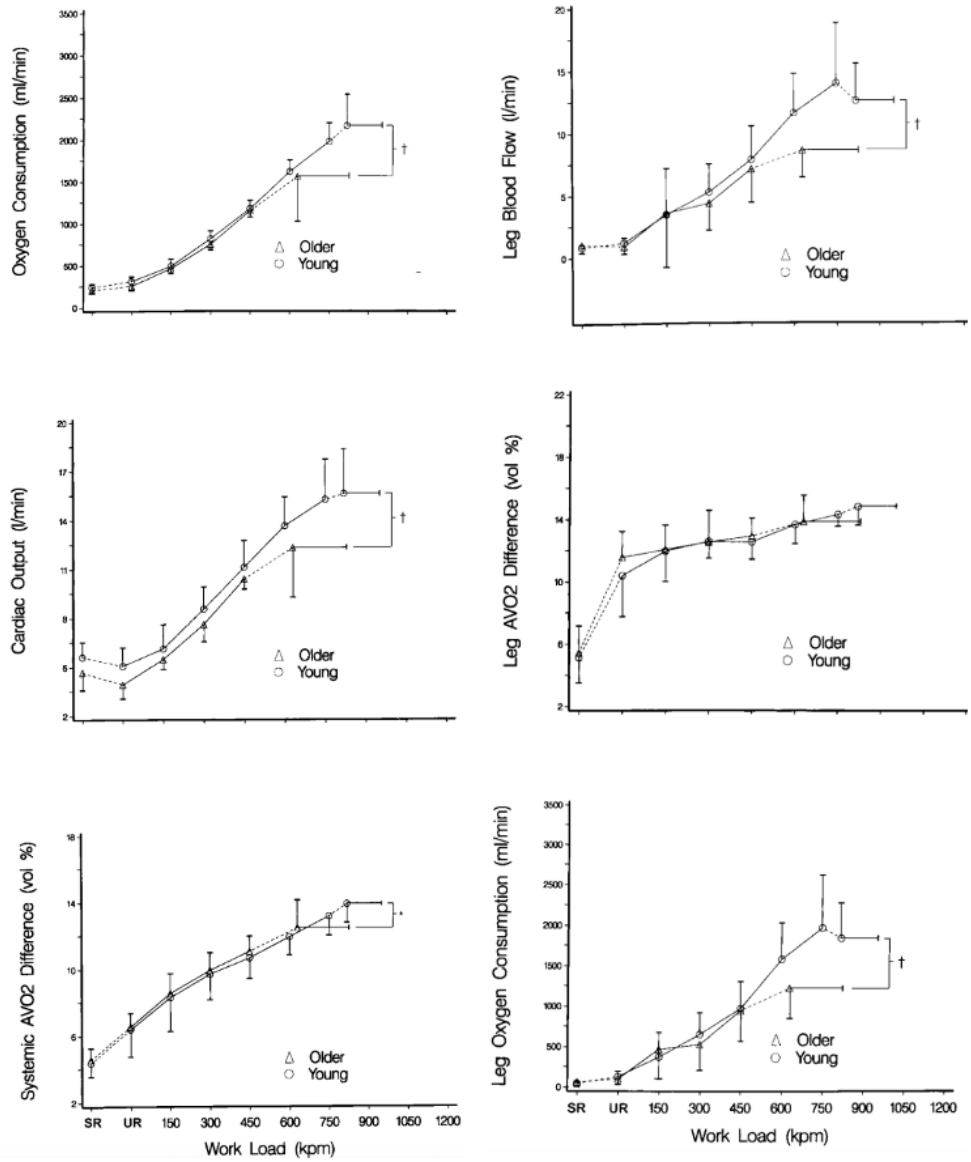

Figure 6. Systemic and local cardiovascular responses to two-legged incremental cycling exercise to exhaustion in healthy, young males (28 ± 7 years) and healthy, elderly males (66 ± 4 years). * $p < 0.05$ and † $p < 0.01$ different from younger cohort. Figure reproduced from (Beere *et al.*, 1999).

On the other component of the Fick equation, arteriovenous oxygen difference may play a role in the age-related depreciation of one's $\dot{V}O_{2\,max}$. Yet, the literature is conflicting, with the majority of studies reporting a similar arteriovenous oxygen difference between older and young participants during submaximal and maximal exercise (Hagberg *et al.*, 1985; Proctor *et al.*, 1998b; Lawrenson *et al.*, 2003; Proctor *et al.*, 2003; Proctor *et al.*, 2004; Mortensen *et al.*, 2012c), whilst others reported a decrease (Houghton *et al.*, 2016; Fuller *et al.*, 2021) or increase during exercise (Wahren *et al.*, 1974; Poole *et al.*, 2003). Interestingly, it appears that studies displaying a maintained cardiac output in elderly participants due to an enhanced stroke volume, reported a diminished arteriovenous oxygen difference (Houghton *et al.*, 2016; Fuller *et al.*, 2021); therefore, suggesting that an enhanced stroke volume could be a compensatory adaptation to maintain adequate blood flow and oxygen delivery to the working muscles. Moreover, in support of a reduced arteriovenous oxygen difference being the prominent factor accounting for the age-related decline in $\dot{V}O_{2\,max}$, are two exercise-intervention studies which reported increases in aged participants' $\dot{V}O_{2\,max}$ primarily due to an enhanced arteriovenous oxygen difference as cardiac output remained unchanged (Beere *et al.*, 1999; McGuire *et al.*, 2001). Though, a counterargument is that these data provide evidence that arteriovenous oxygen difference can be modified with exercise training whereas cardiac output is not; thus, suggesting that the attenuation in cardiac output is truly the effect of age and the primary limitation explaining the age-related decline in $\dot{V}O_{2\,max}$. Other plausible factors that could account for the age-related depreciation of one's exercise capacity are a reduced ability to shunt blood from the viscera to working muscles (i.e., an impaired functional sympatholysis) (Rodeheffer *et al.*, 1984; Koch *et al.*, 2003), a reduction in mitochondrial oxidative capacity in the muscles (Betik & Hepple, 2008), and an impaired response to various vasodilators (e.g., nitric oxide and ATP) and vasoconstrictors (e.g., endothelin-1, α-adrenergic agonists and angiotensin II) (Holowatz & Kenney, 2010; Wray & Richardson, 2015; Hearon & Dinenno, 2016).

In summary, there are a wide range of mechanisms and factors that likely play a role, to some extent, in the age-related decline of one's $\dot{V}O_{2\,max}$. Whilst the exact role and outcome of certain factors are still questioned—particularly cardiac output and arteriovenous oxygen difference—there are definitive trends in the literature. The most apparent and consistent finding is that whilst an age-related effect on cardiac output

and arteriovenous oxygen difference during relative submaximal workloads may be contested, one's maximum oxygen consumption, cardiac output and thus, workload, decline with age, irrespective of health and exercise-trained status.

Limb haemodynamic responses. It is typically recognised that functional limb hyperaemia is reduced in elderly populations during exercise (Betik & Hepple, 2008); though, elderly individuals are still able to produce large increases in limb blood flow of ≥2-fold during low-intensity small muscle mass exercise (Lawrenson *et al.*, 2003; Donato *et al.*, 2006; Mortensen *et al.*, 2012c; Piil *et al.*, 2018) and as high as 9-fold during high-intensity large muscle mass exercise (Wahren *et al.*, 1974; Beere *et al.*, 1999). Nonetheless, the interaction and responses between one's exercise-trained status, exercise modality, and number/mass of recruited muscle groups is rather conflicting. Firstly, regarding small muscle mass exercise, no age-related differences were observed in limb blood flow between normally active participants during low-intensity to submaximal handgrip exercise (Donato *et al.*, 2006). In addition, no age-related leg blood flow differences were observed during low-intensity to submaximal knee-extensor exercise in endurance-trained elderly participants (Mortensen *et al.*, 2012c) and low-intensity knee-extensor exercise in habitually active participants (Piil *et al.*, 2018). Though, an age-related attenuation has been found in sedentary participants during low-intensity to submaximal (Mortensen *et al.*, 2012c) and submaximal to maximal knee-extensor exercise (Lawrenson *et al.*, 2003), and low-intensity to submaximal knee-extensor exercise in normally active participants (Donato *et al.*, 2006; Piil *et al.*, 2018). Furthermore, during large muscle mass two-legged cycling, several studies have reported an attenuation in leg blood flow between young and elderly participants during submaximal to maximal exercise in sedentary (Poole *et al.*, 2003), normally active (Beere *et al.*, 1999; Proctor *et al.*, 2004) and endurance-trained (Wahren *et al.*, 1974; Proctor *et al.*, 1998b) individuals. However, in contradiction, the majority of these studies which plot blood flow responses across various workloads reported a similar magnitude of functional hyperaemia at the lower intensities, with the disparity between aged-groups increasing in parallel to the increasing workload (Wahren *et al.*, 1974; Beere *et al.*, 1999; Poole *et al.*, 2003; Proctor *et al.*, 2003). Despite variations in the literature, the following generalisations can be inferred: (a) elderly individuals remain able to produce large increases in functional hyperaemia, with limb blood flow increasing proportionally to workload and

thus, metabolic demands; (b) there is a trend for elderly participants to have an attenuated limb blood flow during exercise when compared to their younger counterparts; (c) mass of recruited muscles appears to have an effect, with the elderly and young individuals demonstrating a similar limb blood flow when a smaller muscle mass is recruited; and (d) there is little to no difference in limb blood flow between aged cohorts during low-intensity large muscle mass exercise.

The effect of age on the cardiovascular system during hyperthermia

Systemic cardiovascular responses. During whole-body heat stress, central and local thermosensitive mechanisms engage in attempt to dissipate heat from the body, prevent drastic increases in core temperature and therefore, minimise homeostatic disturbances (Johnson & Proppe, 1996; Crandall & Gonzalez-Alonso, 2010). This is true for both young and elderly populations. As shown in Figure 7, during extreme whole-body heating to thermal tolerance (∆ core temperature = + ~2.7 ºC), elderly participants reported an increased cardiac output (∆= ~2 L·min^{-1}) and heart rate (∆= ≥50 beat·min^{-1}), and a reduced stroke volume (∆= ~20 mL·beat^{-1}) (Minson *et al.*, 1998). However, the increase in cardiac output was substantially smaller in comparison to their younger counterparts who displayed an increase of ~4.5 L·min^{-1} (Minson *et al.*, 1998). Other whole-body heating studies found that moderate whole-body hyperthermia (∆ core temperature = + ~0.5 ºC) induced a reduction in stroke volume (∆= ~15 ml·beat^{-1}), an increase in heart rate (~15–20 beat·min^{-1}), but mixed findings regarding cardiac output with one study reporting an increase (∆= ~1 L·min^{-1}) (Sagawa *et al.*, 1988) whilst the other did not (Kenny *et al.*, 2017) in both young and elderly participants, though all values were lower in the elderly cohort. Similarly, during isolated bilateral lower-leg heating where core temperature increased by 0.5 ºC, Romero *et al.* (2017) found that despite increases in cardiac output (∆= ~1 L·min^{-1}) and heart rate (~20 beat·min^{-1}), the responses were attenuated in elderly participants in comparison to their younger counterparts who demonstrated a substantially larger systemic blood flow. Moreover, not all studies compared mean arterial pressure responses during hyperthermia between aged cohorts. Though, those that did, reported that the elderly cohort had a higher baseline mean arterial pressure which then decreased to a greater extent in the elderly cohort (~10–20 mmHg) during moderate whole-body heating (∆ core temperature = + ~0.5 ºC) (Sagawa *et al.*, 1988),

bilateral whole-leg heating (Engelland *et al.*, 2020) and bilateral lower-leg heating (Romero *et al.*, 2017); but surprisingly, no changes were observed during severe whole-body heating (Δ core temperature = + ~2.5 ºC) (Minson *et al.*, 1998). Consequently, whilst some variation exists in the exact interaction among cardiac output, heart rate, stroke volume and mean arterial pressure during hyperthermia, it is apparent that elderly individuals exhibit a diminished central haemodynamic response.

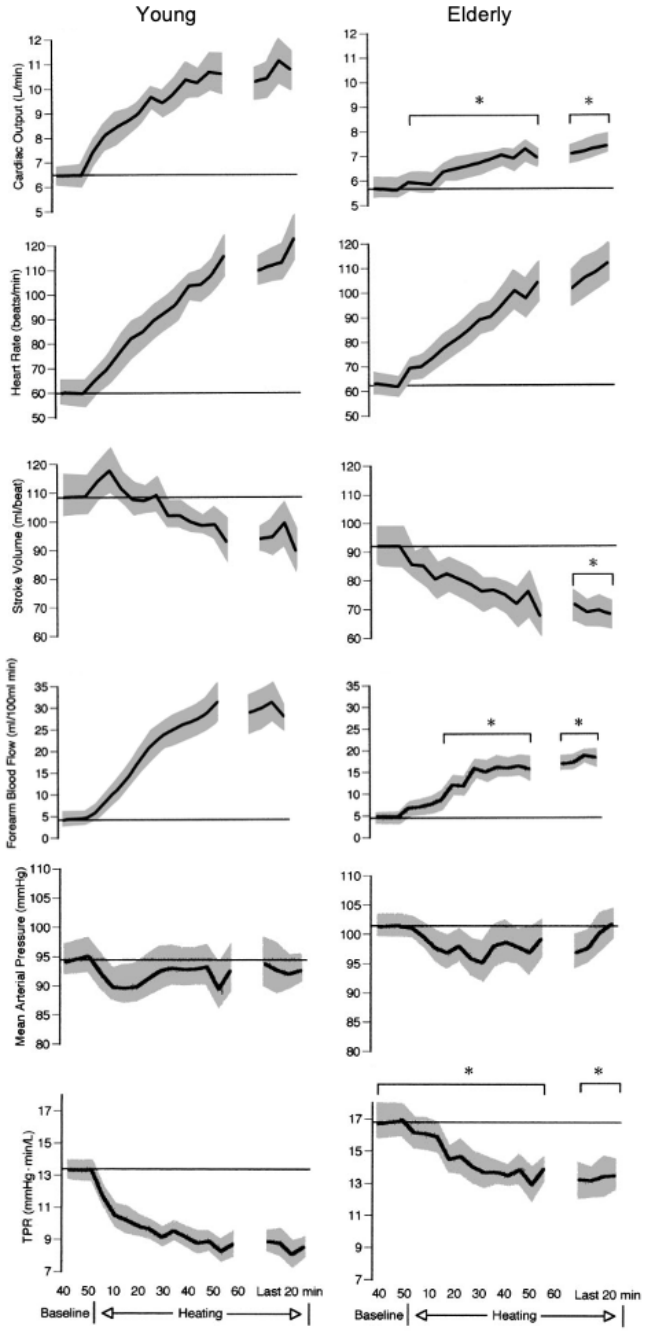

Figure 7. Cardiovascular responses during whole-body hyperthermia in young, healthy males (23 ± 1 years) and elderly, healthy males (70 ± 3 years) individuals. Esophageal temperature increased ~1.5 ºC following 60 min and ~2 ºC during the last 20 min of hyperthermia. * Different from young cohort ($p < 0.05$). Figure reproduced from (Minson *et al.*, 1998).

Peripheral haemodynamic responses. A wealth of literature demonstrates that elderly individuals present an attenuated cutaneous hyperaemic response during local and whole-body hyperthermia, with the aged participants showing a diminished magnitude of skin blood flow for a given core temperature (Kenney, 2001; Van Someren *et al.*, 2002; Kenney & Munce, 2003). Whilst skin blood flow does indeed increase in elderly individuals during hyperthermia, the magnitude of this increase can be close to 2-fold lower in elderly individuals when compared to younger adults (Kenney & Havenith, 1993; Minson *et al.*, 1998). Reasons for this age-related attenuation in hyperthermia-induced cutaneous hyperaemia have been postulated as (a) structural changes in the dermal microcirculation—e.g., reductions in vessel number and function (Montagna & Carlisle, 1979; Weiss *et al.*, 1992; Wehrli *et al.*, 2007)—and (b) mechanistic changes—e.g., diminished axon-reflex and vascular responsiveness to vasodilators (Kenney *et al.*, 1997; Minson *et al.*, 2001)—which ultimately result in a decreased cutaneous vasodilation and reduced skin conductance and thus, attenuated cutaneous blood flow (Martin *et al.*, 1995; Kenney, 2001; Van Someren *et al.*, 2002; Kenney & Munce, 2003). Furthermore, the attenuated skin blood flow observed in elderly participants has also been attributed to a reduced ability to effectively shunt blood from the splanchnic and renal circulations and therefore, *redistribute* blood to the skin (Minson *et al.*, 1998). However, it should be noted that the elderly cohort did also exhibit reductions in splanchnic and renal blood flow in response to whole-body heating and that perhaps, the magnitude of the reductions were smaller than the younger cohort due to a lower absolute cardiac output and reduction in vasodilator capacity as opposed to an inadequate circulatory *redistribution* from the viscera (Minson *et al.*, 1998).

In addition, numerous studies exist which illustrate the limb haemodynamic responses to both isolated-limb and whole-body hyperthermia. Traditionally, studies have used the increase in forearm blood flow as a direct reference of the increase in skin blood flow (Kenney & Havenith, 1993; Martin *et al.*, 1995; Minson *et al.*, 1998); however, as mentioned by Martin *et al.* (1995) this can only be true if muscle and bone blood flow does not change during hyperthermia. One could argue that forearm blood flow is a good indicator of systemic skin blood flow as a strong correlation ($R^2 = 0.93$, $p \leq 0.05$) exists between the hyperthermia-induced changes in forearm blood flow and skin blood flow (Minson *et al.*, 1998). However, this needs to be interpreted with caution as the skin blood flow calculation used in the aforementioned study is estimated as the

sum of cardiac output and *redistributed* splanchnic and renal flow which has inherent shortcomings (see earlier section in literature review) (Minson *et al.*, 1998). Additionally, as discussed earlier, contemporary studies have demonstrated hyperthermia-induced increases in muscle blood flow and thus, invalidating the notion that increases in limb hyperaemia during hyperthermia are direct representations of changes in skin blood flow (Wyper & McNiven, 1976; Heinonen *et al.*, 2011; Pearson *et al.*, 2011; Chiesa *et al.*, 2015; Chiesa *et al.*, 2016; Kalsi *et al.*, 2017). Therefore, for the remainder of this section, the absolute age-related differences in limb hyperaemia during hyperthermia will be discussed without any inference on whether the increases are due to skin, muscle, or bone tissue perfusion. Regarding forearm blood flow, studies have reported increases of ~3-fold following local forearm heating (Rooke *et al.*, 1994; Martin *et al.*, 1995) and 2–4-fold whole-body heating in elderly participants (Sagawa *et al.*, 1988; Armstrong & Kenney, 1993; Minson *et al.*, 1998; Kenny *et al.*, 2017), though the hyperaemic response was attenuated by as much as 50 % in comparison to young adults (Rooke *et al.*, 1994). Similarly, 2-fold increases in common femoral artery blood flow during bilateral leg heating (Engelland *et al.*, 2020), 6-fold increases superficial femoral artery blood flow during bilateral lower-leg heating (Romero *et al.*, 2017), and 2-fold increases in calf blood flow during whole-body heating (Kenny *et al.*, 2017) have been observed in elderly individuals, which typically was lower than their younger counterparts. Changes in vascular conductance mirrored the increases in forearm and/or leg blood flow during both local and whole-body heating (Sagawa *et al.*, 1988; Rooke *et al.*, 1994; Martin *et al.*, 1995; Romero *et al.*, 2017; Engelland *et al.*, 2020). Overall, hyperthermia evokes large increases in both cutaneous and limb hyperaemia in elderly participants, though the increase is attenuated with age.

The effect of age on the cardiovascular system during exercise and hyperthermia

Systemic cardiovascular responses. As demonstrated in the previous sections, both exercise and hyperthermia pose challenges to elderly individuals due to age-related declines in one's physiology (Kenney & Munce, 2003; Betik & Hepple, 2008). Thus, and unsurprisingly, the physiological challenge of defending homeostasis and meeting metabolic demands during the combination of exercise and hyperthermia is

substantially heightened (Kenney *et al.*, 2021). The challenge of meeting the blood flow demands of the exercising muscle as well as the skin to dissipate heat during exercise heat stress is dependent on numerous factors such as exercise intensity, intensity of hyperthermia, and amount of exercising muscle mass (Chiesa *et al.*, 2019). Generally, young adults are able to accommodate the additional burden of heat stress during small muscle mass and/or low-intensity to submaximal exercise large muscle mass exercise by augmenting cardiac output, heart rate and mean arterial pressure (Nielsen *et al.*, 1990; Calbet *et al.*, 2007; Chiesa *et al.*, 2015; Trangmar *et al.*, 2017; Chiesa *et al.*, 2019; Watanabe *et al.*, 2020). However, during maximal whole-body exercise, the addition of hyperthermia can impair performance and push the cardiovascular system to its regulatory limits, due to a more rapid attenuation in cardiac output, mean arterial pressure and functional hyperaemia (Gonzalez-Alonso & Calbet, 2003; Chiesa *et al.*, 2019).

The cardiovascular responses for elderly individuals observed during exercise in heated environments are not too different from what has been reported during exercise in normothermic conditions and passive hyperthermia. In comparison to normothermic conditions, both submaximal and maximal exercise in heated conditions have shown to result in increases in heart rate, decreases in cardiac output, stroke volume, a maintained mean arterial pressure, and in the case of maximal exercise, a reduction in time to exhaustion in middle-aged and elderly individuals (de Paula Viveiros *et al.*, 2012; Best *et al.*, 2014). Nonetheless, elderly cohorts typically demonstrate a diminished ability to increase cardiac output and heart rate to the same level as young adults, during submaximal large muscle mass exercise (Kenney, 1988; Kenney *et al.*, 1990; Tankersley *et al.*, 1991; Ho *et al.*, 1997; Moran *et al.*, 2002; Best *et al.*, 2014). Moreover, whilst one's exercise-trained status can reduce the magnitude of the age-related attenuation in $\dot{V}O_{2\,max}$ (Dehn & Bruce, 1972) which may improve one's capacity for heat dissipation (Meade *et al.*, 2019), trained status did not affect the age-related attenuation in cardiac output and heart rate with trained and sedentary elderly adults showing similar values during relative submaximal cycling in 36 ºC conditions (Ho *et al.*, 1997). However, these measurements are compared between relative workloads despite the trained elderly individuals cycling at a higher absolute workload. This may have implications on the interpretation as the higher absolute workload

would result in a higher metabolic heat production and therefore, pose a higher thermal challenge for the group with a higher $\dot{V}O_{2\,max}$ (Ho *et al.*, 1997).

Peripheral haemodynamic responses. The majority of literature reporting the effect of age on peripheral haemodynamics during exercise in heated conditions are focused on reporting arm limb blood flow (which was not actively engaged during exercise) and changes in regional (e.g., visceral, renal and splanchnic) blood flow as surrogates of cutaneous hyperaemia (Kenney, 1988; Kenney *et al.*, 1990; Tankersley *et al.*, 1991; Kenney & Ho, 1995; Ho *et al.*, 1997; Larose *et al.*, 2013). These studies reported that elderly individuals, albeit significantly lower than young adults, demonstrated continuous increases of ~2-fold in forearm blood flow throughout the exercise protocols—increasing in a time- and/or workload-related manner (Kenney, 1988; Kenney *et al.*, 1990; Tankersley *et al.*, 1991; Kenney & Ho, 1995; Ho *et al.*, 1997). Additionally, though exercise-trained status did not appear to improve the maintenance of central haemodynamics with age, one's exercise-trained status appears to have an effect on the magnitude of cutaneous hyperaemia during exercise heat stress, with trained elderly individuals reporting larger magnitudes in comparison to their sedentary and normally active counterparts (Tankersley *et al.*, 1991; Ho *et al.*, 1997). Furthermore, in line with the traditional hypothesis that blood is *redistributed* from one area of vascular beds to another to meet thermoregulatory and/or metabolic demands, studies have reported that elderly individuals have an attenuated ability to cause vasoconstriction to splanchnic and renal vascular beds during exercise heat stress; thus, reducing the amount of blood *redistributed* to the skin, as demonstrated by the lower forearm blood flow (Kenney & Ho, 1995; Ho *et al.*, 1997).

Following extensive literature searches, and to the best of my knowledge, no studies report direct blood flow measurements of an exercising limb in elderly populations during exercise heat stress. Thus, the haemodynamic responses to exercise heat stress in young adults will be reviewed whilst speculating on what could be expected in elderly individuals. The combination of small muscle mass exercise and heating—such as single-leg knee extensor exercise—has been reported to increase leg blood flow by 0.6–0.7 $L \cdot min^{-1}$, in comparison to normothermic conditions, due to the combination of thermoregulatory and metabolic stimuli (Pearson *et al.*, 2011; Chiesa *et al.*, 2015). Conversely, other studies have reported no differences in leg blood flow

during single-leg knee-extensor exercise between hyperthermic and normothermic conditions (Savard *et al.*, 1988; Ferguson *et al.*, 2006). Whilst discrepancies exist in the literature as to whether hyperthermia has an additive effect on functional hyperaemia during exercise, it should be noted that Ferguson *et al.* (2006) did report a similar ~0.5 $L \cdot min^{-1}$ higher blood flow during the hyperthermic exercise condition. However, these increases were not found to be significant, this could be due to the employment of a lower-intensity of heating: isolated thigh-heating as opposed to whole-leg (Chiesa *et al.*, 2015) and whole-body heating (Pearson *et al.*, 2011). During whole-body (large muscle mass) submaximal exercise in heated conditions, the metabolic and thermoregulatory demands are amplified which ultimately results in the disappearance of the independent hyperthermia-induced additive effect on limb hyperaemia observed during small muscle mass exercise (Nielsen *et al.*, 1990; Trangmar *et al.*, 2017; Chiesa *et al.*, 2019). A linear relationship between core temperature and skin blood flow exists during exercise heat stress; however, this linear relationship disappears when core temperature exceeds 38 ºC, which results in the attenuation of skin blood flow (Brengelmann *et al.*, 1977; Nadel *et al.*, 1979; Fortney *et al.*, 1981; Nose *et al.*, 1990; Kellogg *et al.*, 1993). This is likely due to a limitation of active vasodilator activity, as opposed to an increase in vasoconstrictor tone (Kellogg *et al.*, 1993); thus, explaining, at least in part, why the hyperthermia-induced additive effect on limb hyperaemia disappears with whole-body, large muscle mass submaximal exercise (Chiesa *et al.*, 2019). Additionally, as discussed previously, whole-body exercise to maximal exertion in hot environments puts an extraordinary burden on the cardiovascular system as evidenced by the reductions in stroke volume and thus, cardiac output, mean arterial pressure and functional limb hyperaemia (Gonzalez-Alonso & Calbet, 2003; Gonzalez-Alonso *et al.*, 2004). This attenuation in exercise limb hyperaemia is associated with a reduced perfusion pressure and cardiac output (Chiesa *et al.*, 2019). Consequently, whilst no data currently exists on the effect of age on the functional hyperaemic response of exercising limbs during exercise heat stress, the following could be deduced and hypothesised: (a) elderly populations, much like their younger counterparts, will exhibit significant increases in tissue perfusion to exercising limbs in direct relation to metabolic and thermoregulatory demands; (b) hyperthermia may induce an additive effect on functional limb hyperaemia during small muscle mass and low-intensity exercise but not during large muscle mass submaximal and maximal intensities; and (c) despite large increases in

functional hyperaemia during exercise heat stress, absolute values of perfusion will be lower than observed in young adults, though exercise-trained status may minimise this age-related attenuation.

Summary

Ageing results in significant declines in $\dot{V}O_{2\,max}$ and exercise capacity which are exacerbated by sedentary behaviour. With ageing, individuals experience reductions in both their central and peripheral cardiovascular physiology; however, elderly populations demonstrate similar haemodynamic responses to exercise, hyperthermia, and its combination as their younger counterparts, albeit lower. This is of importance as it supports the notion that exercise, passive hyperthermia or exercise with heating may have the potential to improve vascular health, particularly in elderly population who exhibit a reduced exercise capacity, through various mechanisms associated with higher tissue perfusion.

Literature Review Summary

The present chapter aimed to provide a brief synopsis of the literature surrounding the mechanisms governing and inducing large cardiovascular and haemodynamic adjustments during whole-body hyperthermia, local limb hyperthermia, exercise and how age can influence those adjustments. When exposed to hot environments, the human body engages systemic thermoregulatory mechanisms to dissipate heat and prevent or attenuate the increases in core temperature. These mechanisms include increased sweat rate, cardiac output, and increased cutaneous and peripheral circulation. Research has found that there is a positive correlation between increases in core temperature and an augmented cardiac output, with cardiac output increasing ~2.6 $L \cdot min^{-1} \cdot {}^{o}C^{-1}$.

During whole-body heat stress, cardiac output increases primarily due to an increased heart rate, with a strong positive correlation between the two. This increase is attributed to a direct effect of temperature on the sinoatrial node, increased sympathetic activity, reduced vagal tone, baroreceptor-mediated feedback, and a systemic global hyperadrenergic state. During hyperthermia, the increase in cutaneous hyperaemia is likely achieved through a reduction in sympathetic

vasoconstrictor nerve activity, an increase in sympathetic active cutaneous vasodilation, and/or the activation of local thermosensitive mechanisms due to a heightened skin temperature. Estimates of whole-body skin blood flow are calculated as the difference between cardiac output and visceral blood flow or calculated as a whole-body extrapolation of a directly measured area of skin. Numerous studies have reported increases in limb hyperperfusion during whole-body hyperthermia, with increases as high as 10-fold in the forearm and 3–4-fold in the leg. Similarly, studies have found that local unilateral and bilateral hyperthermia can cause substantial increases in limb blood flow to a similar magnitude as moderate whole-body hyperthermia, without any increases in core temperature or large changes in central haemodynamics. This suggests that local thermosensitive mechanisms are the primary regulators of limb blood flow during local hyperthermia. Contemporary studies favour the role of local thermosensitive mechanisms as the chief mediator of limb hyperaemia; however, the contribution of central vs local mechanisms remains elusive.

Furthermore, ageing is associated with numerous structural and functional alterations in the peripheral vasculature and the myocardium. These typically include increases in arterial diameter, wall thickness and stiffness, and left ventricular hypertrophy, alongside elevations in arterial blood pressure and reductions in maximal cardiac output. Consequently, these modifications produce a diminished maximal aerobic exercise capacity ($\dot{V}O_{2\,max}$) and tolerance to acute hyperthermia. Nonetheless, elderly individuals are still able to produce large increases in functional tissue hyperperfusion during exercise and hyperthermia, though there is a trend for an attenuated response when compared to younger counterparts. The weight of the evidence indicates that elderly adults experience an attenuated cardiovascular, specifically haemodynamic, response during severe whole-body hyperthermia and maximal large muscle mass exercise. However, whether this age-related attenuation in functional hyperaemia is apparent during low-intensity small muscle mass exercise and local limb hyperthermia remains unknown.

Aims

Study 1. The primary aim of this study is to comprehensively investigate the haemodynamic responses in the major arteries of the human leg during prolonged

whole-leg heating and isolated upper-leg and lower-leg heating. Additionally, it aimed to establish relationships among conduit artery hyperaemia, local tissue oxygen saturation, and local temperature. And lastly, it aimed to shed light on the impact of local hyperthermia on local arterial stiffness and distensibility.

Study 2. Building on from the first study, study two aims to delve further into relationship between local limb-segment heating and the ensuing hyperaemia. More specifically, this study will assess the temperature, tissue oxygenation and haemodynamic responses in the major arteries of the human leg during isolated upper-leg heating, quadriceps heating, and partial-quadriceps heating.

Study 3. This study aimed to comprehensively examine and compare the haemodynamic responses between single-leg hyperthermia, one-legged knee-extensor exercise and the combination of both single-leg hyperthermia and one-legged knee-extensor exercise in aged and young adults. In doing so, it aimed to elucidate the impact of age on functional hyperaemia during heat stress and small muscle mass exercise and determine whether local heating has an additive effect on functional hyperaemia.

Hypotheses

Study 1. It was hypothesised that: (a) local hyperthermia would result in profound and sustained increases in blood flow profiles in the arteries supplying the heated leg/leg segment; (b) no changes in the haemodynamic and temperature profiles would be observed in the control leg/adjacent leg segment; (c) local hyperaemia and tissue oxygenation are positively related to regional temperature; and (d) local arterial distensibility would largely remain unchanged during whole leg hyperthermia.

Study 2. It was hypothesised that: (a) all local hyperthermia interventions would increase the blood flow profiles of the femoral arteries which supply the upper leg, with the magnitude of hyperaemia being proportional to the heated tissue surface area/volume, and (b) tissue perfusion will remain unchanged in the adjacent non-heated areas.

Study 3. It was hypothesised that: (a) single-leg hyperthermia and one-legged knee-extensor exercise would increase limb blood flow, which would be tightly coupled to

increases in local limb temperature and/or metabolic demands; and (b) elderly participants would demonstrate an attenuated hyperaemic response to single-leg hyperthermia and one-legged knee-extensor exercise, in comparison to the young adult group.

CHAPTER 3: Methods

Introduction

The following chapter will introduce the general methods utilised in the present thesis' experimental chapters. The specific experimental procedures used in each experimental study will be discussed in depth within the relevant experimental chapter. However, this chapter will aim to provide a general overview of the various methods, discussing their strengths and limitations.

Anthropometry

Participants were asked to weigh themselves in a semi-nude state and then had their height measured (SECA 798 Scale, Germany) and in the case for *Chapters 5 and 6*, had their leg anthropometric measurements recorded. The latter data allowed an estimate of leg composition using the method reported by Jones and Pearson (1969). Seven leg circumferences were taken at the gluteal furrow, one-third subischial (one-third of the distance between the gluteal furrow and the popliteal crease), the minimum circumference above the knee, the maximum circumference at the knee joint, the minimum circumference below the knee, the maximum circumference at the calf, and the minimum circumference at the ankle joint. In addition, the length of these sites in respect to the floor were recorded, along with foot length. Moreover, skinfold measurements were obtained at the following four sites: one-third subischial (anterior and posterior sites) and at the maximum calf circumference (lateral and medial sites) using skinfold callipers (Figure 8) (Jones & Pearson, 1969).

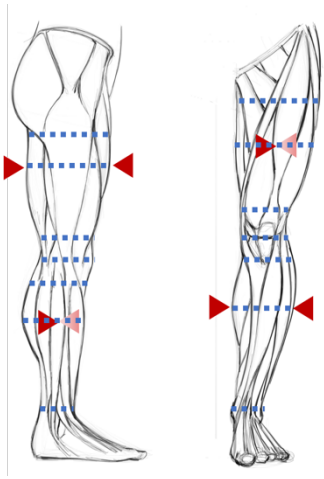

Figure 8. Diagram of circumference (blue dotted lines) and skinfold sites (red triangles) used for anthropometric leg measures.

The anthropometric data was allowed for the calculation of relative tissue perfusion per 100 g of tissue, comparison of muscle mass distribution between elderly and young participants, and to ensure that we had a homogenous population as large differences in adipose tissue could affect the rate of conductive heat transfer from the heated garment to the internal tissues (Ducharme & Tikuisis, 1991; Petrofsky & Laymon, 2009). Whilst Dual Energy X-ray Absorptiometry (DEXA) is generally accepted as the gold standard method for anthropometric measures, there is a good agreement between DEXA and skin-fold measures (Wattanapenpaiboon *et al.*, 1998), which lend support to the validity and reliability of the presently collected data.

Experimental Interventions

Water-perfused trouser

A custom-made water-perfusion trouser was utilised for all heating protocols except for partial-quadriceps heating in *Chapter 5* where the heating was performed via diathermy. The water-perfusion trouser was adjustable and thus, was able to fit all participants securely and be manipulated for the various protocols which required different lengths. The trouser also had custom made opening which allowed one to measure blood flow via duplex Doppler ultrasound without having to remove the trouser, thus minimising heat loss. Once the trouser secured onto the participant's leg, it was wrapped in a survival blanket to limit heat loss (Figure 9). The trouser was connected to a thermostatically controlled water circulator (Julabo F-34, Seelbach, Germany), which for most protocols during *Chapters 4 and 5,* continuously circulated 50 ºC water. However, during the protocol in *Chapter 6*, 58 ºC water was circulated due to a lighter weight version of the trouser being utilised which had a much smaller diameter of tubing and thus, was more susceptible to heat loss. Consequently, to compensate for this, a higher water temperature was used. The water-perfused trouser manipulated leg tissue temperature via conduction where heat energy was transfer from the trouser to the skin and internal tissues through a positive temperature gradient across mediums in direct contact (Incropera *et al.*, 1996).

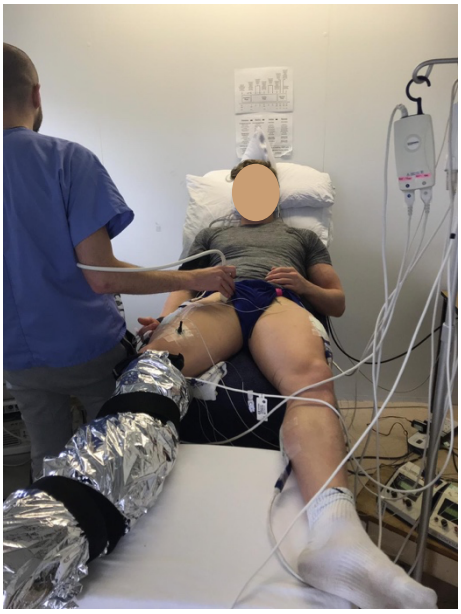

Figure 9. Example of the heated water-perfused trouser being employed to heat the lower-leg during protocol 3 in *Chapter 4*.

Diathermy

During the third protocol in *Chapter* 5, partial-quadriceps heating was conducted using pulsed shortwave diathermy. Diathermy uses electromagnetic currents to heat tissues, transferring heat to the internal tissues via radiation (Beninca *et al.*, 2021). This modality of heating provides a unique opportunity to heat the internal tissues of a limb uniformly at various depths (Sekins *et al.*, 1982; Sekins *et al.*, 1984; Beninca *et al.*, 2021), unlike conductive methods such as the heated water-perfused trouser which slowly heats the tissues in order from superficial to deep. In the present thesis, heating via pulsed shortwave diathermy was performed via the MegaPulse II, EMS Physio, UK which delivers 800 pulses per second, with a pulse duration of 400 μs, and heated a surface area of ~200 cm^2 (Figure 10).

Figure 10. Short-wave diathermy unit (MegaPulse II, EMS Physio, UK) used to heat the quadriceps during the partial-quadriceps heating protocol in *Chapter 5*. Reproduced from EMS Physio.

Knee-extensor exercise

In *Chapter 6*, participants performed incremental one-legged knee-extensor exercise. Though it would have been beneficial to conduct a transitional exercise modality to everyday life such as walking, the isolated nature of the dynamic one-legged knee-extensor exercise model allows one to measure femoral artery blood flow via Doppler ultrasound (Andersen *et al.*, 1985; Radegran, 1997; Rådegran, 1999). The one-legged knee-extensor exercise consisted of two 5 min bouts, the first at 6 W and the second at 12 W. Intensity was controlled by increasing the resistance on the flywheel via metal weights. To account for the minor variations in cadence—i.e., differences in the number of knee extensions per minute—individual work rates were calculated for each stage using the following formula: $Work\ Rate = \overline{Cadence} \times Resistance$. However, participants were recommended to use a cadence of 60 revolutions per minute.

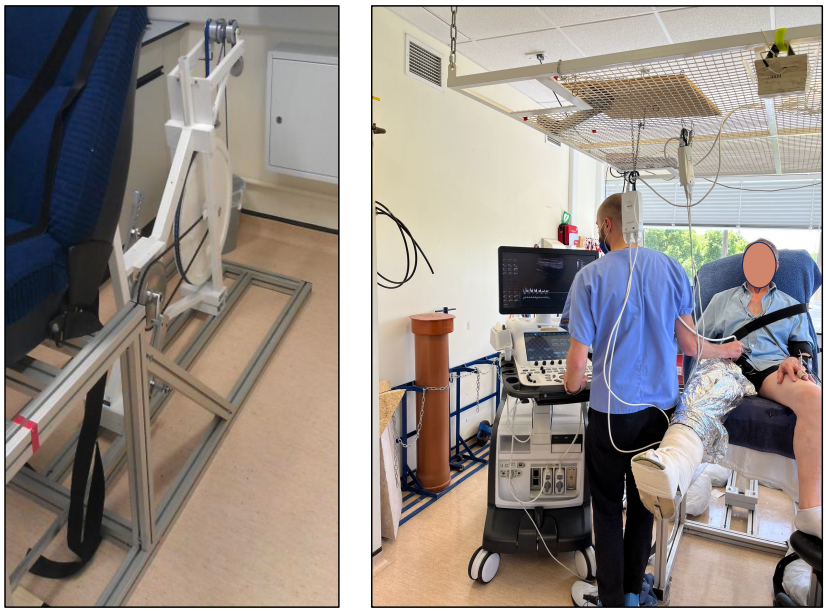

Figure 11. Illustration of the dynamic knee-extensor ergometer. Photo on the left is reproduced from Chiesa (2014) and demonstrates the flywheel and weight basket where resistance could be increased in 6 W increments. Photo on the right demonstrates participant undergoing whole-leg heating and knee-extensor exercise during the experimental protocol in *Chapter 6*. Participants had their leg securely fastened into the ergometer's boot whilst their torso and non-exercising leg were fastened to the ergometer to ensure security.

Temperature Measurements

All temperature measurements were recorded using specialised thermistors (Physitemp, Clifton, NJ, USA) which were connected to a thermocouple meter (TC-2000, Sable Systems International, Las Vegas, NV, USA). The thermocouple meter was connected to a data acquisition system (PowerLab 26T, ADInstruments, New Zealand), which collected the data at a sampling rate of 1000 Hz and exported the data in 1 min bins via its paired data acquisition software (LabChart 7, ADInstruments, New Zealand).

Core temperature

Core temperature (T_c) was measured using a rectal probe (Ret-1 Special, Physitemp, Clifton, NJ, USA) which was self-inserted 15 cm past the sphincter muscle (Figure 12). There is debate in the literature as to what temperature measurements constitute as core temperature, with gold standard being blood temperature at the pulmonary artery and esophageal temperature regarded as the clinical gold standard (Hymczak *et al.*, 2021). Criticisms of rectal core temperature are commonly centred around its poor correlation with pulmonary artery blood temperature and its delayed response (Lefrant *et al.*, 2003; Hymczak *et al.*, 2021). However, in contradiction, a past study has report

close temporal relationships between esophageal, rectal and muscle temperatures during various exercise intensities (Saltin & Hermansen, 1966). Moreover, in support of the present selection to use rectal temperature as a surrogate of core temperature, previous studies from our laboratory have reported significant increases in rectal temperature during whole-body heating in under 30 mins (Pearson *et al.*, 2011; Chiesa *et al.*, 2016; Kalsi *et al.*, 2017; Watanabe *et al.*, submitted); thus, suggesting that it is sufficiently sensitive to observe changes in core temperature.

Figure 12. Image of rectal temperature probe. The probe (Ret-1 Special, Physitemp, Clifton, NJ, USA) was self-inserted 15 cm past the sphincter muscle.

Skin temperature

Skin temperature (T_{sk}) was measured at various locations, dependent on the protocol. Skin temperature was measured using commercially available thermistors (IT-18, Physitemp, Clifton, NJ, USA) which were securely held in place using medical tape (Figure 13).

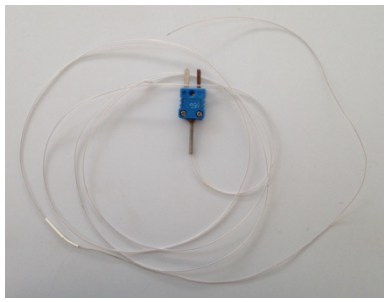

Figure 13. Image of skin temperature thermistors. These thermistors (IT-18, Physitemp, Clifton, NJ, USA)were attached to the participants skin using medical tape.

Muscle temperature

Muscle temperature (T_m) was measured using thermistors (T-204f, Physitemp, Clifton, NJ, USA) inserted via an 18G catheter ~3 cm into the belly of the desired muscle. Depending on the protocol, quadriceps muscle temperature was measured either in the distal, mid-portion or proximal location of the vastus lateralis, hamstrings muscle temperature was measured in the mid-portion of the biceps femoris, and calf muscle temperature was measured in the mid-portion of the gastrocnemius.

Average tissue temperature

Average (mean) leg and segmental leg temperature were calculated using custom-made formulas based on previously reported volume ratios of the different tissue compartments in the leg (Wang *et al.*, 1999). Where both local skin and muscle temperature data were obtained, mean leg temperature ($\overline{T}_{Leg}$) was calculated as a weighted average of skin and muscle temperature: $\overline{T}_{Leg} = (T_{m\,thigh} \times 0.66) + \left(\frac{T_{sk\,thigh} + T_{sk\,hamstring}}{2} \times 0.06\right) + \left(T_{m\,calf} \times 0.25\right) + (T_{sk\,calf} \times 0.03)$; mean upper-leg temperature ($\overline{T}_{Upper-Leg}$) was calculated as: $\overline{T}_{Upper-Leg} = (T_{m\,thigh} \times 0.92) + \left(\frac{T_{sk\,thigh} + T_{sk\,hamstring}}{2} \times 0.08\right)$; and mean lower-leg temperature ($\overline{T}_{Lower-Leg}$) was calculated as: $\overline{T}_{Lower-Leg} = (T_{m\,calf} \times 0.88) + (T_{sk\,calf} \times 0.12)$. However, in circumstances where muscle temperature was not recorded, such as in experimental study detailed in *Chapter 6*, average tissue temperature was represented as mean skin leg temperature ($\overline{T}_{Sk\_Leg}$), and was calculated as an unweighted average of quadriceps, hamstrings, calf, and foot T_{sk}. Similarly, mean skin upper-leg temperature ($\overline{T}_{Sk\_Upper-Leg}$) was calculated as the unweighted average of quadriceps and hamstrings T_{sk}, and mean skin lower-leg temperature ($\overline{T}_{Sk\_Lower-Leg}$) was calculated as the unweighted average of calf and foot T_{sk}. It is important to note that these average tissue temperatures are estimates and due to the small number of data points and complexity in thermodynamic energy exchanges between tissues, their accuracy is somewhat limited. Nonetheless, these average tissues temperatures are useful in providing a depiction of the regional changes in tissue temperature.

Central Haemodynamics

Heart rate

Heart rate was continuously measured via two sets of three-lead echocardiograms. One set was connected to a data acquisition system (PowerLab 26T, ADInstruments, New Zealand), which collected the data at a sampling rate of 1000 Hz and exported the data in 1 min bins via its paired data acquisition software (LabChart 7, ADInstruments, New Zealand). The second set was connected to the ultrasound machine (Vivid 7 Dimension or Vivid E95, 198 GE Medical, Horton, Norway) as the beat-to-beat was required when analysing the ultrasound scans.

Blood pressure

Arterial blood pressure was measured non-invasively using infrared photoplethysmography (Figure 14) (Finometer, FMS, Netherlands). Participants were two pressure-inflated cuffs: one on the middle finger of the left hand (Figure 14) and the other cuff on the upper-left arm. This measurement of arterial blood pressure is centred around the volume-clamp method whereby, in essence, changes in intra-luminal pressure of the arterial diameter within the finger are met by an equal pressure generated from the finger cuff which maintain, and therefore clamp, the arterial diameter at its predetermined “set-point” (Bogert & van Lieshout, 2005). Since the pressure required from the cuff to maintain the arterial diameter at this “set-point” is equal to the intra-luminal pressure of the artery, this method provides an accurate, continuous and non-invasive assessment of arterial pressure (Wesseling *et al.*, 1993; Bogert & van Lieshout, 2005).

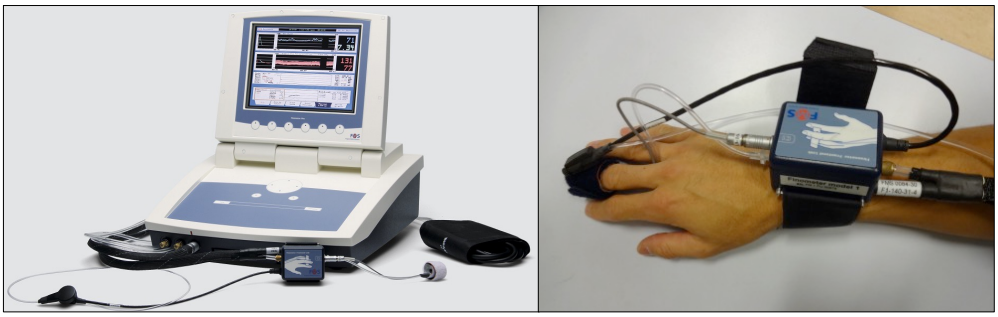

Figure 14. Finometer equipment. Image on the left illustrates the Finometer infrared photoplethysmography device (reproduced from Finapres.com, FMS, Netherlands). Image on the right demonstrates the fitting of the device on the participant’s wrist and middle finger.

Stroke volume

Stroke volume was directly estimated using the ModelFlow method (Beatscope, FMS) (Wesseling *et al.*, 1993). The ModelFlow method utilises the participant's age, height and weight to estimate an individual pressure-area relationship for the aorta. This calculation is then combined with the measured arterial pressure tracings (as described above) to estimate beat-by-beat stroke volume (Wesseling *et al.*, 1993; Bogert & van Lieshout, 2005).

Cardiac output

Cardiac output was automatically calculated by the Finometer (FMS, Netherlands) as the instantaneous product of the heart rate and stroke volume (Figure 14). This method has been validated during resting, control conditions (Wesseling *et al.*, 1993; Bogert & van Lieshout, 2005); however, cardiac output calculated via the ModelFlow method has been shown to underestimate cardiac output during passive hyperthermia (Shibasaki *et al.*, 2011). As such, caution must be taken when interpreting these data.

Local Haemodynamics

Arterial haemodynamics

Modality. There are several methods to measure limb blood flow, including duplex Doppler ultrasound, microdialysis, positron-emission tomography and venous occlusion plethysmography (Taylor *et al.*, 1984; Johnson *et al.*, 1986; Hussain, 1997; Radegran, 1997; Rådegran, 1999; Heinonen *et al.*, 2011; Richey *et al.*, 2023). Each method has advantages and disadvantages ranging from running costs to distribution of tissue perfusion to volume of examined tissue. For example, positron-emission tomography is known to provide extremely rich detail on the perfusion between different tissues—i.e., skin, muscle, bone (Heinonen *et al.*, 2011). However, it requires extremely expensive equipment and only provides data on a small cross-section; thus, it is known to underestimate perfusion values. For the present thesis, duplex Doppler ultrasound was selected as the mode to assess blood flow as it (i) provides data on both velocity and arterial diameter, (ii) illustrates the distribution of arterial blood flow within a limb at the macrocirculatory level, (iii) can provide haemodynamic data during both rest and exercise, and (iv) is relatively in expensive in comparison to some of the

other aforementioned methods. However, since duplex Doppler ultrasound is sensitive to movement artifacts, validity and reliability are highly dependent on the skills of the sonographer, with coefficient of variation for blood flow at the common femoral artery typically ranging between 3 and 12 % (Shoemaker *et al.*, 1996; Rådegran, 1999; Pearson *et al.*, 2011). Consequently, to ensure reliability of measures, repeat testing was carried out during resting conditions in the common femoral artery where a coefficient of variation of 7.9 % was obtained which is in line with the literature (Pearson *et al.*, 2011; Chiesa *et al.*, 2016).

Vascular ultrasound. Vascular duplex Doppler ultrasound involves the emission of high-frequency sound waves into a medium to create a greyscale image. Anatomical mediums such as adipose, muscle and bone tissue have different acoustic impedances. As such, when the transducer emits ultrasound waves, it causes the local displacement of particles, with these waves reflecting off the medium at different energy proportions in relation to the medium's acoustic impedance (Thrush & Hartshorne, 1999). The reflected ultrasound waves then return and result in the vibration of the ultrasound transducer which generates a voltage and ultimately produces a 2D greyscale B-scan image (Thrush & Hartshorne, 1999). This B-scan then allows one to measure arterial diameter offline which will be discussed in greater detail below. To obtain a measurement for blood velocity, the ultrasound unit utilises the Doppler effect whereby the transmitted ultrasound beams are back-scattered from the flowing blood cells at a different frequency pitch. The reflected waves are then received by the ultrasound transducer which provides a numerical value for blood velocity (Thrush & Hartshorne, 1999).

Figure 15. Duplex Doppler ultrasound machines used for the collection of local haemodynamic data. Vivid 7 ultrasound device (right) was used for data collection in *Chapter 4* whilst Vivid E95 was used for data collection in *Chapter 5 and 6*. Photos reproduced from respective User Manuals (GE Healthcare, UK). *Collection of scans.* Blood velocity and arterial diameter were measured at set time points throughout the protocols in the various arteries. In experimental *Chapter 4*, blood flow was measured using a duplex Doppler ultrasound system (Vivid 7 Dimension, 198 GE Medical, Horton, Norway) with a 10 MHz linear array transducer probe (GE Medical Systems, UK) at an insonation angle of ≤ 60 º, with sample volume positioned in the centre of the artery (Figure 15). For experimental *chapters 5 and 6*, the same method was used but with a different duplex Doppler ultrasound system (Vivid E95, GE Medical Systems, UK) alongside a 9 MHz linear array transducer probe (GE Medical Systems, UK) (Figure 15). Before commencing baseline blood velocity and diameter measures, arterial sites for the common, superficial and profunda femoral arteries and in the popliteal artery in both legs were located and marked to ensure blood flow measures were consistently measured at the same site (Figure 16). Superficial and profunda femoral artery blood flow measurements were acquired at a distance of ≥ 2 cm from the femoral bifurcation to prevent turbulent flow disruption to the measurements, and thus improve validity of measures. Blood velocity and arterial diameter were analysed offline using a commercially available software (EchoPAC, GE Medical, Horton, Norway).

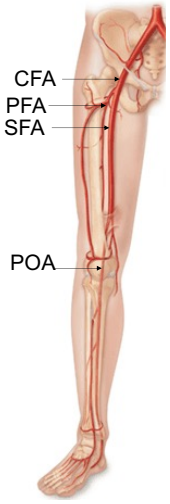

Figure 16. Schematic illustrating the different anatomical landmarks for the common (CFA), superficial (SFA) and profunda (PFA) femoral arteries and the popliteal artery (POA). *Blood velocity.* Blood velocity was averaged over two 12 s duplex Doppler and B-mode scans and reported in cm·s^{-1} (Figure 17).

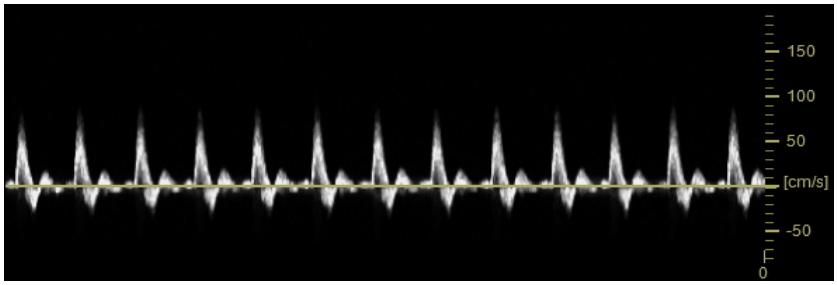

Figure 17. Example of a Doppler pulse-wave velocity trace measured at the common femoral artery at baseline. The trace was taken over 12 s and is expressed in cm·s^{-1}. *Arterial diameter.* To measure arterial diameter, the B-mode scans (Figure 18) were used to measure the distance between the arterial walls at systole and diastole where the arterial diameter is at its largest and smallest, respectively. Once these lengths were obtained, average arterial diameter—D_{mean} (cm)—was calculated using: $D_{mean} = \frac{1}{3}\left(D_{systole}\right) + \frac{2}{3}\left(D_{diastole}\right)$ (Radegran, 1997). Moreover, during protocol 1 in *Chapter* 4, arterial diameter was also determined using CAROLAB (Zahnd et al., 2013) which was a prerequisite for the assessment of arterial distensibility and wave intensity parameters. CAROLAB (Zahnd et al., 2013) uses block matching to provide an accurate measurement of the vessel diameter at each frame. A comparison of arterial diameter between the two methods was performed to ensure validity and reliability. On average, the continuous arterial wall tracking software revealed diameter values 1.9 ± 2.0 % (p

< 0.0001) higher than those obtained via the previously described weighted average of peak systolic and diastolic diameter.

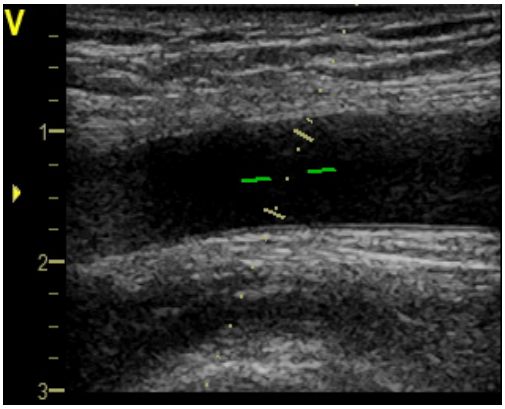

Figure 18. Example of a B-mode scan taken at the common femoral artery at baseline. *Blood flow.* Blood flow (mL·min^{-1}) was calculated using the following equation: $BF = V_{mean} \times \pi \times (\frac{D_{mean}}{2})^2 \times 60$, where V_{mean} is the average blood velocity (cm·s^{-1}) D_{mean} is the average arterial diameter (cm).

Shear rate. Shear rate (SR) was calculated using: $SR = \frac{4 \times V_{mean}}{D_{mean}}$, where V_{mean} is mean blood velocity.

Vascular conductance. Vascular conductance (VC) was calculated using the following formula: $VC = BF \div MAP$, where it is represented as mL·min^{-1}·mmHg^{-1}, BF is blood flow (mL·min^{-1}) and MAP is mean arterial pressure (mmHg).

Wave intensity and local arterial distensibility

Wave intensity analysis was conducted to assess whether prolonged single-leg hyperthermia (*Chapter 4*) had an impact on wave intensity parameters and arterial distensibility. Whilst other techniques which measure pulse wave velocity between two arterial sites via tonometry or Doppler ultrasound are commonly employed to assess arterial stiffness, they provide a regional estimate of stiffness—e.g., the entire arterial network from the carotid to the femoral artery (Millasseau *et al.*, 2005; Xu *et al.*, 2022). Thus, highlighting the advantage of the present method as it can directly estimate local arterial distensibility/compliance of the measured artery (Feng & Khir, 2010; Negoita *et al.*, 2018). Additionally, for clarity, arterial distensibility is directly related to arterial compliance where arterial distensibility is associated to the elasticity of the arterial wall

while arterial compliance refers to the buffering function of the artery (Van Bortel *et al.*, 1995). Both these functions are inversely related to arterial stiffness—i.e., the higher the arterial distensibility/compliance, the lower the arterial stiffness. Moreover, while arterial vasodilation and distensibility/compliance are related in that a less stiff artery has greater capacity to dilate, it is important to note that these indices of arterial stiffness were collected to provide data on the potential health benefits of local hyperthermia rather than to provide insight into the mechanisms regulating tissue perfusion.

Following the obtainment of the ultrasound B-mode scans, images were exported as DICOM files for offline analysis. Wave speed determination and wave intensity analysis were only performed on the CFA of both the experimental and control leg as the DICOM image quality for the other arteries, particularly that of the PFA and POA, was not sufficient for diameter block matching. Diameter waveform extraction was performed using CAROLAB (Zahnd *et al.*, 2013), which uses block matching to provide an accurate measurement of the vessel diameter at each frame. Extracted diameter waveforms were saved as Excel files (Microsoft Corporation, Redmond, WA, USA) for later analysis. Doppler ultrasound DICOM files were analysed in Matlab (version R2019b, The MathWorks, Inc., Natick, Massachusetts, USA), to extract the flow velocity waveforms, using custom designed algorithms as previously reported (Negoita *et al.*, 2018). Diameter waveform data were obtained for the CFA at every 30 min.

This diameter and flow velocity waveforms were then used to calculate wave speed (c) using the ln(*D*)*U*-loop method (Feng & Khir, 2010); the following equation was used: $c = \pm\frac{1}{2}\frac{\mathrm{d}U_{\pm}}{\mathrm{d}(\ln D)_{\pm}}$ where $\mathrm{d}U$ and $\mathrm{d}(\ln D)$ are the incremental differences between adjacent data of velocity (U) and diameter (D). Moreover, forward compression waves (FCW) and forward expansion waves (FEW), which reflect left ventricular performance in early and late systole respectively, were calculated using previously documented techniques (Pomella *et al.*, 2018). Data outputs were averaged over two scans for the same time point, with three waveforms analysed per scan. Subsequently, with the determination of c, distensibility (D_s) was calculated using the following Bramwell and Hill (1922) equation: $D_s = p^{-1} \times c^{-2}$, where p represents blood density which was assumed equal to 1050 kg·m^{-3} (Pomella *et al.*, 2018).

Skin blood flow

Skin blood flow was measured in the quadriceps during the various protocols via laser-Doppler flowmetry (Periflux 4001 Flowmetry System, Jarfalla, Sweden), reported in perfusion units (PU). The probe was a single-point 780 nm wavelength laser-Doppler probe which operated at a power output of 1 mV and a fibre-separation of 0.25 nm which was attached to the skin of the quadriceps, specifically on the vastus lateralis. The probe transmits infrared light ≤ 1 mm deep into the dermis where the frequency of the light is changed by the moving red blood cells (Fredriksson *et al.*, 2009). The use of the Doppler effect allows the quantification of the cutaneous microcirculatory tissue perfusion as the magnitude of change in wavelength and frequency of the returning light is directly related to the number and velocity of present red blood cells (Fredriksson *et al.*, 2009). Calculating skin blood flow via laser Doppler has numerous advantages: it is a simple, direct measure which reports perfusion data directly under the probe and gives a reliable indication of cutaneous and/or microcirculatory blood flow (Holloway & Watkins, 1977; Nilsson *et al.*, 1980a; Nilsson *et al.*, 1980b; Leahy *et al.*, 1999). Yet, it is limited in the fact that (a) it does not have quantitative units, thus making it challenging to compare data, (b) it is extremely sensitive to movement artifacts, (c) lack of precise knowledge on the depth of measurement although likely ≤1 mm, (d) discrepancies surrounding *instrument zero* and *biological zero, and (e)* these measurements are only accurate for the specific location where the measurements took place as cutaneous circulation is heterogenous (Leahy *et al.*, 1999; Braverman, 2000). Nonetheless, despite these limitations, the use laser-Doppler flowmetry does indeed provide additional, important data on the changes in cutaneous tissue perfusion.

Tissue oxygen saturation

Direct, continuous and non-invasive measures of regional tissue haemoglobin (venous) oxygen saturation (% rSO_2) were obtained in the experimental and control legs using two near-infrared spectroscopy units with four optode pads each (NIRS; INVOS 5100C Cerebral Oximeter; Somanetics Corp, Troy, MI, USA). The optode pads were placed on the skin surrounding the quadriceps, hamstrings, calf and foot, depending on the protocol, and were taped to reduce interference from external light sources. NIRS provides data on tissue oxygen saturation ranging from 0 to 99 %;

however, during conditions where metabolic demands and $\dot{V}O_2$ are maintained, NIRS provides a surrogate measure of microcirculatory tissue perfusion (Ferrari *et al.*, 2004; Harel *et al.*, 2008). The optodes employed contain a light source and two photodetectors which are 3 and 4 cm away from the light source, and measure tissue oxygen saturation at different depths (Marin & Moore, 2011). The emitted light penetrates the limb tissue and returns following a banana-shaped path to the surface where it is detected by optode's photodetectors, with the shallower-penetrating light emerging closer to the light source and the deeper-penetrating light emerging further away from the light source (Figure 19). While NIRS provides can provide data on microcirculatory perfusion in the deeper tissues, especially when compared to laser-Doppler spectroscopy, care needs to be taken when interpreting the results. In particular, when referring to any changes in tissue oxygen saturation as a sole change in muscle perfusion as the measurement can be affected by the thickness of adipose tissue (Ferrari *et al.*, 2004) and has been shown to be contaminated by changes in skin blood flow (Davis *et al.*, 2006). Irrespectively, while care should be taken in the interpretation of results, when combined with direct arterial blood flow measurements via duplex Doppler ultrasound and laser-Doppler spectroscopy, tissue oxygen saturation data via NIRS, provides further data on the distribution and changes in local tissue perfusion.

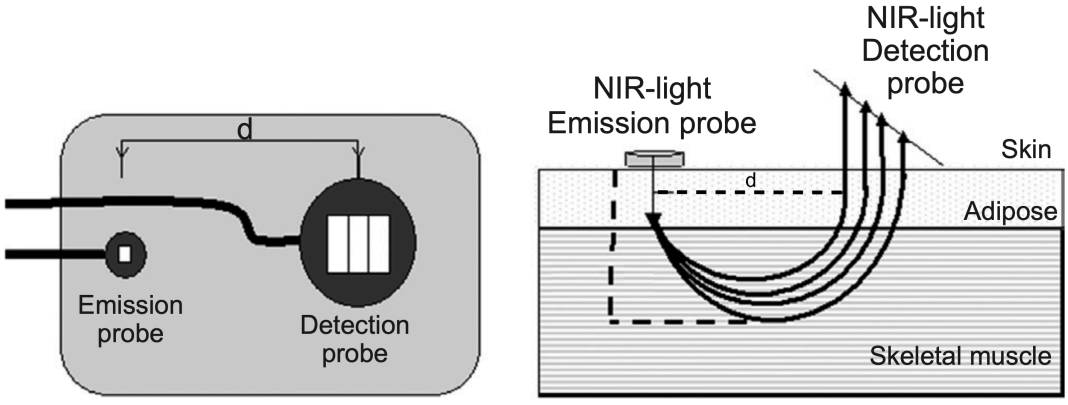

Figure 19. Schematic of a Near-Infrared Spectrometry optode (left) and the banana-shaped path that the light follows before being detected by the photodetectors (right). *d* is the length between the light source and photodetectors and is usually between 3 to 4 cm. Figure reproduced from Lima and Bakker (2011).

CHAPTER 4

Study 1: Regional thermal hyperaemia in the human leg: evidence of the importance of thermosensitive mechanisms in the control of the peripheral circulation

Contextual and relevant particulars for Study 1

Study 1 was published in *Physiological Reports* in 2021. The published article can be found in the appendices.

> Koch Esteves, N., Gibson, O. R., Khir, A. W., & González-Alonso, J. (2021). Regional thermal hyperemia in the human leg: Evidence of the importance of thermosensitive mechanisms in the control of the peripheral circulation. *Physiological Reports*, 9, e14953. https://doi.org/10.14814/phy2.14953.

Author contributions. This study was performed at Brunel University London, Uxbridge, UK as part of the present PhD thesis with NKE as the primary researcher and author. NKE and JGA conceived and designed the research. NKE, ORG, AWK and JGA acquired the data. NKE analysed the data. NKE, AWK and JGA interpreted the data.

Abstract

Hyperthermia is thought to increase limb blood flow through the activation of thermosensitive mechanisms within the limb vasculature, but the precise vascular locus in which hyperthermia modulates perfusion remains elusive. We tested the hypothesis that local temperature-sensitive mechanisms alter limb haemodynamics by regulating microvascular blood flow. Temperature and oxygenation profiles and leg haemodynamics of the common (CFA), superficial (SFA) and profunda (PFA) femoral arteries and popliteal artery (POA) of the experimental and control leg were measured in healthy participants during: (1) 3 h of whole-leg heating (WLH) followed by 3 h of recovery (n = 9); (2) 1 h of upper-leg heating (ULH) followed by 30 min of cooling and 1 h ULH bout (n = 8); and (3) 1 h of lower-leg heating (LLH) (n = 8). WLH increased experimental leg temperature by 4.2 ± 1.2 ºC and blood flow in CFA, SFA, PFA and POA by ≥3-fold, whilst core temperature essentially remained stable. Upper- and lower-leg blood flow increased exponentially in response to leg temperature and then declined during recovery. ULH and LLH similarly increased the corresponding segmental-leg temperature, blood flow and tissue oxygenation without affecting these responses in the non-heated leg segment, or perfusion pressure and conduit artery diameter across all vessels. Findings demonstrate that whole-leg hyperthermia induces profound and sustained elevations in upper- and lower-limb blood flow and that segmental hyperthermia matches the regional thermal hyperaemia, without causing thermal or haemodynamic alterations in the non-heated limb segment. These observations support the notion that heat-activated thermosensitive mechanisms in the microcirculation regulate limb tissue perfusion during hyperthermia.

Keywords: Heat, blood flow, haemodynamics, thermal mechanisms.

Introduction

Passive whole and segmental limb hyperthermia increases local tissue perfusion in association with elevations in calculated limb vascular conductance (Keller *et al.*, 2010; Pearson *et al.*, 2011; Kalsi *et al.*, 2017; Romero *et al.*, 2017). Nevertheless, the exact vascular locus in which hyperthermia increases blood flow and tissue perfusion within the limb vascular tree remains elusive. Classic whole-body hyperthermia studies suggest that central haemodynamic factors—i.e., changes in mean arterial pressure and cardiac output—modulate the regulation of peripheral blood flow during hyperthermia (Edholm *et al.*, 1957; Blair *et al.*, 1960). Recent evidence, however, reveals that single-leg heating elicits similar leg blood flow responses to moderate whole-body hyperthermia (core temperature Δ = +1 ºC), despite large differences in systemic haemodynamics and temperature responses (Pearson *et al.*, 2011; Chiesa *et al.*, 2016). During local hyperthermia, limb blood flow is elevated in a local tissue temperature-dependent manner, suggesting that local thermosensitive mechanisms in the limb vasculature rather than central haemodynamic factors play a crucial role in limb tissue blood flow regulation during hyperthermia (Heinonen *et al.*, 2011; Chiesa *et al.*, 2016; van Mil *et al.*, 2016). Nonetheless, it remains unknown whether heating a limb segment—i.e., the upper- or lower-leg—solely increases temperature and blood flow in the heated region or whether it would also evoke responses in the adjacent non-heated limb segment.

Conduit artery blood flow is the product of vascular conductance and perfusion pressure gradient (Laughlin, 1999). In local limb hyperthermic conditions where perfusion pressure remains stable, an increase in vascular conductance—and thus an increase in arterial diameter and/or blood velocity—could explain the hyperthermia-induced hyperaemia. Nevertheless, whether local hyperthermia increases conduit artery diameter remains equivocal, with studies reporting increases (Kalsi *et al.*, 2017), decreases (Thomas *et al.*, 2017), or no changes in arterial diameter (Pearson *et al.*, 2011; Chiesa *et al.*, 2016; Teixeira *et al.*, 2017; Coombs *et al.*, 2021). Moreover, hyperthermia may directly act on the conduit artery supplying blood to the heated region by increasing arterial stiffness/decreasing arterial distensibility rather than altering diameter, as seen during incremental exercise (Pomella *et al.*, 2018). The current literature suggests that central and/or regional arterial stiffness is unchanged during acute hyperthermia (Ganio *et al.*, 2011; Moyen *et al.*, 2016; Schlader *et al.*,

2019), and declines following the cessation of heating (Thomas *et al.*, 2017; Lee *et al.*, 2018; Sugawara & Tomoto, 2021). To our knowledge, however, no study has explored the effects of hyperthermia on arterial distensibility using local techniques—such as the *PU*-loop or ln(*D*)*U*-loop (Khir *et al.*, 2001; Feng & Khir, 2010). This could provide further evidence to support the therapeutic potential of local hyperthermia for treatment of circulatory diseases and/or rehabilitation (Brunt *et al.*, 2016; Thomas *et al.*, 2017; Coombs *et al.*, 2019), as arterial stiffness—which might rise during long-term bed rest, leg immobilisation and sedentary behaviour (Bleeker *et al.*, 2005; van Duijnhoven *et al.*, 2010; Mortensen *et al.*, 2012b; Bohn *et al.*, 2017)—is commonly associated with increases in cardiovascular mortality (Vlachopoulos *et al.*, 2010).

The aim of the present study was three-fold. Firstly, to comprehensively investigate the tissue temperature and oxygenation profiles and the haemodynamic responses in the major arteries of the human leg during prolonged whole-leg heating and the subsequent recovery, and during isolated upper-leg and lower-leg heating. Secondly, to establish the relationships among conduit artery hyperaemia, local tissue oxygenation and local hyperthermia. And thirdly, to determine the impact of local hyperthermia on local arterial stiffness and distensibility. It was hypothesised that: (a) local hyperthermia would result in profound and sustained increases in blood flow profiles in the arteries supplying the heated leg/leg segment; (b) no changes in the haemodynamic and temperature profiles would be observed in the control leg/adjacent leg segment; (c) local hyperaemia and tissue oxygenation are positively related to regional temperature; and (d) local arterial distensibility would largely remain unchanged during whole-leg hyperthermia.

Materials and methods

Participants

This study consisted of three protocols: 1) whole-leg heating, 2) upper-leg heating, and 3) lower-leg and foot heating. In total, eight healthy men and one healthy woman (mean ± SD: age 28 ± 11 years; height 177 ± 8 cm; mass 79.7 ± 9.1 kg) participated in protocol 1, five healthy men and three healthy women (age 32 ± 14 years; height 174 ± 10 cm; mass 72.7 ± 13.9 kg) in protocol 2, and five healthy men and three healthy women (age 29 ± 11 years; height 176 ± 9 cm; mass 72.3 ± 11.2 kg) in protocol 3. Three participants completed all three protocols whereas four completed two. Prior

to the start of the study, informed written consent was obtained from all participants following a detailed written and verbal explanation of the experimental protocol. Participants were considered healthy following the completion of a health questionnaire. All procedures were approved by the Brunel University London Research Ethics Committee and are in agreement with the ethical principles stated in the Declaration of Helsinki (2013). Participants refrained from heavy exercise for 48 h, alcohol consumption for 24 h and caffeine consumption for 12 h before the commencement of the protocols.

Experimental protocols

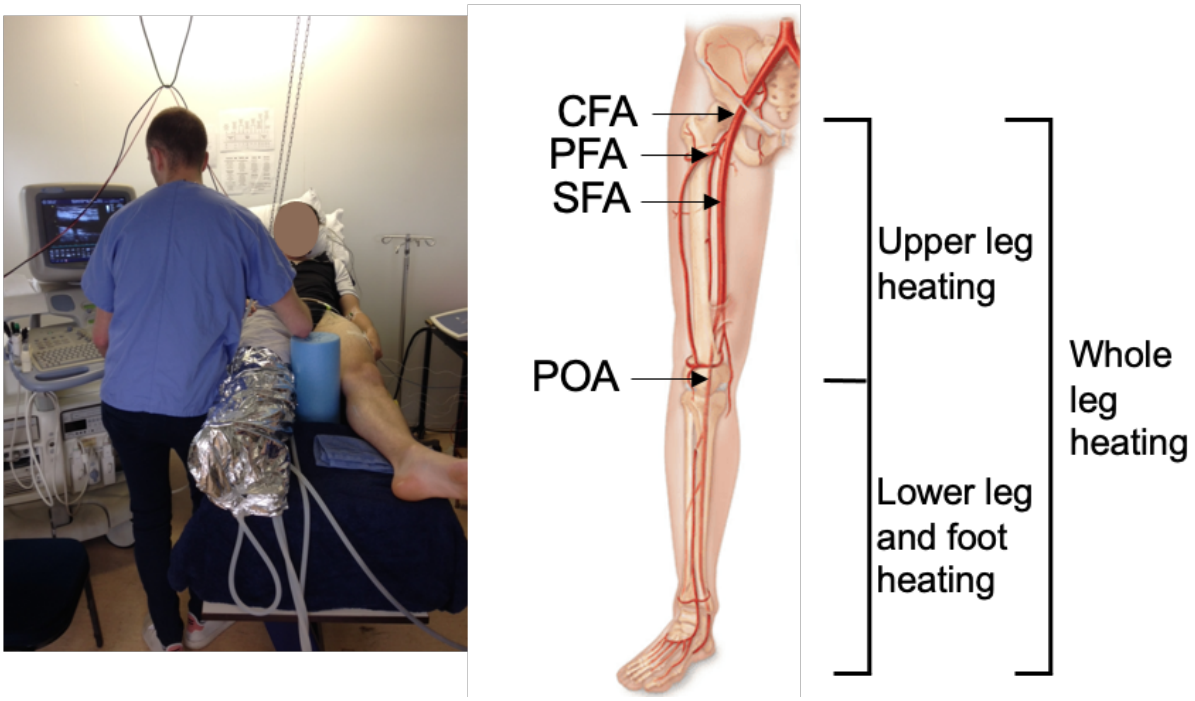

Figure 20. Example of experimental set up. Illustration of the experimental set up—participant was undergoing whole-leg heating in the present example. The diagram shows the segments where blood flow was measured for the CFA, SFA, PFA and POA, respectively, and which segments were heated for the various protocols.

For all three protocols, participants were asked to consume their usual breakfast and report to the laboratory between 08h00 and 09h00, whereby they fasted until the completion of the protocol. They were weighed in a semi-nude state and had their height measured (SECA 798 Scale, Germany) and then asked to rest in a supine position on a custom-built bed within a climate chamber set at 21 ºC, where they remained for the entire duration of the study.

Protocol 1: effects of prolonged whole-leg heating on thermal, haemodynamic and tissue oxygenation responses. Protocol 1 consisted of 3 h of whole-leg heating, followed by 3 h of passive recovery. Following instrumentation—ECG electrodes, intravenous cannulation at the antecubital vein, and temperature thermistors (described below)—participants were fitted with a custom-made water-perfusion trouser on their right leg, which was then wrapped in a survival blanket to limit heat loss. The trouser was connected to a thermostatically controlled water circulator (Julabo F-34, Seelbach, Germany), which continuously circulated 50 ºC water for the first 1.5 h of heating. The water was later reduced to 48 ºC to prevent a large increase in core temperature. Blood flow was measured every 30 min in the common (CFA), superficial (SFA) and profunda (PFA) femoral arteries and popliteal artery (POA) of the experimental and control legs (Figure 20). A 5 ml-venous blood sample and subjective perceptual measures were also collected at the same time points. Haemoglobin and haematocrit concentrations were measured using a commercially available Hb analyser (Hb 201^{+} system, HemoCue AB, Ängelholm, Sweden), and a microscope and a ruled apparatus were used after centrifuging blood samples for 5 min (Billett, 1990), respectively. Haemoglobin and haematocrit values were then used to calculate blood volume, red blood cell volume, and plasma volume changes and estimate their absolute values, using previously described methods (Dill & Costill, 1974; Sawka *et al.*, 1992). Subjective perceptual measures were obtained with a 5-point thermal comfort scale (T_{comf}) (1 representing comfortable and 5 very uncomfortable, respectively) (Willmott *et al.*, 2017) and an 8-point thermal sensation (T_{sens}) scale (0 representing unbearably cold and 8 unbearably hot, respectively) (Toner *et al.*, 1986).

Protocol 2: effects of upper-leg heating on thermal, haemodynamic and tissue oxygenation responses. Following instrumentation of ECG electrodes and temperature thermistors, much like protocol 1, participants were fitted with a custom-made water-perfusion trouser on their right upper-leg, which was then wrapped in a survival blanket to prevent heat loss (Figure 20). The trouser was connected to a thermostatically controlled water circulator, which continuously circulated 50 ºC water for the first 1 h of heating, before circulating 20 ºC water for the 30 min of cooling, and lastly circulated 50 ºC water for the last 1 h of heating. Blood flow was measured every

10 min in the CFA and POA of the experimental leg, and in the control leg at baseline and 150 min of the protocol.

Protocol 3: effects of lower-leg and foot heating on thermal, haemodynamic and tissue oxygenation responses. Following instrumentation of ECG electrodes and temperature thermistors, much like the previous protocols, participants were fitted with a custom-made water-perfusion trouser on their right lower-leg and foot, which was then wrapped in a survival blanket to limit heat loss. The trouser was connected to a thermostatically controlled water circulator, which continuously circulated 50 ºC water for the 1 h of heating. Blood flow was measured every 20 min in the CFA, SFA, PFA and POA of the experimental leg (Figure 20), and in the control leg at baseline and 60 min of the protocol.

Temperature measurements

Core temperature (T_c) was measured using a rectal probe (Ret-1 Special, Physitemp, Clifton, NJ, USA) which was self-inserted 15 cm past the sphincter muscle. Skin temperature (T_{sk}) in the thigh and calf for both the experimental and control legs were measured using commercially available thermistors (IT-18, Physitemp, Clifton, NJ, USA) which were securely held in place using medical tape. Muscle temperature (T_m) in the vastus lateralis muscle and in the gastrocnemius muscle of the experimental and control leg in protocol 1, and only of the experimental leg in protocols 2 and 3, were measured using thermistors (T-204f, Physitemp, Clifton, NJ, USA) inserted via an 18G catheter ~3 cm into the mid-portion of the muscle. T_c, T_m and T_{sk} were recorded online using a commercially available thermocouple meter (TC-2000, Sable Systems International, Las Vegas, NV, USA) connected to a data acquisition system (PowerLab 26T, ADInstruments, New Zealand). Additionally, mean leg temperature ($\overline{T}_{Leg}$) was calculated as a weighted average: $\overline{T}_{Leg} = (T_{m\ thigh} \times 0.66) + \left(\frac{T_{sk\ thigh} + T_{sk\ hamstring}}{2} \times 0.06\right) + \left(T_{m\ calf} \times 0.25\right) + (T_{sk\ calf} \times 0.03)$; mean upper-leg temperature ($\overline{T}_{Upper-Leg}$) was calculated as: $\overline{T}_{Upper-Leg} = (T_{m\ thigh} \times 0.92) + \left(\frac{T_{sk\ thigh} + T_{sk\ hamstring}}{2} \times 0.08\right)$; and mean lower-leg temperature ($\overline{T}_{Lower-Leg}$) was calculated as: $\overline{T}_{Lower-Leg} = (T_{m\ calf} \times 0.88) + (T_{sk\ calf} \times 0.12)$. Mean leg and segmental leg temperature formulas were

created using previously reported volume ratios of the different tissue compartments in the leg (Wang *et al.*, 1999).

Haemodynamic measurements

Heart rate was continuously measured using a three-lead echocardiogram. Moreover, arterial blood pressure, stroke volume and cardiac output were measured non-invasively—at the same time points as the arterial blood flow measurements—using infrared photoplethysmography (Finometer, FMS, Netherlands), through a cuff on the middle finger of the left hand. Cardiac output was calculated as heart rate × stroke volume, where stroke volume was directly estimated using the ModelFlow method, which incorporated corrections for age, height, and weight (Beatscope, FMS) (Wesseling *et al.*, 1993). Blood flow was measured at set time points—recording two 12 s Doppler scans—throughout the protocols in the various arteries using a duplex Doppler ultrasound system (Vivid 7 Dimension, 198 GE Medical, Horton, Norway) with a 10 MHz linear array transducer probe (GE Medical Systems, UK) at an insonation angle of ≤ 60 °, with sample volume positioned in the centre of the artery. The water-perfusion heated trouser had custom-made openings which allowed the probe to be placed on the skin with minimal heat loss. Before commencing baseline blood flow measures, arterial sites for the CFA, SFA, PFA and POA in both legs were located and marked to ensure blood flow measures were consistently measured at the same site. SFA and PFA blood flow measurements were acquired at a distance of ≥ 2 cm from the femoral bifurcation to prevent turbulent flow disruption to the measurements, and thus improve validity of measures. Blood flow (mL·min^{-1}) was calculated using the following equation: $BF = V_{mean} \times \pi \times (\frac{D_{mean}}{2})^2 \times 60$, where V_{mean} is the average blood velocity (cm·s^{-1}) and D_{mean} (cm) is the average diameter calculated using: $D_{mean} = \frac{1}{3}(D_{systole}) + \frac{2}{3}(D_{diastole})$ (Radegran, 1997). Moreover, arterial diameter was determined using CAROLAB (Zahnd *et al.*, 2013) which was a prerequisite for the assessment of arterial distensibility and wave intensity parameters—outlined in the subsequent section. CAROLAB (Zahnd *et al.*, 2013) uses block matching to provide an accurate measurement of the vessel diameter at each frame. A comparison of arterial diameter between the two methods was performed to ensure validity and reliability. On average, the continuous arterial wall tracking software revealed diameter values 1.9 ± 2.0 % (p < 0.0001) higher than those obtained via the previously

described weighted average of peak systolic and diastolic diameter. Additionally, blood flow was expressed in terms relative to tissue mass—i.e., mL·min^{-1}·100 g^{-1}—and was calculated using the participants' body mass and previously reported segment mass to body mass ratios (Clauser *et al.*, 1969).

Shear rate (SR) was calculated using: $SR = \frac{4 \times V_{mean}}{D_{mean}}$, where V_{mean} is mean blood velocity. Additionally, vascular conductance (VC) was calculated using the following formula: $VC = BF \div MAP$, where it is represented as mL·min^{-1}·mmHg^{-1}, BF is blood flow (mL·min^{-1}) and MAP is mean arterial pressure (mmHg). Blood flow was analysed offline using a commercially available software (EchoPAC, GE Medical, Horton, Norway). Blood velocity was averaged over two 12 s Doppler scans, and average diameter was determined from four 2D B-mode images. Furthermore, blood pressure and temperature data were collected at 1000 Hz using a commercially available data acquisition system (PowerLab 26T, ADInstruments, New Zealand) and exported in 1 min bins using a commercially available data acquisition software (LabChart 7, ADInstruments, New Zealand). Following exportation, data were imported and analysed in Microsoft Excel software (Microsoft Corporation, Redmond, WA, USA). Data are reported as 2 min averages throughout the three protocols. Additionally, quadriceps skin blood flow was measured in all three leg heating protocols via laser-Doppler flowmetry (Periflux Flowmetry System, Jarfalla, Sweden), reported in perfusion units (PU). The probe was attached to the skin of the thigh, specifically on the vastus lateralis.

Wave intensity and local arterial distensibility

Following the obtainment of the ultrasound B-mode scans, as described above, images were exported as DICOM files for offline analysis. Wave speed determination and wave intensity analysis were only performed on the CFA of both the experimental and control leg as the DICOM image quality for the other arteries, particularly that of the PFA and POA, was not sufficient for diameter block matching. Diameter waveform extraction was performed using CAROLAB (Zahnd *et al.*, 2013), which uses block matching to provide an accurate measurement of the vessel diameter at each frame. Extracted diameter waveforms were saved as Excel files (Microsoft Corporation, Redmond, WA, USA) for later analysis. Doppler ultrasound DICOM files were

analysed in Matlab (version R2019b, The MathWorks, Inc., Natick, Massachusetts, USA), to extract the flow velocity waveforms, using custom designed algorithms as previously reported (Negoita *et al.*, 2018). Diameter waveform data were obtained for the CFA at every 30 min.

This diameter and flow velocity waveforms were then used to calculate wave speed (c) using the ln(*D*)*U*-loop method (Feng & Khir, 2010); the following equation was used: $c = \pm\frac{1}{2}\frac{\mathrm{d}U_{\pm}}{\mathrm{d}(\ln D)_{\pm}}$ where $\mathrm{d}U$ and $\mathrm{d}(\ln D)$ are the incremental differences between adjacent data of velocity (U) and diameter (D). Moreover, forward compression waves (FCW) and forward expansion waves (FEW), which reflect left ventricular performance in early and late systole respectively, were calculated using previously documented techniques (Pomella *et al.*, 2018). Data outputs were averaged over two scans for the same time point, with three waveforms analysed per scan. Subsequently, with the determination of c, distensibility (D_s) was calculated using the following Bramwell and Hill (1922) equation: $D_s = p^{-1} \times c^{-2}$, where p represents blood density which was assumed equal to 1050 kg·m^{-3} (Pomella *et al.*, 2018).

Tissue oxygenation measures

Direct and continuous measures of regional tissue haemoglobin (venous) oxygen saturation (% rSO_2) were obtained in the experimental and control legs using two near-infrared spectroscopy units with four optode pads each (NIRS; INVOS 5100C Cerebral Oximeter; Somanetics Corp, Troy, MI, USA). The optode pads were placed on the skin surrounding the quadriceps, hamstrings, calf, and foot of both the control and experimental legs and taped to reduce interference from external light sources.

Statistical analysis

Statistical analysis was conducted using R (version 3.5.1, Team (2013)). For protocol 1, linear mixed effects models (two-way) were performed to investigate differences in haemodynamics, flow profiles and temperature between and within the experimental and control leg over time. In addition, linear mixed effects models (one-way) were conducted to investigate differences over time in systemic variables—i.e., heart rate, cardiac output, stroke volume, blood pressure, perceptual measures, and non-leg temperatures. For protocols 2 and 3, linear mixed effects models (one-way) were

conducted to investigate differences over time in all variables—i.e., haemodynamics, flow profiles and temperature. Following the linear mixed effects models, once a significant effect was found, a Tukey's *post-hoc* test was conducted to locate the specific time points at which those changes occurred. Additionally, linear, exponential and polynomial regression curve fit tests were performed using GraphPad Prism (version 8, GraphPad Software, La Jolla, California, USA) to assess the relationship between various key data. Subsequently, Akaike's Information Criterion was used to evaluate which model provides the most appropriate fit. Where an exponential curve fit was appropriate, the equation $y = y_0 \cdot e^{-K \cdot x}$ was used, where y_0 is the y value when x (time) is zero and k is the rate constant. Significance of this fit is reported through 95 % confidence intervals on the estimated value of k with the null value being $k = 0$. Moreover, a MANOVA test was conducted using SPSS (Version 26; IBM, Armonk, NY, USA), to compare the difference in blood flow between the three heating protocols. After, if a significant effect was found, a Bonferroni's *post-hoc* test was conducted to locate the specific time points at which those changes occurred.

Results

Protocol 1: effects of prolonged whole-leg heating on thermal, haemodynamic and tissue oxygenation responses

Regional and core temperatures, and thermal perception. Full leg and core temperature responses are illustrated in Figure 21. As per design, quadriceps, hamstring, calf and foot T_{sk}, and quadriceps and calf T_m, and thus, $\overline{T}_{Leg}$ of the experimental leg increased progressively during the 3 h whole-leg heating protocol, whereas it remained unchanged or declined in the control leg (Figure 21). Specifically, experimental $\overline{T}_{Leg}$ increased by 3.6 ± 0.3 ºC (p < 0.0001) following 3 h of heating, whilst the control $\overline{T}_{Leg}$ steadily declined throughout the protocol (Δ = -2.2 ± 1.5 ºC, p < 0.0001). Experimental $\overline{T}_{Leg}$ remained elevated during the first 1 h of recovery (p = 0.008), before declining towards baseline. However, T_c was only significantly elevated at 3 h (0.2 ± 0.2 ºC; p < 0.0001), reaching a peak temperature of 37.1 ± 0.2 ºC (Figure 21). Perceptual responses—thermal comfort (T_{comf}) and thermal sensation (T_{sens})—remained low and stable throughout the 6 h protocol. T_{comf} averaged 1.2 ± 0.8 units (p

= 0.214), whilst T_{sens} averaged 4.4 ± 0.7 units (p = 0.041) increasing by 1 unit between 1.5–2.5 h of whole-leg heating (p = 0.029).

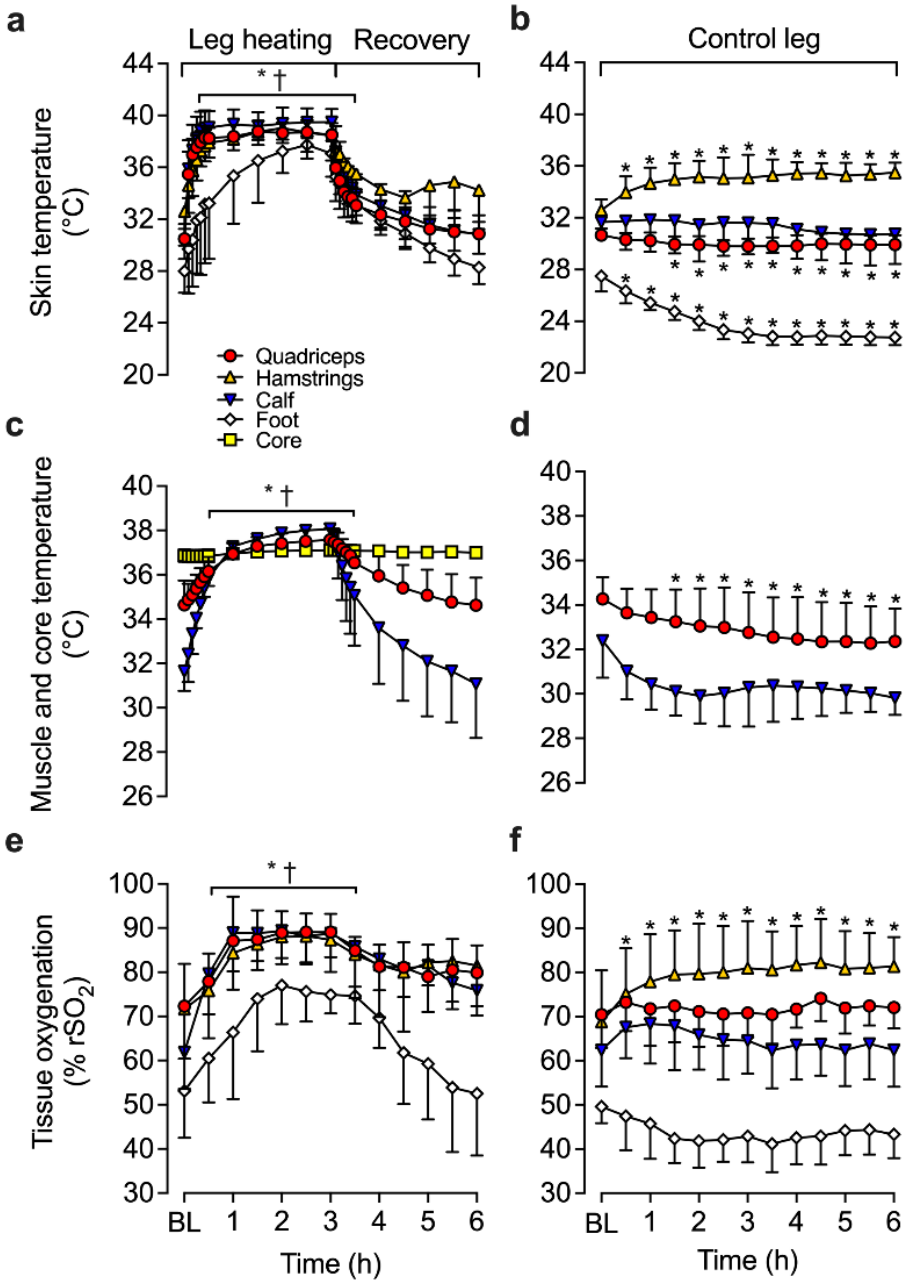

Figure 21. Core and leg temperatures (a, b, c, d) and regional tissue oxygenation (e, f) during whole-leg hyperthermia and recovery in the experimental (heated; a, c, e) and control (contralateral; b, d, f) legs. Data represented as mean ± SD (n = 9). BL signifies baseline measurements. ∗ Different from baseline, $p < 0.05$. † Different from respective control (contralateral) leg, $p < 0.05$. All variables below half-tick down line reported significant differences.

Leg blood flow, tissue oxygenation and systemic haemodynamics. Complete blood flow responses for the CFA, SFA, PFA and POA are illustrated in Figure 22a. In the experimental leg, CFA, SFA and POA blood flow increased ≥ 3-fold and PFA blood

flow increased ~ 2-fold within the first hour of whole-leg heating ($p < 0.0001$) (Figure 22a). Thereafter, arterial blood flow remained elevated and plateaued around 1.5–2 h until the cessation of heating (Figure 22a). During the subsequent 3 h recovery period, blood flow in the CFA, SFA and POA remained elevated for the first 30 min (CFA and SFA: $p < 0.0001$; POA: $p = 0.010$) and then steadily decreased towards baseline values (Figure 22a). No changes in blood flow were observed in control leg for all four arteries throughout the entirety of the 6 h protocol ($p > 0.05$) (Figure 22b).

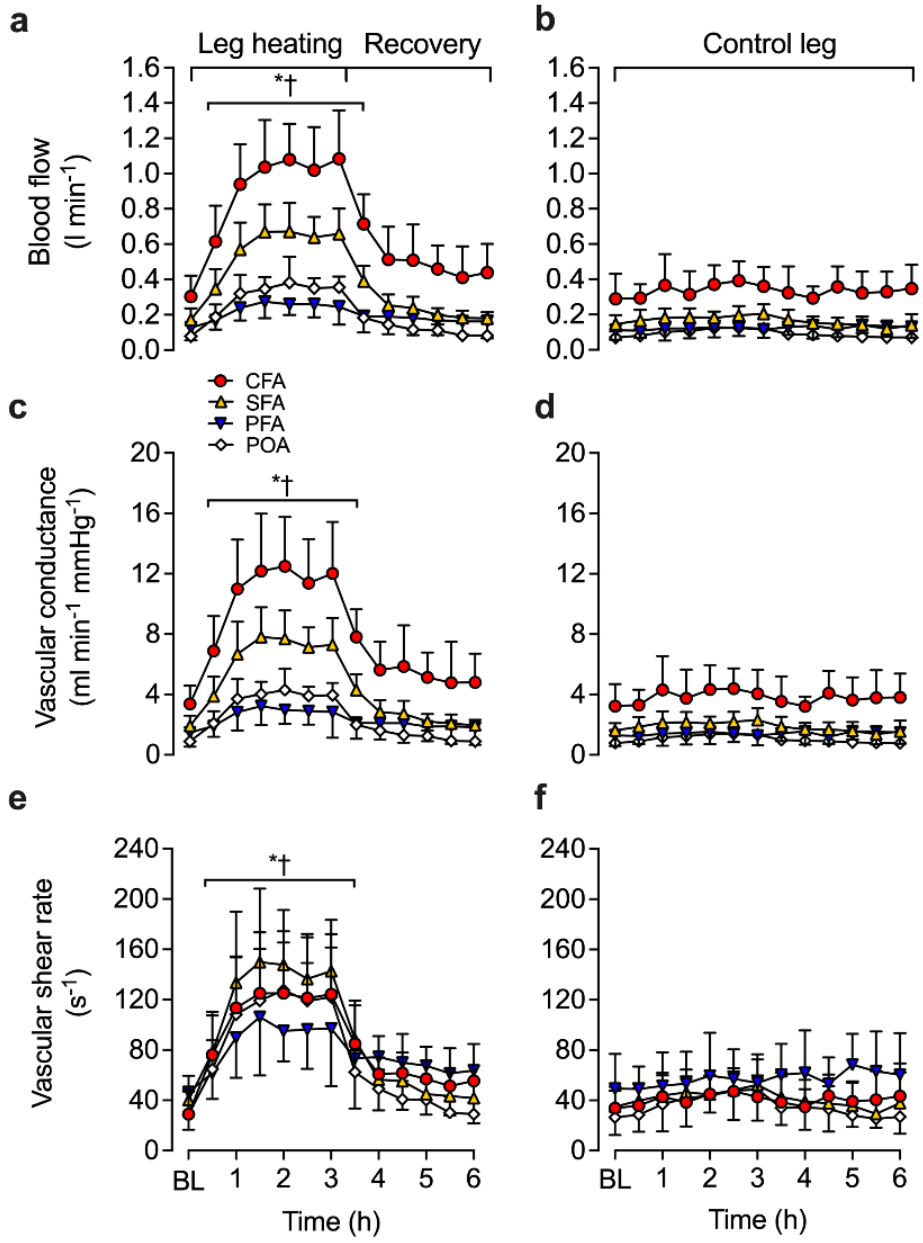

Figure 22. Blood flow (a, b), vascular conductance (c, d) and shear rate (e, f) during whole-leg hyperthermia and recovery in CFA, SFA, PFA and POA of the experimental (heated; a, c, e) and control (contralateral; b, d, f) legs. Data represented as mean ± SD ($n = 9$). BL signifies baseline measurements. ∗ Different from baseline, $p < 0.05$. † Different from respective control (contralateral)

leg, $p < 0.05$. All variables below half-tick down line reported significant differences.In line with the previously mentioned arterial responses, average upper- and lower-leg blood flow were ~ 3-fold higher in the experimental leg than the control leg following the whole-leg heating protocol ($p < 0.0001$). Additionally, upper-leg blood flow was higher than lower-leg blood flow at all times ($p < 0.0001$). On average, experimental upper-leg blood flow was 305 ± 249 $mL \cdot min^{-1}$ higher than experimental lower-leg blood flow during heating. However, when accounted for estimated tissue mass, upper- and lower-leg blood flow were similar following 3 h of whole-leg heating (9.3 ± 2.8 vs 10.5 ± 2.0 $mL \cdot min^{-1} \cdot 100\ g^{-1}$, $p = 0.369$). Similar responses were observed in shear rate and vascular conductance in all four arteries of the experimental leg (Figure 22c & Figure 22e). Experimental leg CFA, SFA, PFA and POA shear rate and vascular conductance increased during the whole-leg heating protocol, with ~ 4-fold increases being observed in the CFA, SFA and POA at 3 h ($p < 0.0001$), whilst a ~ 2-fold increase was observed in the PFA ($p < 0.0001$). In contrast, no changes were observed in the control leg (Figure 22d & Figure 22f). Moreover, whole-leg heating increased upper-leg tissue oxygenation by 16 ± 9 % rSO_2 units ($p < 0.0001$), and lower-leg tissue oxygenation by 24 ± 9 % rSO_2 units ($p < 0.0001$) of the experimental leg (Figure 21e). On the other hand, tissue oxygenation remained unchanged in the control leg ($p > 0.05$, respectively) with the exception of hamstring tissue oxygenation which increased by 12 ± 7 % rSO_2 units at 6 h ($p < 0.0001$; Figure 21f). However, when all the control leg sites were evaluated together, a close linear relationship was observed between the changes in local temperature and tissue oxygenation ($R^2 = 0.88$, $p < 0.0001$).

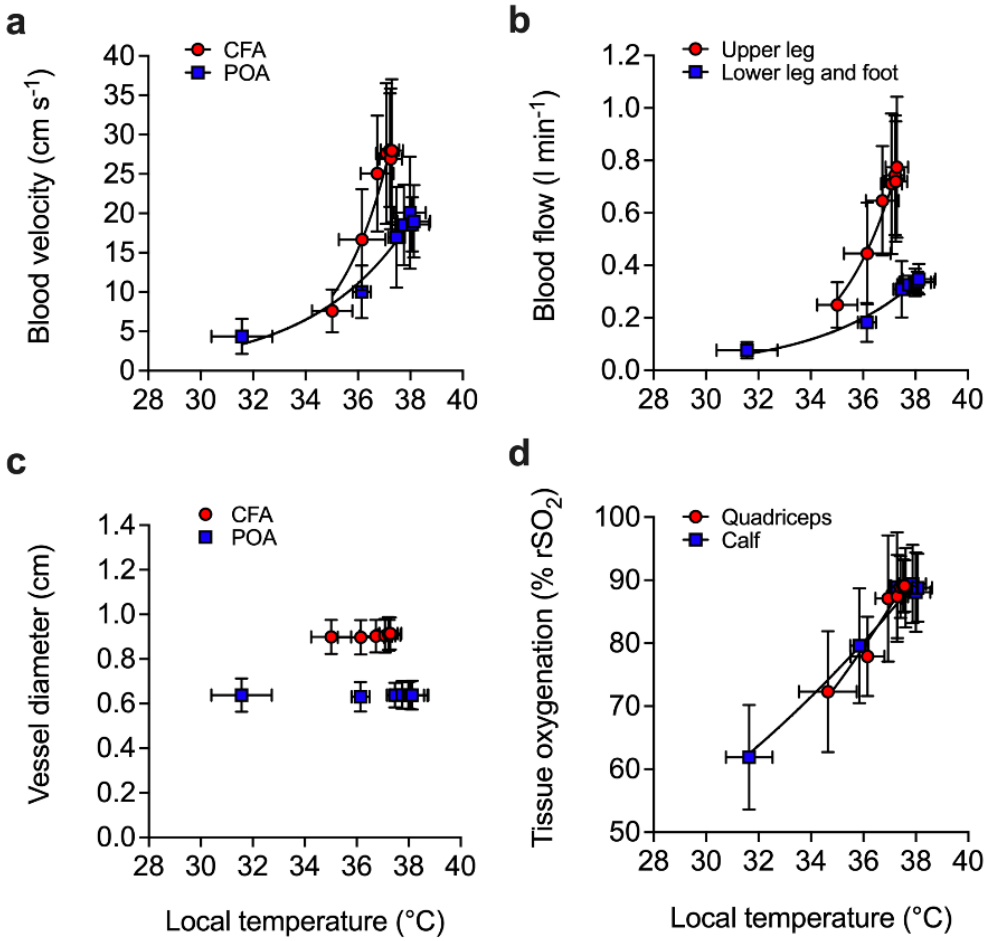

Figure 23. Relationship between the local temperature ($\overline{T}_{Leg}$) and local blood velocity (a), blood flow (b), vessel diameter (c) and tissue oxygenation (d) values during whole-leg hyperthermia and recovery. Data represented as mean ± SD (*n* = 9). Vertical error bars signify dependent variable SD, whilst horizontal error bars signify $\overline{T}_{Leg}$ SD, respectively.

Increases in arterial blood flow were exponentially related to increases in local temperature (upper-leg: R^2 = 0.98, k = 0.46 [0.37,0.57]; lower-leg: R^2 = 0.98; k = 0.26 [0.20,0.35]) (Figure 23b) and were attributed to an increased blood velocity (all $p \leq$ 0.012) (Figure 23a), as arterial diameter remained constant throughout the heating protocol (p > 0.1) (Figure 23c). In agreement with the global blood flow dynamic responses, increases in regional tissue oxygenation were exponentially related to increases in local temperature (upper-leg: R^2 = 0.96, k = 0.08 [0.06,0.10]; lower-leg: R^2 = 0.98, k = 0.06 [0.05,0.07]) (Figure 23d). Experimental leg quadriceps skin blood flow increased during whole-leg heating from 3 ± 2 to 78 ± 37 PU (p = 0.006) before slowly returning to baseline (3 ± 1 PU) following 3 h of recovery (p = 1.00). At the systemic level, no significant changes were observed for systemic blood volume, red cell volume, plasma volume and cardiac stroke volume (all p > 0.5; Table 1). However,

increases of 8 ± 7 bpm in heart rate (p = 0.002) and 1.2 ± 0.7 L·min^{-1} in cardiac output (p = 0.038) were observed at 3 h of whole-leg heating (Table 1).

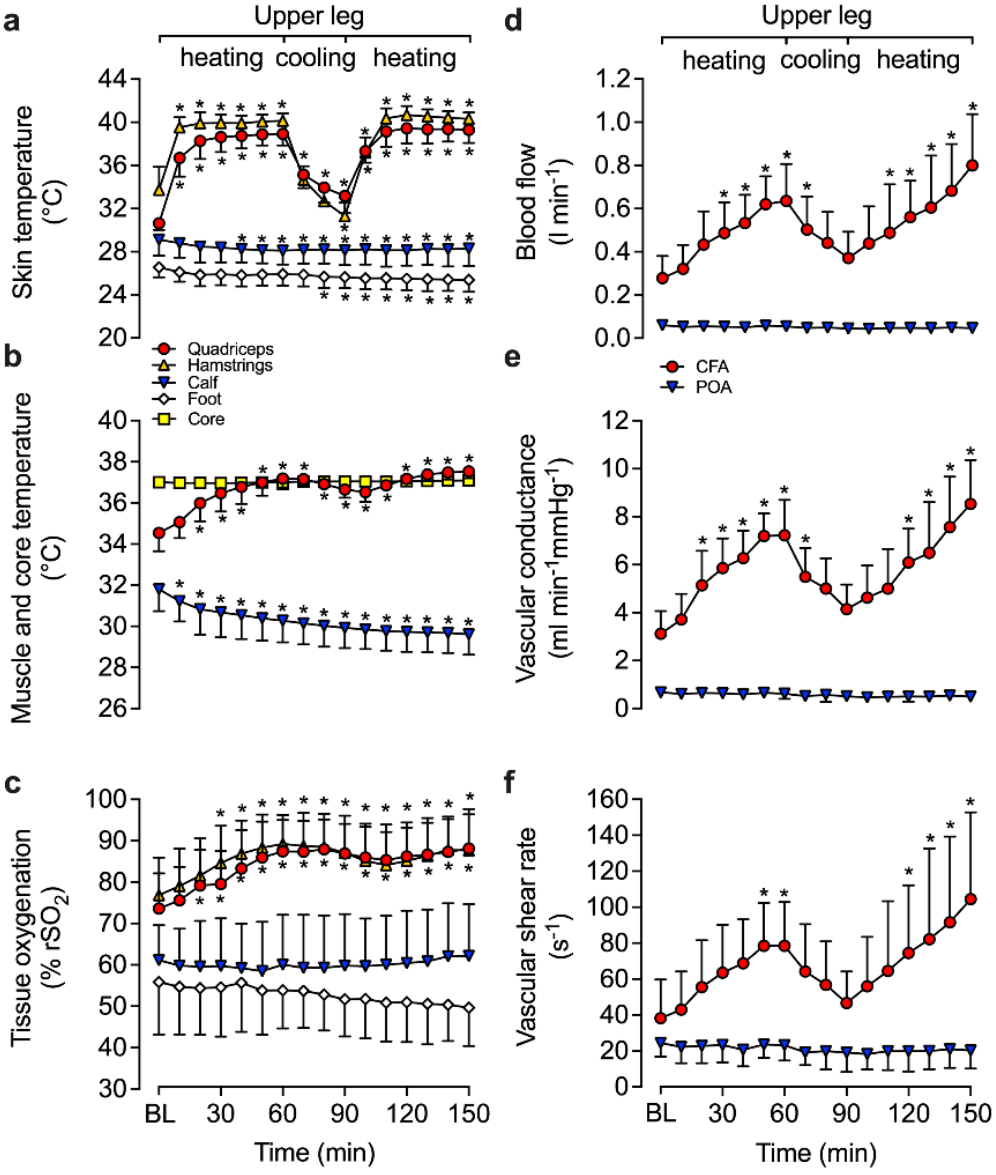

Figure 24. Core and leg temperatures (a, b), regional tissue oxygenation (c), blood flow (d), vascular conductance (e) and shear rate (f) in CFA and POA during upper-leg heating and cooling. Data represented as mean ± SD (n = 8). BL signifies baseline measurements. ∗ Different from baseline, p < 0.05.

Table 1. Influence of prolonged whole-leg heating and subsequent recovery on central haemodynamics, common femoral artery blood flow, wave speed, arterial distensibility and wave intensity parameters.

		Time (h)						
Variables	Intervention	Baseline	1	2	3	4	5	6
MAP (mmHg)		89 ± 7	88 ± 10	88 ± 9	91 ± 9	91 ± 8	90 ± 4	92 ± 5
$\dot{Q}$ ($L \cdot min^{-1}$)		6.3 ± 0.6	6.8 ± 1.0	7.0 ± 0.8	7.5 ± 0.7*	6.7 ± 1.3	6.7 ± 1.7	6.7 ± 1.0
SV (mL)		111 ± 24	107 ± 19	111 ± 24	118 ± 30	116 ± 35	115 ± 41	114 ± 39
HR ($beats \cdot min^{-1}$)		58 ± 10	64 ± 8*	65 ± 10*	66 ± 13*	60 ± 10	61 ± 11	63 ± 14
CFA blood flow ($mL \cdot min^{-1}$)	Heated leg	320 ± 98	940 ± 226*†	1080 ± 202*†	1122 ± 250*†	513 ± 184	459 ± 134	438 ± 163
	Control leg	289 ± 142	365 ± 177	371 ± 108	360 ± 110	293 ± 67	323 ± 121	347 ± 136
POA blood flow ($mL \cdot min^{-1}$)	Heated leg	78 ± 30	319 ± 106*†	382 ± 147*†	356 ± 60*†	145 ± 23	112 ± 33	82 ± 22
	Control leg	70 ± 34	101 ± 58	110 ± 40	120 ± 23	84 ± 19	74 ± 18	70 ± 30
Wave speed ($m \cdot s^{-1}$)	Heated leg	20.4 ± 7.8	21.6 ± 10.6	20.6 ± 8.1	21.4 ± 10	22.1 ± 9.1	18.2 ± 6.2	19.3 ± 5.8
	Control leg	16.5 ± 9.3	16.1 ± 7.3	16.4 ± 6.3	16.9 ± 9.1	17.2 ± 3.4	15.4 ± 2.4	17.2 ± 4.4
Distensibility ($x10^{-3} \cdot mmHg^{-1}$)	Heated leg	0.3 ± 0.2	0.3 ± 0.3	0.3 ± 0.3	0.3 ± 0.2	0.2 ± 0.2	0.3 ± 0.1	0.2 ± 0.1
	Control leg	0.3 ± 0.2	0.3 ± 0.3	0.3 ± 0.2	0.4 ± 0.2	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
FCW ($cm^2 \cdot s^{-1}$)	Heated leg	1.6 ± 1.1	2.1 ± 0.8	2.5 ± 0.8	2.0 ± 0.7	1.9 ± 0.7	2.2 ± 0.9	2.6 ± 1.5
	Control leg	2.0 ± 0.8	2.2 ± 0.6	2.5 ± 1.1	1.8 ± 0.5	1.6 ± 0.6	1.7 ± 0.4	1.9 ± 1.0
FEW ($cm^2 \cdot s^{-1}$)	Heated leg	0.5 ± 0.3	0.7 ± 0.3	0.7 ± 0.3	0.7 ± 0.3	0.8 ± 0.2	0.8 ± 0.4	0.8 ± 0.4
	Control leg	0.5 ± 0.3	0.6 ± 0.3	0.6 ± 0.3	0.7 ± 0.4	0.4 ± 0.1	0.6 ± 0.2	0.4 ± 0.2

Values are means ± SD for 9 participants, except for the wave intensity derived paraments (n = 6). MAP: mean arterial pressure; $\dot{Q}$: cardiac output; SV: stroke volume; HR: heart rate; CFA: common femoral artery; POA: popliteal artery; FCW: forward compression waves; FEW: forward expansion waves. First 3 h represent whole-leg heating responses, whilst hours 4 to 6 represent responses during the subsequent 3 h passive recovery. ∗Different from baseline, $p < 0.05$. † Different from respective control (contralateral) leg, $p < 0.05$.

Protocol 2: effects of upper-leg heating on thermal, haemodynamic and tissue oxygenation responses

Regional temperature responses. Full leg and core temperature responses are illustrated in Figure 24. As per design, substantial increases in quadriceps and hamstrings T_{sk}, quadriceps T_m and $\overline{T}_{Upper-Leg}$ were observed following the two bouts of upper-leg heating (all $p < 0.0001$). During the 30 min bout of upper-leg cooling, however, upper-leg T_{sk} dropped, with hamstring T_{sk} declining below baseline (Δ = -2.4 ± 2.5 ºC, $p = 0.0004$) whereas quadriceps T_{sk} remained elevated from baseline (Δ = +2.6 ± 1.7 ºC, $p = 0.002$) (Figure 24a). Similar to quadriceps T_{sk}, T_m remained elevated above baseline during the cooling bout (Δ = +2.1 ± 1.0 ºC, $p = 0.0001$) (Figure 24b). Consequently, $\overline{T}_{Upper-Leg}$ was elevated throughout the entirety of the protocol, increasing from 34.4 ± 0.8 ºC to 37.7 ± 0.4 ºC at 150 min ($p < 0.0001$). In contrast, in the lower-leg, calf T_{sk} tended to decline ($p = 0.054$), whilst calf T_m and foot T_{sk} decreased -2.2 ± 0.4 ºC and -1.2 ± 1.0 ºC, respectively (both $p < 0.0001$) at 150 min. T_c and control leg quadriceps T_{sk} remained unaltered throughout the protocol ($p = 0.105$ and $p = 0.214$, respectively) (Figure 24b).

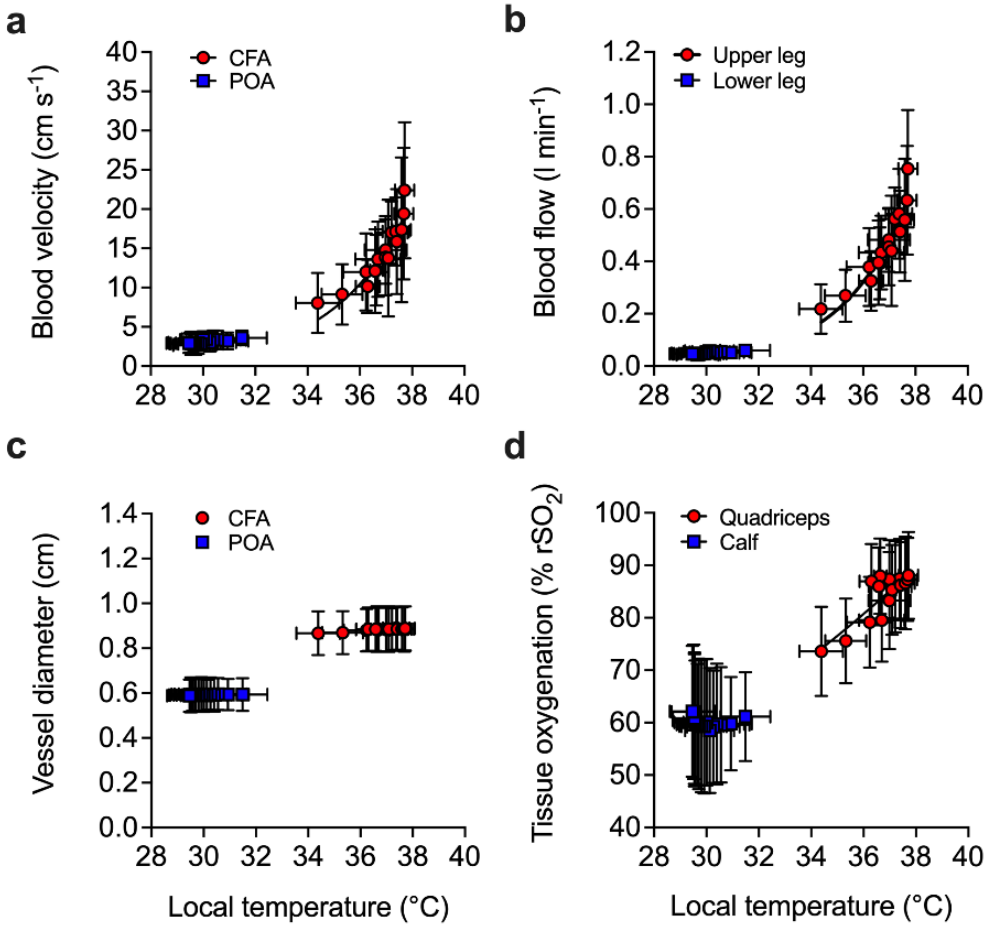

Figure 25. Relationship between the local temperature ($\overline{T}_{Leg}$) and local blood velocity (a), blood flow (b), vessel diameter (c) and tissue oxygenation (d) values during upper-leg heating and cooling. Data represented as mean ± SD (*n* = 8). Vertical error bars signify dependent variable SD, whilst horizontal error bars signify $\overline{T}_{Leg}$ SD, respectively.

Leg blood flow, tissue oxygenation and systemic haemodynamics. Complete blood flow responses for the CFA and POA are illustrated in Figure 24d. Following the first bout of 1 h upper-leg heating, CFA blood flow increased 2.3-fold ($p < 0.0001$) and then declined towards baseline during the 30 min bout of upper-leg cooling ($p = 0.639$) (Figure 24d). In the second bout of 1 h upper-leg heating, CFA blood flow surpassed the magnitude obtained in the first bout of heating—increasing 2.9-fold and peaking at 801 ± 237 mL·min^{-1} ($p < 0.0001$) (Figure 24d). These increases in CFA blood flow were exponentially related to an increasing $\overline{T}_{Upper-Leg}$ ($R^2 = 0.89$, $k = 0.40$ [0.31,0.51]) (Figure 25b) and were the result of an increased blood velocity ($p = 0.001$) (Figure 25a), as CFA diameter remained unchanged ($p = 0.065$) (Figure 25c). Throughout the protocol, POA blood flow remained stable ($p = 0.149$) (Figure 24d). Accordingly, the calculated upper-leg blood flow increased by 536 ± 243 mL·min^{-1} following the second

1 h heating bout ($p < 0.0001$). Upper-leg blood flow was higher than lower-leg blood flow at all time points, ranging from a 3.6-fold difference at baseline to 16-fold difference following the second bout of upper-leg heating ($p < 0.0001$). In the control leg, however, CFA and POA blood flow pre- and post-protocol remained stable ($p = 0.369$ and $p = 0.150$, respectively).

Shear rate and vascular conductance mirrored the blood flow responses in both the CFA and POA: CFA shear rate and vascular conductance increased ~ 2-fold in the first bout and almost 3-fold in the second 1 h bout of upper-leg heating (both $p < 0.0001$) (Figure 24). Conversely, POA shear rate and vascular conductance remained stable throughout the entire protocol (shear rate: $p = 0.153$; vascular conductance: $p = 0.107$) (Figure 24e & Figure 24f). Furthermore, quadriceps skin blood flow increased from 7 ± 9 to 58 ± 27 PU during the first 1 h bout of upper-leg heating. Quadriceps skin blood flow declined towards baseline at the end of the 30 min upper-leg cooling bout ($p = 0.996$ vs. baseline), before increasing to 43 ± 28 PU ($p = 0.003$) once again during the second bout of upper-leg heating. Tissue oxygenation responses are illustrated in Figure 24c. At all-time points, upper-leg tissue oxygenation was higher than lower-leg tissue oxygenation ($p < 0.0001$). During the first 1 h upper-leg heating bout, upper-leg tissue oxygenation increased by 13 ± 4 % rSO_2 units ($p < 0.0001$), increasing exponentially in response to $\overline{T}_{Upper-Leg}$ ($R^2 = 0.71$, $k = 0.05$ [0.03,0.07]) (Figure 25d). Subsequently, upper-leg tissue oxygenation plateaued and remained elevated during the succeeding upper-leg cooling and upper-leg heating bouts ($p < 0.0001$) (Figure 24c). Conversely, calf and foot tissue oxygenation remained unchanged ($p = 0.350$ and $p = 0.074$, respectively) (Figure 24c). At the systemic level, heart rate, cardiac output, stroke volume, and mean arterial pressure remained stable with averages of 57 ± 5 bpm, 5.1 ± 0.8 $L \cdot min^{-1}$, 91 ± 15 ml and 89 ± 14 mmHg, respectively (all $p > 0.05$) (Table 2).

Table 2. Influence of upper-leg heating and cooling on central haemodynamics and common femoral artery blood flow.

		Time (min)					
Variables	Intervention	Baseline	30	60	90	120	150
MAP (mmHg)		88 ± 16	83 ± 14	88 ± 14	88 ± 11	92 ± 12	94 ± 18
$\dot{Q}$ ($L \cdot min^{-1}$)		4.9 ± 0.8	5.0 ± 0.7	5.0 ± 0.8	5.2 ± 0.9	5.2 ± 0.7	5.5 ± 1
SV (mL)		89 ± 13	92 ± 15	91 ± 17	93 ± 19	92 ± 16	101 ± 24
HR ($beats \cdot min^{-1}$)		57 ± 5	57 ± 6	58 ± 9	57 ± 3	58 ± 5	56 ± 6
CFA blood flow ($mL \cdot min^{-1}$)	Heated leg	278 ± 102	487 ± 142*	636 ± 170*	371 ± 122	561 ± 168*	801 ± 237*
	Control leg	299 ± 133	—	—	—	—	277 ± 127
POA blood flow ($mL \cdot min^{-1}$)	Heated leg	60 ± 18	53 ± 18	54 ± 18	45 ± 19	47 ± 23	47 ± 18
	Control leg	61 ± 19	—	—	—	—	49 ± 23

Values are means ± SD for 8 participants, except for $\dot{Q}$ and SV values where $n = 6$. MAP: mean arterial pressure; $\dot{Q}$: cardiac output; SV: stroke volume; HR: heart rate; CFA: common femoral artery; POA: popliteal artery. Baseline to 60 min represents upper-leg heating responses, 90 min represents upper-leg cooling, and 120 min to 150 min represents the second bout of upper-leg heating. ∗Different from baseline, $p < 0.05$.

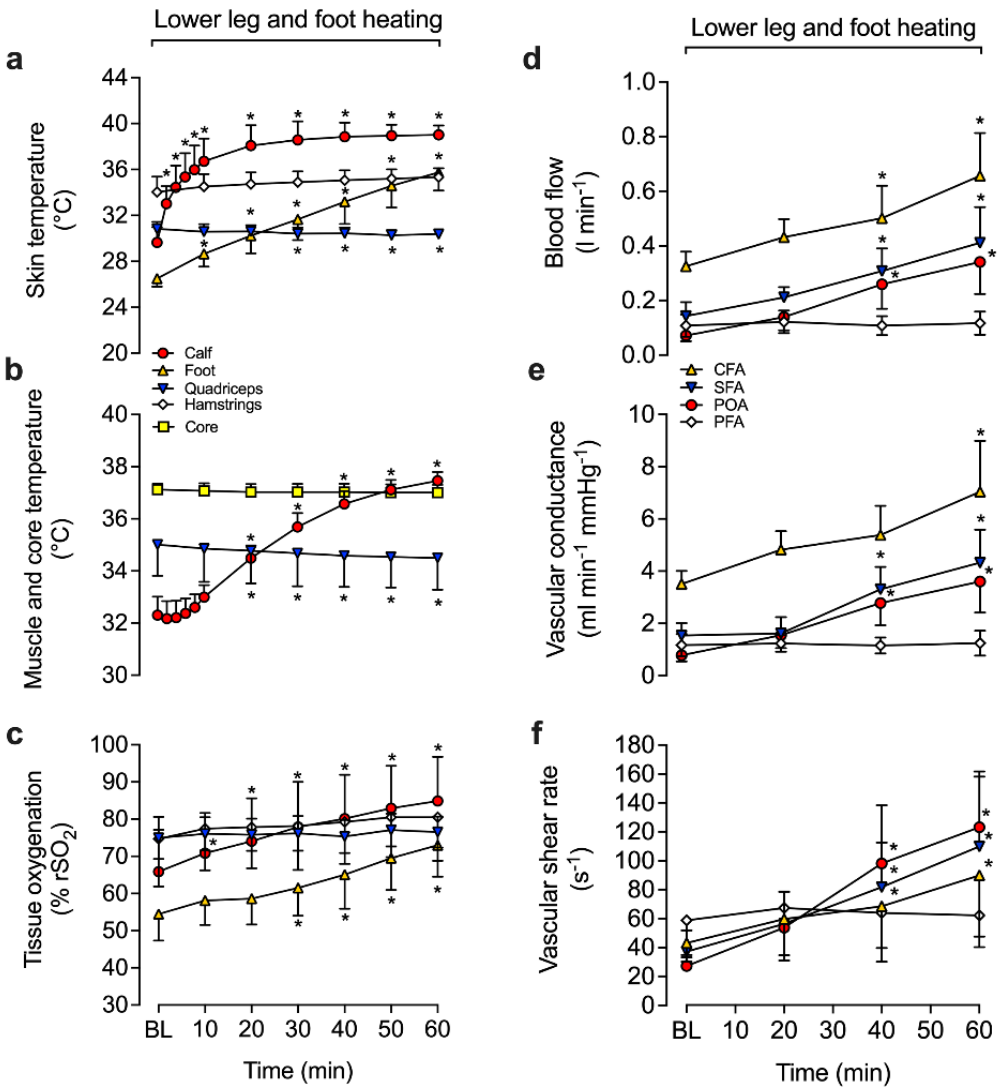

Figure 26. Core and leg temperatures (a, b), regional tissue oxygenation (c), blood flow (d), vascular conductance (e) and shear rate (f) in CFA, SFA, PFA and POA during lower-leg and foot heating. Data represented as mean ± SD (n = 8). BL signifies baseline measurements. ∗ Different from baseline, $p < 0.05$.

Protocol 3: effects of lower-leg and foot heating on thermal, haemodynamic and tissue oxygenation responses

Regional temperature responses. Full leg and core temperature responses are illustrated in Figure 26. As per design, substantial increases in calf and foot T_{sk}, calf T_m and $\overline{T}_{Lower-Leg}$ were observed following the 1 h bout of lower-leg and foot heating with $\overline{T}_{Lower-Leg}$ increasing from 32.0 ± 0.7 ºC to 37.7 ± 0.3 ºC ($p < 0.0001$). In contrast, $\overline{T}_{Upper-Leg}$ slowly declined throughout the protocol (Δ = -0.4 ± 0.2 ºC, p = 0.003). T_c remained unaltered (p = 0.081) (Figure 26b).

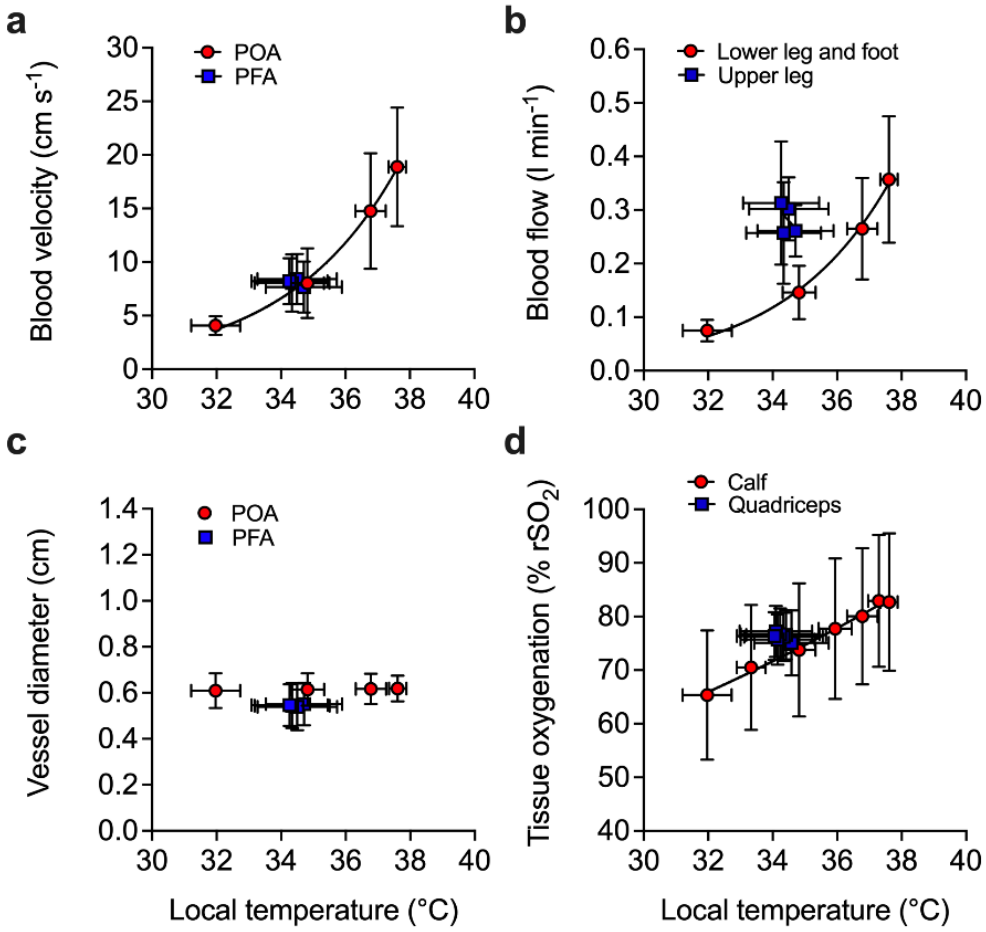

Figure 27. Relationship between the local temperature ($\overline{T}_{Leg}$) and local blood velocity (a), blood flow (b), vessel diameter (c) and tissue oxygenation (d) values during lower-leg and foot heating. Data represented as mean ± SD (*n* = 8). Vertical error bars signify dependent variable SD, whilst horizontal error bars signify $\overline{T}_{Leg}$ SD, respectively.

Leg blood flow, tissue oxygenation and systemic haemodynamics. Complete blood flow responses for the CFA, SFA, PFA and POA are illustrated in Figure 26. In response to lower-leg and foot heating, CFA, SFA and POA blood flow increased, whilst PFA blood flow remained stable ($p = 0.474$). Following 1 h of lower-leg heating, CFA and SFA blood flow increased > 2-fold ($p < 0.0001$) and POA blood flow increased 4.7-fold ($p < 0.0001$) (Figure 26d). The increase in POA blood flow was exponentially related to increases in $\overline{T}_{Lower-Leg}$ ($R^2 = 0.99$, $k = 0.30$ [0.22,0.41]) (Figure 27b), and was attributed to an increased blood velocity ($p < 0.001$) (Figure 27a) as diameter remained unchanged ($p > 0.1$) (Figure 27c). Upper-leg blood flow remained constant throughout the entire protocol at 276 ± 84 mL·min^{-1} ($p = 0.257$) as the increases in CFA and SFA blood flow were proportional to the increase in lower leg blood flow. Similarly, CFA, SFA and POA shear rate and vascular conductance

increased during the lower-leg and foot heating protocol, with over 2-fold increases being observed at 1 h in both the CFA and SFA ($p < 0.0001$ for shear rate and vascular conductance in both arteries, respectively), whilst POA shear rate and vascular conductance increased ~ 4.5-fold at 1 h (both $p < 0.0001$). In contrast, no changes in PFA shear rate and vascular conductance were observed (shear rate: $p = 0.549$; vascular conductance: $p = 0.476$). In response to lower-leg and foot heating, lower-leg and foot tissue oxygenation increased by 18 ± 6 % rSO_2 units, respectively ($p < 0.0001$). These increases in lower-leg tissue oxygenation were exponentially related to increases in $\overline{T}_{Lower-Leg}$ ($R^2 = 0.99$, $k = 0.04$ [0.04,0.05]), respectively (Figure 27d). No changes in quadriceps tissue oxygenation ($p = 0.210$; Figure 26c) or quadriceps skin blood flow ($p = 0.462$) were observed during lower-leg and foot heating. However, similar to the control leg during whole-leg heating, hamstrings tissue oxygenation increased slightly by 6 ± 3 % rSO_2 units ($p = 0.015$; Figure 26c). At the systemic level, heart rate, cardiac output, stroke volume, and mean arterial pressure remained stable with averages of 59 ± 8 bpm, 5.2 ± 0.9 $L \cdot min^{-1}$, 89 ± 18 ml, and 93 ± 43 mmHg, respectively (all $p > 0.05$) (Table 3). In addition, no changes in control leg CFA, SFA, PFA and POA blood flow pre- and post-protocol were observed (all $p > 0.05$).

Table 3. Influence of lower-leg and foot heating on central haemodynamics and common femoral artery blood flow.

Variables	Intervention	Time (min)			
		Baseline	20	40	60
MAP (mmHg)		93 ± 10	90 ± 9	93 ± 11	96 ± 18
$\dot{Q}$ ($L \cdot min^{-1}$)		5.1 ± 0.9	5.2 ± 0.8	5.0 ± 1.0	5.3 ± 1.1
SV (mL)		89 ± 20	88 ± 15	89 ± 24	89 ± 14
HR ($beats \cdot min^{-1}$)		59 ± 8	59 ± 9	58 ± 8	59 ± 8
CFA blood flow ($mL \cdot min^{-1}$)	Heated leg	325 ± 53	432 ± 66	502 ± 119*	656 ± 157*
	Control leg	327 ± 85	—	—	314 ± 39
POA blood flow ($mL \cdot min^{-1}$)	Heated leg	72 ± 21	140 ± 49	259 ± 89*	341 ± 117*
	Control leg	74 ± 22	—	—	64 ± 11

Values are means ± SD for 7 participants, except for blood flow values where $n = 8$. MAP: mean arterial pressure; $\dot{Q}$: cardiac output; SV: stroke volume; HR: heart rate; CFA: common femoral artery; POA: popliteal artery. Baseline to 60 min represents lower-leg and foot heating responses. ∗Different from baseline, $p < 0.05$.

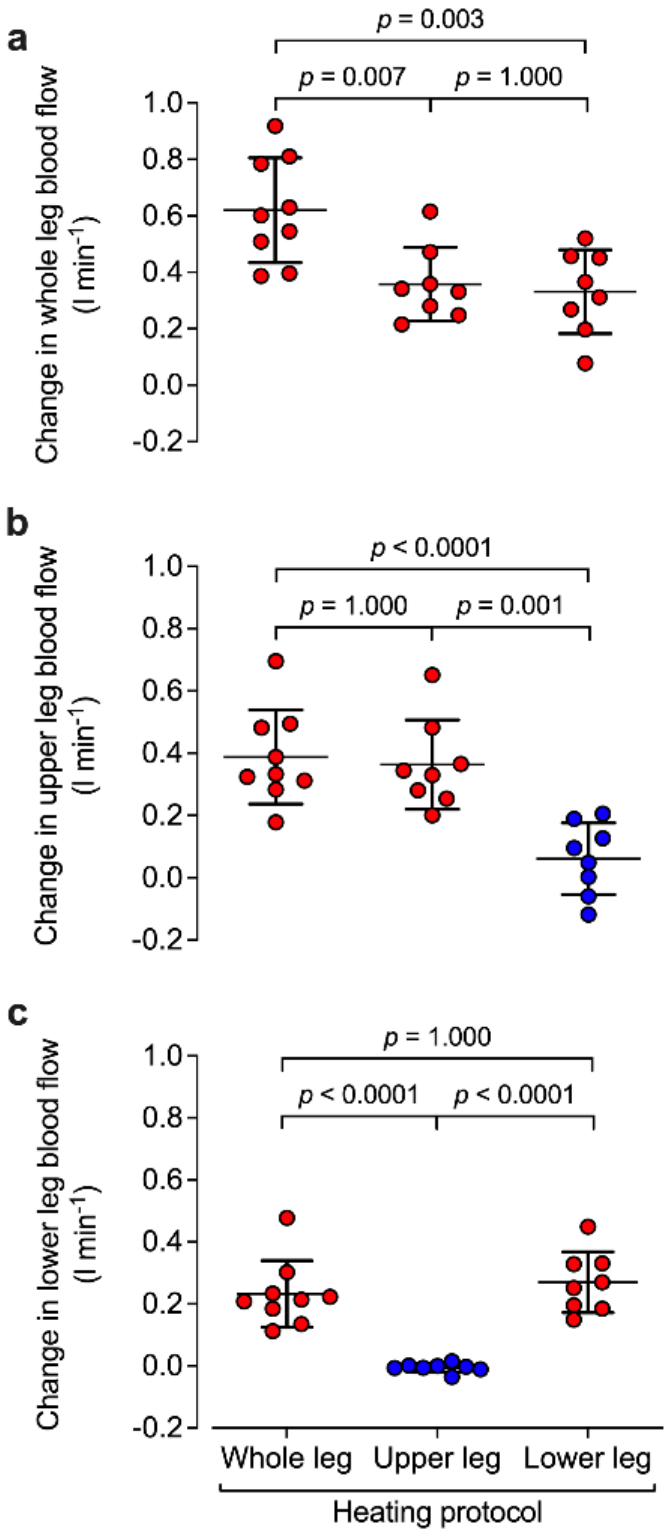

Figure 28. Changes in regional blood flow during 1 h whole leg and segmental leg heating. Circles depict the individual data points whilst the lines illustrate mean ± SD (*n* = 25). Red circles represent heated segments whilst blue circles represent control segments, respectively. The figure reports three levels of comparisons: whole-leg vs upper-leg blood flow, whole-leg vs lower-leg blood flow and upper-leg vs lower-leg blood flow, respectively, with *p* values and half-tick down lines illustrating the differences. Note that increases in segmental blood low reflect the changes in local temperature, regardless of the heating protocol.

All protocols: comparison of changes in regional blood flow with whole-leg, upper-leg, and lower-leg and foot hyperthermia

Individual and mean changes in whole-leg, upper-leg and lower-leg blood flow during the first hour of the three heating protocols are illustrated in Figure 28. The increase in whole-leg blood flow was greater following 1 h of whole-leg heating than during 1 h of upper- (p = 0.007) and lower-leg heating *(*p = 0.003); however, upper-leg and lower-leg blood flow were not different from one another during whole-leg heating (p = 0.155). During the upper-leg heating protocol, the increase in whole-leg blood flow was not different from the increase in upper-leg blood flow (p = 1.00) but the elevation in upper-leg blood flow was higher than that of lower-leg blood flow (p < 0.0001). Lastly, during lower-leg and foot heating, the increase in lower-leg blood flow was similar to the increase in whole-leg blood flow (p = 0.982), such that the change in lower-leg blood flow was greater than that of upper-leg blood flow (p = 0.008). Moreover, these changes in upper-leg and lower-leg blood flow occurred in an exponential fashion to the change in temperature (Figure 29). Strong relationships between the change in upper-leg blood flow and the change in upper-leg temperature exist during whole-leg heating (R^2 = 0.97, k = 0.67 [0.57,0.79]) and upper-leg heating (R^2 = 0.89, k = 0.85 [0.65,1.08]). Similarly, strong exponential relationships between the change in lower-leg blood flow and the change in lower-leg temperature exist during whole-leg heating (R^2 = 0.98, k = 0.40 [0.33,0.49]) and lower-leg and foot heating (R^2 = 0.99, k = 0.49 [0.21,0.94]).

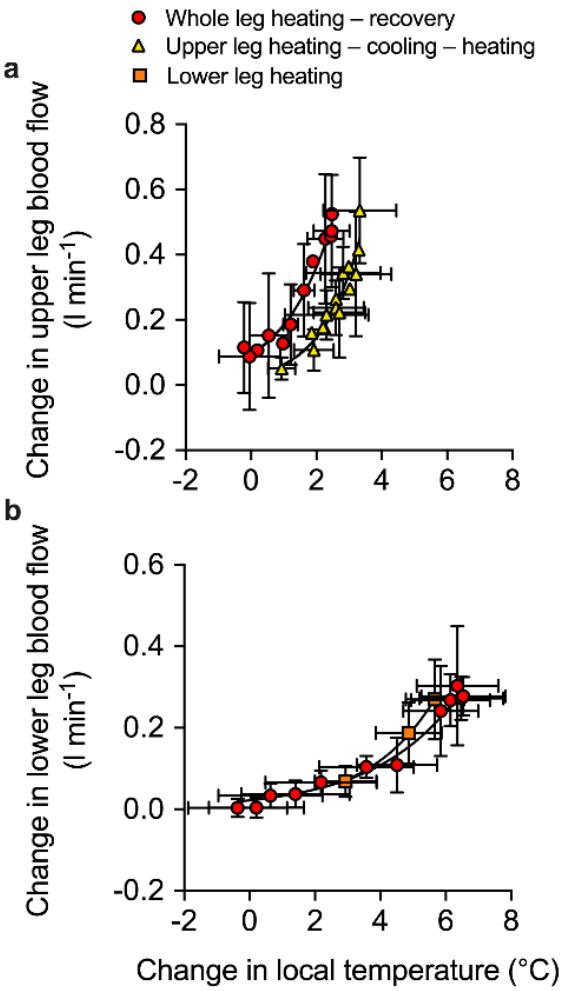

Figure 29. Relationship between the change in local temperature ($\overline{T}_{Leg}$) and the change in local blood flow during whole-leg, upper-leg, and lower-leg and foot heating. Data represented as mean ± SD (*n* = 25). Vertical error bars signify Δ blood flow SD, whilst horizontal error bars signify Δ $\overline{T}_{Leg}$ SD, respectively.

Wave speed, local arterial distensibility and wave intensity parameters

Wave speed, distensibility and wave intensity parameters measured at the CFA during prolonged whole-leg heating and its subsequent recovery are reported in Table 1. Wave speed remained stable throughout the protocol in both legs (p = 0.908) with no differences being observed between the experimental and control leg (p = 0.324). Consequently, as arterial distensibility is calculated from wave speed, no changes were observed throughout the protocol (p = 0.841) or between legs (p = 0.329). Similarly, wave intensity parameters, forward compression, and forward expansion waves, did not change in response to whole-leg heating (p = 0.218 and 0.860, respectively), nor were there any differences between legs (p = 0.371 and 0.097, respectively).

Discussion

This study explored the relationships between local hyperthermia and the haemodynamic profiles of the leg major arteries and the oxygenation of the tissues they perfuse, comparing the responses of experimental and control legs as well as the upper and lower leg segments during prolonged whole-leg and segmental-leg heating. In line with the study's hypotheses, local macro- and microvascular blood flows were closely related to local temperature across all the experimental conditions producing large variations in local temperature, but essentially no changes in core temperature. Whole-leg hyperthermia markedly increased blood flow and vascular conductance in the four major arteries during the 3 h heating protocol, then slowly declined during the subsequent recovery in association with the fall in local temperature. Segmental-leg hyperthermia elicited comparable increases in regional blood flow to that of the regional hyperaemia observed during whole-leg hyperthermia. Additionally, increases in blood flow of the heated leg and leg segment occurred without noticeable changes to perfusion pressure or mean conduit artery diameter, whilst local tissue oxygenation, blood velocity and blood flow were positively related with local temperature. Together, these findings support the notion that heat activates thermosensitive mechanisms in the leg microcirculation, thereby regulating the flow of blood through the human leg during local hyperthermia.

Impact of regional hyperthermia on leg tissue perfusion

Local hyperthermia, whether it be through prolonged whole- or segmental-leg heating, resulted in sustained ≥ 3-fold increases in regional tissue perfusion. In all hyperthermic conditions, arterial blood flow was closely coupled with changes in local temperature—increasing in an exponential fashion with the rise in local hyperthermia. Of note is the strikingly similar increases in blood flow in the upper and lower leg during 1 h of segmental- and whole-leg hyperthermia (Figure 28). Previous studies have characterised the significant effects of segmental-leg hyperthermia on global and local limb blood flow (Heinonen *et al.*, 2011; Kuhlenhoelter *et al.*, 2016; Romero *et al.*, 2017; Thomas *et al.*, 2017; Walsh *et al.*, 2019), and the blood flow differences between the upper and lower leg under normothermia (Klein *et al.*, 2003), and during whole-body hyperthermia with lower leg occlusion (Chiesa *et al.*, 2016). The present study is the first to comprehensively and directly compare the responses of the major leg arteries

and the regional leg blood flow distribution during segmental- and whole-leg hyperthermia, in attempt to isolate the effects of local thermosensitive regulatory mechanisms on thermal hyperaemia. At baseline, we observed a ~ 3:1 distribution in blood flow between the upper and lower leg, in agreement with previous literature (Klein *et al.*, 2003; Chiesa *et al.*, 2016). However, when upper- and lower-leg blood flow are expressed per 100 g of tissue, the blood flow values are similar, both at baseline and following 3 h of whole-leg heating (2–3 and 9–10 $mL \cdot min^{-1} \cdot 100\ g^{-1}$, respectively). This suggests that the approximately 3 times greater mass (Wang *et al.*, 1999) and, by extension, more abundant muscle, skin, fat and bone vasculature of the upper leg in comparison to the lower leg, might largely account for the higher absolute blood flow of the upper leg. Notwithstanding, we consistently observed lower baseline tissue oxygenation in the lower leg and foot compared to the upper leg, suggestive of a greater basal oxygen extraction from the circulation in response to lower local perfusion and tissue temperature (Davis *et al.*, 2006). These indications of a coupling between lower temperature and blood perfusion in the lower leg and foot are consistent with observations that the distal regions of the leg are more susceptible to ischemia in disease conditions such as peripheral arterial disease and diabetes (Ouriel, 2001; Hirsch *et al.*, 2006; Hills *et al.*, 2009). Yet, in the present study, all leg segments were found to be highly responsive to prolonged leg hyperthermia, with the relative increase in blood flow being greater in the lower leg than the upper leg (3.8- vs. 2.6-fold, respectively). Thus, the degree of tissue perfusion and temperature heterogeneity will therefore diminish with leg hyperthermia compared to control conditions (Figure 21 and Figure 22). Nonetheless, the presently observed close temporal relationships between blood flow and local temperature and between regional tissue oxygenation and local temperature in both the experimental and control leg during the 6 h leg heating and recovery protocol, strongly supports a causal link between local hyperthermia and hyperaemia.

The blood flow responses of the major leg arteries to segmental-leg heating reveal new insight into the regulation of tissue blood flow in human limbs during hyperthermia. Upper-leg heating induced profound increases in thigh temperature and CFA blood flow (~ 3-fold), yet lower-leg temperature and POA blood flow decreased somewhat or remained unchanged (Figure 24). The observed upper-leg hyperaemia during the first hour of upper-leg heating elicited similar increases in upper-leg blood flow to those

observed during whole-leg hyperthermia (~ 370 mL·min^{-1}) and those reported in the literature during isolated-limb heating and whole-body heating (Heinonen *et al.*, 2011; Pearson *et al.*, 2011; Chiesa *et al.*, 2016). In congruence with the findings during upper-leg heating, lower-leg and foot heating resulted in substantial increases in lower-leg temperature and CFA, SFA and POA blood flow, whilst PFA blood flow and upper-leg temperature remained stable. The observation that the increase in CFA and SFA blood flow—which feeds into the POA—is similar to the increase in POA blood flow, lends additional support to the argument that upper-leg blood flow remained unchanged. Furthermore, the magnitude of the lower-leg hyperaemia was similar to that evoked by 1 h of whole-leg hyperaemia (241–270 mL·min^{-1}; Figure 28). Moreover, increased tissue oxygenation—an index of microcirculatory blood flow—of the heated leg segment occurred in parallel to the regional hyperaemia; whilst much like blood flow, no changes were observed in the non-heated adjacent leg segment. Collectively, the similar magnitude of regional blood flow and tissue oxygenation, regardless of whether the heating intervention is being applied to a leg segment or the whole leg, provides additional compelling evidence supporting local hyperthermia as the putative stimulus for the greatly enhanced hyperaemia.

Tissue perfusion regulation during local hyperthermia

Local hyperaemia was associated with increases in local vascular conductance in all hyperthermic protocols, whilst perfusion pressure remained unchanged. A major aim of the present study was to identify the precise vascular locus in which hyperthermia modulates blood flow and tissue perfusion and in doing so, address the contribution between peripheral and central regulatory mechanisms. The primary forces that cause the movement of blood through a vessel are thought to be a positive pressure gradient and an increase in vascular conductance. Throughout all three heating protocols, no noticeable changes in perfusion pressure were observed—much like the majority of studies investigating the haemodynamic responses to local limb heating (Pearson *et al.*, 2011; Chiesa *et al.*, 2016; Kuhlenhoelter *et al.*, 2016; Kalsi *et al.*, 2017). In this study, segmental-leg heating resulted in ≥ 3-fold increases in tissue perfusion, and considerable increases in regional tissue oxygenation and skin blood flow; whilst the adjacent leg segment displayed no changes in these variables. Moreover, we observed no changes in cardiac stroke volume or wave intensity derived forward

compression and forward expansion waves in the heated and control legs. This suggests that left ventricular contractility and late systolic flow deceleration were unchanged throughout the whole-leg heating protocol even though cardiac output did increase to a similar extent as the rise in heated leg blood flow during the 3 h whole-leg heating protocol (0.7–0.8 $L \cdot min^{-1}$). Hence, activation of regulatory mechanisms and pathways in the peripheral circulation rather than augmented central haemodynamic forces (Edholm *et al.*, 1957; Roddie *et al.*, 1957; Blair *et al.*, 1960; Rowell, 1974) must explain the robust increases in blood velocity and flow in the vasculature of the limb hyperthermic tissues.

The increase in blood flow to the heated leg region occurred in the face of a maintained diameter in all examined conduit arteries, including those of the control leg and/or control leg segment where blood velocity and flow did not change. The present findings agree with studies reporting no changes in conduit artery diameter during limb heating (Pearson *et al.*, 2011; Chiesa *et al.*, 2016; Teixeira *et al.*, 2017; Coombs *et al.*, 2019; Coombs *et al.*, 2021), but are at odds with studies showing decreases in POA diameter (Thomas *et al.*, 2017). The unchanged conduit artery diameter, together with the general increases in local vascular conductance with whole-leg and segmental-leg hyperthermia, suggest that vasodilatation might have instead occurred in the downstream small arteries and resistance arterioles, and/or alternatively thermosensitive physical and chemical mechanisms governing blood's rheological properties and kinetic energy permitted the increase in microvascular blood velocity and flow.

Interestingly, the studies assessing the impact of temperature variations in *in vitro* skeletal muscle vessel preparations show, for the most part, that temperature *per se* does not exert a direct effect on smooth muscle contractile function (Vanhoutte & Shepherd, 1970; Ives *et al.*, 2011). This lends support to the view that heat is predominantly acting indirectly via changes in temperature-dependent blood viscosity, red blood cell deformability and dispersion and/or intravascular vasodilatory mechanisms (Akyurekli *et al.*, 1997; Artmann *et al.*, 2008; Heinonen *et al.*, 2011; Binzoni *et al.*, 2012; Stadler *et al.*, 2012; Chiesa *et al.*, 2015; Chiesa *et al.*, 2016). Although not investigated herein, strong relationships exist between 1) increases in blood and tissue temperatures (Chiesa *et al.*, 2015; Kalsi *et al.*, 2017), 2) elevations

in temperature and reductions in blood viscosity and frictional resistance (Snyder, 1971; Cinar *et al.*, 2001; Shin *et al.*, 2004; Lim *et al.*, 2010), and 3) increases in temperature and rises in red cell deformability and dispersion (Manteuffel-Szoege, 1960; Manteuffel-Szoege, 1969; Cinar *et al.*, 2001; Pinho *et al.*, 2016). Hence, heat *per se* may reduce blood viscosity and vascular resistance, and increase red cell deformability, red cell dispersion, blood velocity and blood kinetic energy. It therefore seems plausible that heat-modulated blood rheology explains at least part of the ≥3-fold elevation in thermal hyperaemia.

Another likely possibility is that local hyperthermia induces downstream vasodilatation via heat-sensitive biochemical signals that (1) activate intravascular signalling-transduction mechanisms in the microvasculature such as shear-mediated nitric oxide release and cellular oxidative stress (Kellogg *et al.*, 1999; Minson *et al.*, 2001; Paniagua *et al.*, 2001; Laughlin *et al.*, 2008; Gifford *et al.*, 2014; Romero *et al.*, 2017), and/or (2) stimulate the release of vasoactive molecules from the circulating erythrocytes such as ATP (Pearson *et al.*, 2011; Kalsi & Gonzalez-Alonso, 2012; Kalsi *et al.*, 2017). In support of the involvement of red blood cell signalling mechanisms, close relationships between increases in temperature and erythrocyte-derived ATP release, but not other blood constituents (Etulain *et al.*, 2011; Kalsi & Gonzalez-Alonso, 2012; Kalsi *et al.*, 2017), and between increases in plasma ATP and local limb hyperthermia have been reported (Pearson *et al.*, 2011; Kalsi & Gonzalez-Alonso, 2012; Gonzalez-Alonso *et al.*, 2015; Kalsi *et al.*, 2017). Furthermore, increases in plasma ATP with intra-arterial infusion cause profound elevations in limb blood velocity and tissue perfusion, independent of temperature, metabolic or perfusion pressure changes (González-Alonso *et al.*, 2008; Kalsi & Gonzalez-Alonso, 2012; Kalsi *et al.*, 2017). Taken together, the present and previous experimental evidence suggests that local hyperthermia likely increases local perfusion through activation of vascular thermosensitive mechanisms that cause microvessel vasodilatation.

Hyperthermia influence on local arterial distensibility

Another finding of the present study is that prolonged whole-leg hyperthermia did not alter CFA distensibility in either the experimental or control leg. To our knowledge, this is the first study to directly assess the influence of limb hyperthermia on local arterial stiffness and distensibility using the ln(*D*)*U*-loop method. Despite the ≥ 3-fold

increases in blood velocity, no changes were seen in wave speed or arterial distensibility. The present findings of local stiffness are in agreement with past whole-body and two-leg hyperthermia studies which reported no changes in regional (carotid–radial) arterial stiffness (Ganio *et al.*, 2011; Moyen *et al.*, 2016; Schlader *et al.*, 2019). However, studies exploring the recovery following hyperthermia, reported decreases in peripheral and/or leg (femoral–ankle region) arterial stiffness alongside an elevated core temperature (Caldwell *et al.*, 2017; Thomas *et al.*, 2017; Lee *et al.*, 2018; Cheng *et al.*, 2021; Sugawara & Tomoto, 2021). The latter observations contrast with the unaffected or small changes in arterial stiffness/distensibility, core temperature and arterial pressure observed in the present and previous single-leg heating studies (Takahashi *et al.*, 2011; Chiesa *et al.*, 2015; Chiesa *et al.*, 2016; Engelland *et al.*, 2020). Previous studies have alluded to the possibility that a certain threshold of hyperthermic intensity—such that can initate profound increases in core temperature and/or alterations in sympathetic activity—may be required to elecit reductions in arterial stiffness and associated increases in distensibility (Kaldur *et al.*, 2016; Caldwell *et al.*, 2017). Therefore, although further studies are warranted, the present findings indicate that acute single-leg hyperthermia does not alter CFA stiffness and distensibility and thus, conduit artery vascular tone in conditions evoking no or negligible elevations in core temperature.

Perspectives and significance

The present study provides substantial evidence to indicate that limb tissue blood flow during regional hyperthermia is controlled by highly localised events in the microcirculation, as opposed to central haemodynamic forces or thermal reflexes responding to increases in core temperature. In this construct, the heart accommodates to the increase in leg tissue perfusion by augmenting cardiac output, rather than playing a major role in the control of tissue blood flow and its distribution, as demonstrated here by 1) the unchanged PFA blood flow, quadriceps tissue oxygenation and skin blood flow during lower-leg and foot heating, despite the substantial increases in CFA, SFA and POA blood flow (Figure 26), and 2) the unchanged POA blood flow and calf and foot tissue oxygenation during upper-leg heating in the face of markedly elevated CFA (Figure 24). Future studies can use the approach employed by Watanabe *et al.* (2020) during exercise to directly test this

hypothesis, by simultaneously measuring vascular and cardiac function during passive regional and systemic hyperthermia.

The profound and sustained increases in blood flow and shear rate with prolonged leg heating, may serve as effective haemodynamic stimuli for improving vascular health. Shear stress—which increased ≥ 3-fold in the present study—is widely accepted as an important stimulus for vascular remodelling (Zarins *et al.*, 1987; Girerd *et al.*, 1996; Vita *et al.*, 2008; Green *et al.*, 2017). Numerous intervention studies have investigated the effects of repeated two-leg or whole-body heating and the associated hyperaemia on vascular health, reporting improvements in endothelial function (Imamura *et al.*, 2001; Kihara *et al.*, 2002; Ohori *et al.*, 2012; Carter *et al.*, 2014; Brunt *et al.*, 2016). The advantage of the present local leg hyperthermia approach—as opposed to repeated two-leg or whole-body heating—is that the ensuing haemodynamic stimuli can be applied over prolonged periods without significant systemic physiological strain or thermal discomfort. Therefore, local limb heating may provide an effective alternative to promote beneficial arterial adaptations that improve vascular health in people with reduced or limited exercise capacity.

Experimental considerations

Some experimental considerations should be acknowledged when interpreting the present findings. As reported in the methods, different individuals participated in the three protocols which makes this study a between-subjects design as opposed to the gold standard within-subjects design. Nevertheless, the data from the three participants completing all three protocols and published data (Keller *et al.*, 2010; Pearson *et al.*, 2011; Chiesa *et al.*, 2015; Chiesa *et al.*, 2016) during 1 h whole-leg heating interventions in young healthy adults reveal comparable and reproducible leg blood flows to those observed in the present study (i.e., 0.5–0.6 ± 0.2 $L \cdot min^{-1}$ increase in CFA blood flow). Whilst the present protocols were different to address specific but complementary aims, the inter-protocol comparisons were conducted during the first hour of each protocol when the methodology was identical, other than the obvious distinction of the heated region. Consequently, the highly reproducible haemodynamic responses to local leg and segmental hyperthermia and the fact that the responses were compared during the same timeframe, strongly support that our data and interpretations are valid and robust.

Summary

In conclusion, the present study provides comprehensive and compelling evidence on the effects of local hyperthermia in the human leg circulation. Prolonged whole-leg hyperthermia produces a profound and sustained elevation in upper- and lower-leg blood flow; whilst segmental-leg hyperthermia induces hyperaemia to a magnitude that matches the regional hyperaemia during whole-leg heating without affecting blood flow, temperature, or tissue oxygenation of the non-heated limb segment. Increases in local tissue oxygenation, blood flow, vascular conductance and blood velocity were positively related with the rise in local temperature, yet these increases occurred without any changes to mean perfusion pressure, conduit artery diameter or wave intensity-derived parameters. Collectively, these findings support the notion that local hyperthermia increases peripheral tissue perfusion through the activation of local thermosensitive mechanisms. These mechanisms are proposed to increase microvascular blood flow by inducing blood rheology-mediated increases in vascular conductance and/or vasodilatation in the microcirculation. The markedly enhanced hyperaemia and tissue oxygenation strongly support the therapeutic potential of local hyperthermia for treatment of circulatory diseases and/or rehabilitation.

CHAPTER 5

Study 2: Tight thermo-haemodynamic coupling during regional thigh hyperthermia in humans: insight into the importance of local thermosensitive mechanisms on blood circulation

Contextual and relevant particulars for Study 2

Study 2 was published in *Experimental Physiology* in 2024. The published article can be found in the appendices.

Koch Esteves, N., McDonald, J., & González-Alonso, J. (2024). Thermo-haemodynamic coupling during regional thigh heating: Insight into the importance of local thermosensitive mechanisms in blood circulation. *Experimental Physiology*, 1–14. https://doi.org/10.1113/EP091556

Author contributions. This study was performed at Brunel University London, Uxbridge, UK as part of the present PhD thesis and a BSc dissertation for Jeneil McDonald. The BSc dissertation included a small sample of the data collected. NKE, JGA and JM conceived and designed the research. NKE, JM, and JGA acquired the data. NKE analysed the data. NKE and JGA interpreted the data. NKE wrote the manuscript.

Abstract

A positive relationship between local tissue temperature and perfusion exists, with isolated limb-segment hyperthermia stimulating hyperaemia in the heated region without affecting the adjacent, non-heated limb segment. However, whether partial-limb segment heating evokes a heightened tissue perfusion in the heated region without directly or reflexly affecting the non-heated tissues of the same limb segment remains unknown. This study investigated, in eleven healthy young adults, the lower limb temperature and haemodynamic responses to three levels of 1 h upper-leg heating, none of which alter body core temperature: (1) whole-thigh (WTH; water-perfused wrapping), (2) quadriceps (QH; water-perfused wrapping), and (3) partial-quadriceps (PQH; pulsed shortwave diathermy) heating. It was hypothesised that perfusion will only increase in the heated regions. WTH, QH and PQH increased local heated tissue temperature by 2.9 ± 0.6, 2.0 ± 0.7 and 2.9 ± 1.3 ºC ($p < 0.0001$), respectively, whilst remaining unchanged in the non-heated hamstrings and quadriceps tissues during QH and PQH. WTH induced a 2-fold increase in common femoral artery blood flow ($p < 0.0001$) whereas QH and PQH evoked a similar ~1.4-fold elevation ($p \leq 0.0018$). During QH and PQH, however, tissue oxygen saturation and laser-Doppler skin blood flow in the adjacent non-heated hamstrings or quadriceps tissues remained stable ($p > 0.5000$). These findings in healthy young humans demonstrate a tight thermo-haemodynamic coupling during regional thigh heating, providing further evidence of the importance of local heat-activated mechanisms on the control of blood circulation.

Keywords: Heat, thermal mechanisms, blood flow, haemodynamics.

Introduction

A well-established, positive relationship exists between local tissue temperature and blood flow during whole-body or local-limb hyperthermia (Johnson & Proppe, 1996; Heinonen *et al.*, 2011; Pearson *et al.*, 2011; Chiesa *et al.*, 2016; Kalsi *et al.*, 2017; Koch Esteves *et al.*, 2021). Recent research from our laboratory demonstrated that segmental-limb heating—i.e., isolated upper-leg and lower-leg heating—produces comparable magnitudes of conduit artery hyperaemia for their respective limb segment during whole-leg heating, without affecting tissue temperature or perfusion in the adjacent non-heated segment (Koch Esteves *et al.*, 2021). These findings suggest that during segmental-limb heating, adjacent limb segments are not affected by central neural reflexes (Barcroft *et al.*, 1947; Johnson *et al.*, 1976) or indirect heating, whether it be through conductive or convective heat transfer via the tissues and blood, respectively (Incropera *et al.*, 1996; Xiang & Liu, 2008; Gonzalez-Alonso, 2012).

Explanations for the observed maintenance of adjacent, non-heated segment perfusion could be due to (*a*) an insufficient hyperthermic stimulus to evoke reflex drives (Taylor *et al.*, 1984) and (*b*) an excessively steep positive temperature gradient between the heated and non-heated segments, whereby heat—whether via conductance in the tissues or convection in the blood—is being quickly dissipated in the normothermic limb segment as per Pennes' bioheat equation (Pennes, 1948; Arkin *et al.*, 1994; Incropera *et al.*, 1996; Fiala & Havenith, 2015). However, whether this phenomenon would hold true within a limb segment where the heated and non-heated areas are in proximity remains unclear. For instance, would solely heating the whole or a portion of the quadriceps increase temperature and thus, blood flow in the hamstrings and non-heated quadriceps section or would the hyperaemia be solely confined in the heated area? Research exploring the clinical application of targeted hyperthermia on tumours have found large increases in tissue perfusion and oxygenation of the heated tissue (Song, 1984; Elming *et al.*, 2019; Bosque *et al.*, 2021), while temperature of the adjacent healthy tissue remained unaltered (LeVeen *et al.*, 1976; Kim *et al.*, 1977; Suit & Gerweck, 1979). Although the unchanged temperature of the adjacent tissue is indicative of a maintained tissue perfusion, direct measures of macro- and microcirculation tissue perfusion in healthy limb tissue are required to verify or refute this assumption.

Consequently, the aim of the present study is to comprehensively assess the relationship between local limb-segment heating and the ensuing hyperaemia, specifically investigating temperature, tissue oxygenation and haemodynamic responses in the major arteries of the human leg during whole-thigh heating, quadriceps heating, and partial-quadriceps heating. It was hypothesised that: (*a*) all local hyperthermia interventions would increase the blood flow profiles of the femoral arteries which supply the upper leg, with the magnitude of hyperaemia being proportional to the volume of heated tissue, and (*b*) muscle and skin temperature, tissue perfusion, oxygen saturation and skin blood flow will remain unchanged in the adjacent non-heated areas.

Materials and methods

Ethical approval

The study was approved by the Brunel University London Research Ethics Committee (38109-MHR-Oct/2022-41690-2) and was performed in accordance with the Declaration of Helsinki. All participants provided informed written consent prior to their participation in the present study.

Participants

Eleven healthy, physically active adults (4 women) participated in the present study. Participants had a mean ± SD age of 22 ± 6 years, a height of 174.4 ± 8.8 cm and body mass of 76.1 ± 13.1 kg (Table 4). Prior to the start of the study, informed written consent was obtained from all participants following a detailed written and verbal explanation of the experimental protocol. Participants were considered healthy and physically active following the completion of a health questionnaire and a basic cardiovascular screening. Participants refrained from heavy exercise for 48 h, alcohol consumption for 24 h and caffeine consumption for 12 h before the commencement of the protocols. Moreover, female participants were requested to avoid scheduling their laboratory visit during their menses.

Table 4. Participant demographic and anthropometric characteristics.

Variables	Participants
Age (years)	22 ± 6
Sex	
Female	4 (36%)
Male	7 (64%)
Height (cm)	174.4 ± 8.8
Mass (kg)	76.1 ± 13.1
Right leg volume (litres)	12.2 ± 2.4
Right leg lean volume (%)	74.7 ± 9.1
Right leg non-lean volume (%)	25.3 ± 9.1
Left leg volume (litres)	12.2 ± 2.3
Left leg lean volume (%)	74.9 ± 9.1
Left leg non-lean volume (%)	25.1 ± 9.1

Values are means ± SD for 11 participants.

Experimental protocols

The present study consisted of three protocols: (i) whole-thigh heating, (ii) quadriceps heating, and (iii) partial-quadriceps heating, which were conducted over two visits (Figure 30). Protocol 1 was completed during visit A whilst protocols 2 and 3 were completed during another visit B, with the order being counterbalanced among participants. Upon arriving at the laboratory, participants were asked to weigh themselves in a semi-nude state and then had their height measured (SECA 798 Scale, Germany) and in the case for visit one only, had their leg anthropometric measurements recorded. The latter data allowed an estimate of leg composition using the method reported by Jones and Pearson (1969). Seven leg circumferences were taken at the gluteal furrow, one-third subischial (one-third of the distance between the gluteal furrow and the popliteal crease), the minimum circumference above the knee, the maximum circumference at the knee joint, the minimum circumference below the knee, the maximum circumference at the calf, and the minimum circumference at the ankle joint. Additionally, skinfold measurements were obtained at the following four

sites: one-third subischial (anterior and posterior sites) and at the maximum calf circumference (lateral and medial sites) using skinfold callipers (Jones & Pearson, 1969). Subsequently, participants sat on a custom-built bed within a climate chamber set at an ambient temperature and humidity of 22 ºC and 30–40 %, respectively.

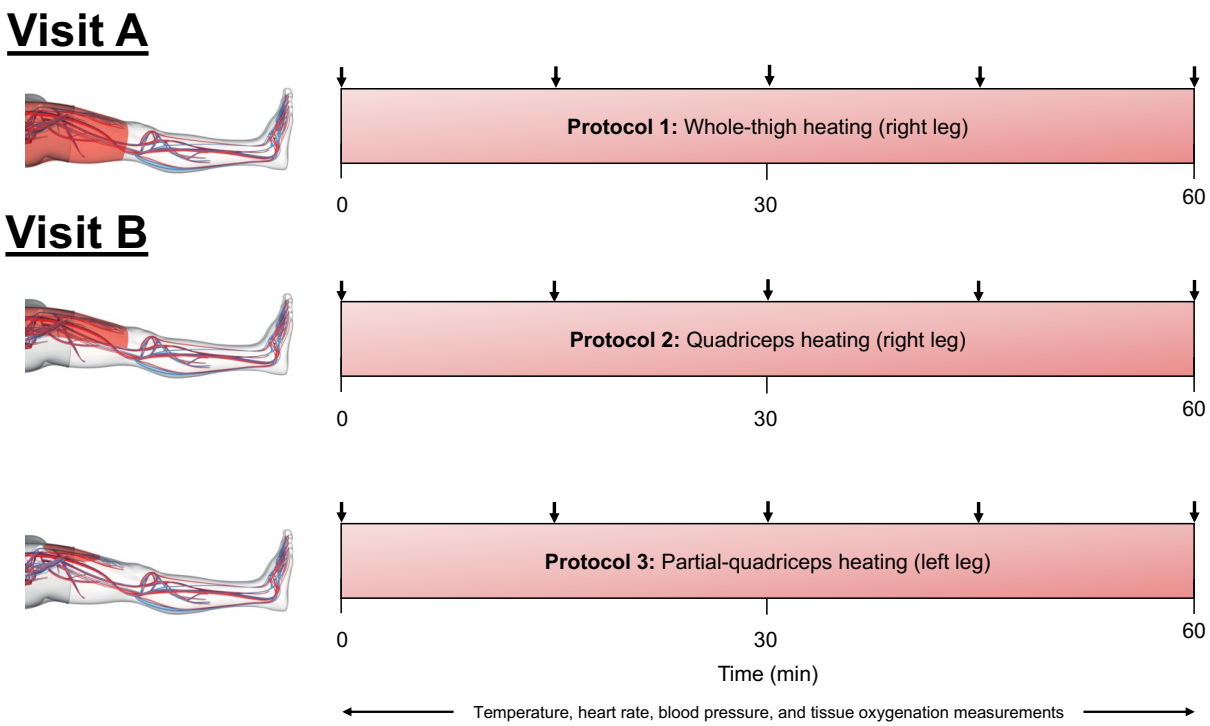

Figure 30. Schematic of experimental protocol. Downward arrows illustrate the times in which an ultrasound blood flow measurement was conducted. Blood flow was measured at the common, superficial and profunda femoral arteries and popliteal artery. Core temperature, leg muscle and skin temperatures, leg tissue oxygen saturation, leg skin blood flow, and central haemodynamics were measured continuously throughout the protocol. Protocol 1 was conducted during visit A, and protocol 2 and 3 on visit B. The respective visits were randomised and counterbalanced.

Protocol 1: effects of whole-thigh heating. Participants were instrumented with ECG electrodes, temperature thermistors, tissue oxygenation optode pads, and the finometer upper-arm and middle finger cuffs (described below). The experimental protocol initiated with baseline haemodynamic measurements of the common femoral artery (CFA), superficial femoral artery (SFA), profunda (deep) femoral artery (PFA) and popliteal artery (POA) in the right leg. Next, participants were fitted with a custom-made water-perfusion trouser on their right upper leg, which was then wrapped in a survival blanket to optimise the heating procedure by limiting heat loss from the trouser to the surrounding environment. The trouser was connected to a thermostatically controlled water circulator (Julabo F-34, Seelbach, Germany), which continuously circulated water at a temperature of 50 ºC. During the 1 h heating protocol, blood flow

was measured every 15 min at the CFA, SFA, PFA and POA of the right, experimental leg (Figure 30).

Protocol 2: effects of quadriceps heating. Following instrumentation of ECG electrodes, temperature thermistors, tissue oxygenation optode pads, and finometer cuffs—much like protocol 1—participants had baseline haemodynamic measurements of the CFA, SFA, PFA and POA in both the right and left legs. Subsequently, participants were fitted with a custom-made water-perfusion trouser which solely covered the top of their right upper leg (i.e., the quadriceps). A survival blanket was then placed on top of the heated trouser to limit heat loss; however, care was taken to not to cover the remainder of upper leg (i.e., the hamstrings) which was left exposed. The trouser was heated as described in protocol 1. During the 1 h heating protocol, blood flow was measured every 15 min at the CFA, SFA, PFA and POA of the right, experimental leg (Figure 30).

Protocol 3: effects of partial-quadriceps heating. This protocol followed immediately after the completion of protocol 2. Baseline haemodynamic measurements of the CFA, SFA, PFA and POA were recorded again in left leg. Partial-quadriceps heating was conducted using a pulsed shortwave diathermy (MegaPulse II, EMS Physio, UK) at 800 pulses per second, with a pulse duration of 400 μs. The heating drum has a surface area of ~200 cm^2. Thus, if one assumes that whole-thigh heating and quadriceps hyperthermia heat 100 % and 50 % of total upper-leg surface area, respectively, using the anthropometric measures calculated from the present cohort, partial-quadriceps hyperthermia heated ~8 % of the upper-leg (i.e., 16 % of the quadriceps). During the 1 h heating protocol, blood flow was measured every 15 min at the CFA, SFA, PFA and POA of the left, experimental leg (Figure 30).

Temperature measurements

Core temperature (T_c) was measured using a rectal probe (Ret-1 Special, Physitemp, Clifton, NJ, USA) which was self-inserted 15 cm past the sphincter muscle. Tympanic temperature (T_{Tym}) was measured using commercially available thermometer (Thermoscan 7, Braun, Germany). Skin temperature (T_{sk}) was measured in the quadriceps at two different locations (one proximal and one distal) and the hamstrings of both legs using commercially available thermistors (IT-18, Physitemp, Clifton, NJ,

USA) which were securely held in place using medical tape. Muscle temperature (T_m) in the proximal portion of the vastus lateralis muscle (during all three protocols), the distal portion of the vastus lateralis muscle (during protocol 3), and in the middle of the biceps femoris muscle of the right leg (during protocol 1 and 2) was measured using thermistors (T-204f, Physitemp) inserted via an 18G catheter ~3 cm deep into the muscle. T_c, T_{sk} and T_m were recorded online using a commercially available thermocouple meter (TC-2000, Sable Systems International, Las Vegas, NV, USA) collected at 1000 Hz through a data acquisition system (PowerLab 26T, ADInstruments, New Zealand), and exported in 30 s bins using a commercially available data acquisition software (LabChart 7, ADInstruments, New Zealand). Data were then imported and analysed in Microsoft Excel software (Microsoft Corporation, Redmond, WA, USA), reported as 2 min averages. Temperature data are reported every 5 min for the first 15 min of protocol 1 and 2 to better characterise the sharp increase in tissue temperatures and then every 15 min, in parallel with haemodynamic measurements, for the remainder of the protocols. During protocol 3, temperature data is reported at baseline, 30 min and 60 min due to interference between the thermocouple meter and diathermy unit. In addition, mean tissue temperature for the heated regions were calculated using:

$\overline{T}_{Whole-thigh} = \left(\frac{T_{m\ quad\ 1}+T_{m\ ham}}{2} \times 0.92\right) + \left(\frac{T_{sk\ quad\ 1}+T_{sk\ quad\ 2}+T_{sk\ ham}}{3} \times 0.08\right)$,

$\overline{T}_{Quadriceps} = \left(T_{m\ quad\ 1} \times 0.92\right) + \left(\frac{T_{sk\ quad\ 1}+T_{sk\ quad\ 2}}{2} \times 0.08\right)$, and $\overline{T}_{Partial-quadriceps} = \left(T_{m\ quad\ 1} \times 0.92\right) + \left(T_{sk\ quad\ 1} \times 0.08\right)$, where *quad* 1, *quad 2,* and *ham* represent the proximal and distal portions of the quadriceps and hamstrings, respectively. Mean tissue temperature formulas were created using previously reported volume ratios of the different tissue compartments in the leg (Wang *et al.*, 1999).

Haemodynamic measurements

Heart rate was continuously measured using a three-lead echocardiogram. Also, arterial blood pressure was measured non-invasively—at the same time points as arterial blood flow measurements—using infrared photoplethysmography (Finometer, FMS, Netherlands), through a cuff on the middle finger of the left hand. Blood flow was measured at set time points—recording two 12 s Doppler images—throughout the protocols in the various arteries using a duplex Doppler ultrasound system (Vivid E95,

GE Medical Systems, UK) with a 9 MHz linear array transducer probe (GE Medical Systems, UK) at an insonation angle of ≤ 60º, with sample volume positioned in the centre of the artery. Before commencing baseline blood flow measures, arterial sites for the CFA, SFA, PFA and POA, in the right leg during protocol 1 and both legs for protocols 2 and 3, were located and marked to ensure blood flow measures were consistently measured at the same site. SFA and PFA blood flow measurements were acquired at a distance of ≥ 2 cm from the femoral bifurcation to prevent turbulent flow disruption to the measurements, and thus improve validity of measures. Blood flow (mL·min^{-1}) was calculated using the following equation: $BF = V_{mean} \times \pi \times (\frac{D_{mean}}{2})^2 \times 60$, where V_{mean} is the average centreline blood velocity (cm·s^{-1}), and D_{mean} (cm) is the average internal diameter calculated using: $D_{mean} = \frac{1}{3}(D_{systole}) + \frac{2}{3}(D_{diastole})$ (Radegran, 1997). Furthermore, upper-leg blood flow was calculated as the difference between whole-leg blood flow (CFA) and lower-leg blood flow (POA).

Shear rate (SR) was calculated using: $SR = \frac{4 \times V_{mean}}{D_{mean}}$, where V_{mean} is mean blood velocity. Additionally, vascular conductance (VC) was calculated using: $VC = BF \div MAP$, where it is represented as mL·min^{-1}·mmHg^{-1}, BF is blood flow (mL·min^{-1}) and MAP is mean arterial pressure (mmHg). Blood flow was analysed offline using a commercially available software (EchoPAC, GE Medical, Horton, Norway). Blood velocity was averaged over two 12 s Doppler images, and average diameter was determined from four 2D B-mode images. Furthermore, central haemodynamic data were collected at 1000 Hz using a commercially available data acquisition system (PowerLab 26T, ADInstruments, New Zealand) and exported in 30 s bins using a commercially available data acquisition software (LabChart 7, ADInstruments, New Zealand). Following exportation, data were imported and analysed in Microsoft Excel software (Microsoft Corporation, Redmond, WA, USA). Data are reported as 2 min averages. Data are reported as 2 min averages throughout the three protocols. Furthermore, quadriceps skin blood flow was measured in all three protocols via laser-Doppler flowmetry (PeriFlux Flowmetry System), reported in perfusion units (PU). The probe was attached to the skin of the thigh, specifically on the distal portion of the vastus lateralis. During protocols 1 and 2, skin blood flow was measured under the heated region; however, during protocol 3, it was measured in the non-heated area of the quadriceps.

Tissue oxygen saturation measures

Direct and continuous measurements of regional tissue haemoglobin oxygen saturation were obtained in the experimental upper legs using a near-infrared spectroscopy unit (NIRS; INVOS 5100C Cerebral Oximeter; Somanetics Corp, Troy, MI, USA). The optodes were placed on the skin surrounding the quadriceps and hamstrings of the experimental upper leg, same positioning as the T_{sk} thermistors, and taped to reduce interference from external light sources. It was not possible to measure tissue oxygen saturation at the heated region during protocol 3—i.e., proximal quadriceps under the diathermy unit—due to interference between the near-infrared spectroscopy unit and diathermy unit.

Statistical analysis

Statistical analysis was conducted using R Studio (version 2022.07.1+554, Team (2022)). An independent *t*-test was conducted to discover any differences in anthropometric data between the right and left leg. In addition, linear mixed-effects models were employed to investigate differences between protocols and over time in all measured variables—that is, central and local haemodynamics, temperature and tissue oxygenation—during the three heating protocols. The linear mixed-effects models were conducted following the confirmation of the data's normality via Shapiro-Wilk test and Mauchly's test of sphericity. Following the linear mixed-effects models, once a significant interaction between protocol and time was found, a Bonferroni post hoc test was conducted to locate the specific time points at which those changes occurred. Significance is set at $p < 0.05$. Note: if no time points or protocols are specified, *p* values are in reference to the interaction (time x protocol) following the linear mixed-effects model, if time points or protocols are specified, then *p* values are obtained from the post hoc test. Results are expressed as mean ± SD, with all data corresponding to the experimental legs. Moreover, linear, exponential, and polynomial regression curve fit tests were performed using GraphPad Prism (version 8, GraphPad Software, La Jolla, California, USA) to assess the relationships among various key data, with R2 representing the goodness of fit. Subsequently, Akaike's Information Criterion was used to evaluate which model provides the most appropriate fit.

Results

Demographic and anthropometric characteristics

Demographic and anthropometric data for the participants are reported in Table 4. No differences were observed in leg volume (p = 0.748) or proportion of lean to non-lean mass (p = 0.797) between the right and left legs.

Regional and core temperature responses to whole-thigh, quadriceps, and partial-quadriceps heating

Leg muscle and skin temperatures are illustrated in Figure 31, whilst core and tympanic temperatures are reported in Table 5. As per experimental design, all three heating protocols induced significant increases in tissue temperature of their respective heated regions (p = 0.0145), whilst the measured unheated regions during quadriceps and partial-quadriceps heating remained unchanged (Figure 31). Specifically, whole-thigh heating increased $\overline{T}_{Whole-thigh}$ to 37.3 ± 0.4 ºC (∆ = +2.9 ± 0.6 ºC; p < 0.0001), quadriceps heating increased $\overline{T}_{Quadriceps}$ to 37.2 ± 0.3 ºC (∆ = +2.0 ± 0.7 ºC; p < 0.0001), and partial-quadriceps heating increased $\overline{T}_{Partial-quadriceps}$ to 36.8 ± 1.3 ºC (∆ = +2.9 ± 1.3 ºC; p < 0.0001), with these temperatures representing the average tissue temperature of the respective heated regions. No between-protocol differences were observed in tissue temperature of the heated regions. Moreover, core temperature was stable during quadriceps and partial-quadriceps heating but decreased marginally following 1 h of whole-thigh heating protocol (∆ = -0.2 ± 0.3; p < 0.001) (Table 5). No between-protocol differences in core temperature were observed (Table 5). Lastly, no differences across time or between protocols were observed in tympanic temperature (p = 0.2664) (Table 5).

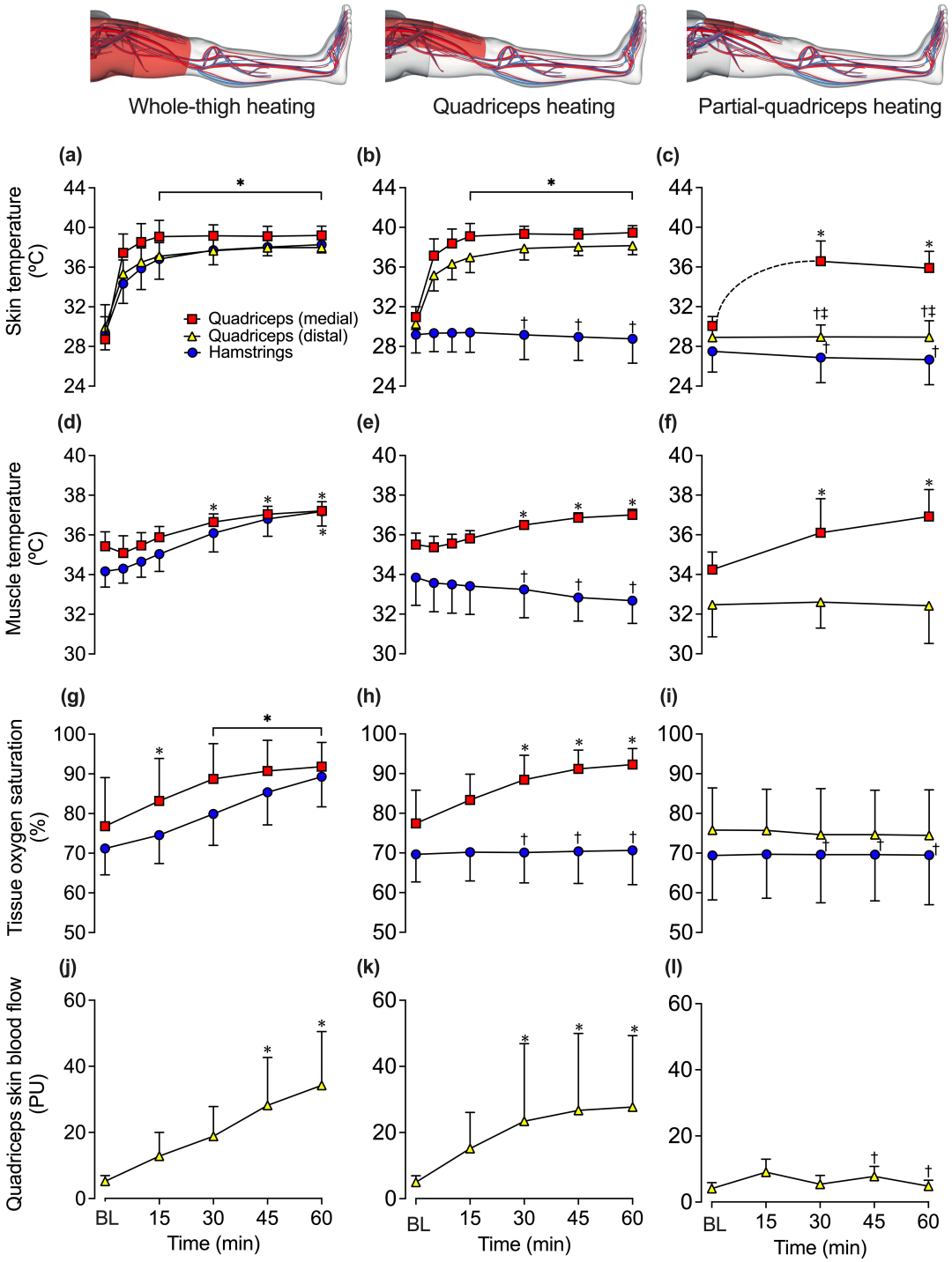

Figure 31. Skin and muscle leg temperatures (a–f), regional tissue oxygen saturation (g–i), and skin blood flow (j–l) during whole-thigh (a, d, g, j), quadriceps (b, e, h, k), and partial-quadriceps (c, f, i, l) hyperthermia. BL signifies baseline measurements. Notes: firstly, due to the fewer data points recorded during partial-quadriceps heating, a curved dotted line between BL and 30 min *Quadriceps (medial)* skin temperature data points was plotted to illustrate the predicted change in temperature over time based on the literature (Hafen *et al.*, 2018). Secondly, quadriceps regional tissue oxygenation and skin blood flow were measured within the heated area during protocol 1 and 2; however, it was only measured outside the heated proximal quadriceps area during protocol 3. ∗ Different from baseline across time within the same protocol, $p < 0.05$. † Different from whole-thigh heating, $p < 0.05$. ‡ Different from quadriceps heating, $p < 0.05$.

Table 5. Influence of whole-thigh, quadriceps, and partial-quadriceps heating on body temperature, and central haemodynamics.

		Time (min)				
Variables	*Intervention*	0	15	30	45	60
T_c *(ºC)*	*Whole-thigh heating*	37.2 ± 0.2	37.0 ± 0.2	37.0 ± 0.2*	37.0 ± 0.3*	37.0 ± 0.3*
	Quadriceps heating	37.1 ± 0.2	36.9 ± 0.2	36.9 ± 0.2	36.9 ± 0.2	36.9 ± 0.2
	Partial-quadriceps heating	36.9 ± 0.2	—	36.9 ± 0.2	—	37.1 ± 0.2
T_{Tym} *(ºC)*	*Whole-thigh heating*	36.9 ± 0.2	36.8 ± 0.2	36.8 ± 0.2	36.7 ± 0.2	36.7 ± 0.2
	Quadriceps heating	36.7 ± 0.4	36.7 ± 0.3	36.7 ± 0.4	36.7 ± 0.4	36.6 ± 0.4
	Partial-quadriceps heating	36.7 ± 0.4	—	36.7 ± 0.4	—	36.7 ± 0.4
HR *(bpm)*	*Whole-thigh heating*	64 ± 7	65 ± 8	64 ± 8	65 ± 7	66 ± 6
	Quadriceps heating	60 ± 8	59 ± 7	60 ± 7	59 ± 8	59 ± 8
	Partial-quadriceps heating	59 ± 9	57 ± 6	59 ± 8	58 ± 8	62 ± 8
MAP *(mmHg)*	*Whole-thigh heating*	87 ± 8	89 ± 8	89 ± 10	88 ± 14	90 ± 13
	Quadriceps heating	92 ± 13	88 ± 13	90 ± 11	92 ± 11	90 ± 10
	Partial-quadriceps heating	93 ± 10	93 ± 11	97 ± 11	98 ± 14	95 ± 13

Values are means ± SD for 11 participants. T_c*:* core temperature*;* T_{Tym}*:* tympanic temperature; HR: heart rate; MAP: mean arterial pressure. ∗Different from baseline, $p < 0.05$. † Different from whole-thigh heating, $p < 0.05$.

Leg haemodynamics, tissue oxygen saturation and systemic haemodynamics during whole-thigh, quadriceps, and partial-quadriceps heating

Complete haemodynamic responses during 1 h of whole-thigh, quadriceps, and partial-quadriceps heating for the CFA, SFA, PFA and POA are reported in Table 6 and Figure 32. Femoral artery blood flow increased steadily during whole-thigh heating with CFA, SFA and PFA blood flow increasing ~2-fold above baseline (Δ = +0.31 ± 0.16, +0.13 ± 0.12, and +0.13 ± 0.07 L·min^{-1}, respectively) following 1 h (all $p <$ 0.0001). One hour of quadriceps heating resulted in a smaller magnitude of upper-leg tissue perfusion in comparison to whole-thigh heating ($p = 0.0080$) with CFA blood flow increasing ~1.4-fold (Δ = +0.15 ± 0.09 L·min^{-1}, $p < 0.0001$); however, no significant changes were observed in SFA ($p = 0.0836$) and PFA ($p = 0.5546$) blood flow. Partial-quadriceps heating increased CFA and PFA blood flow by ~1.4–1.6-fold (Δ = +0.15 ± 0.12 and +0.07 ± 0.06 L·min^{-1}, $p = 0.0006$ and $p = 0.0002$, respectively) whilst SFA blood flow remained unchanged ($p = 1.000$). These increases in upper-leg blood flow

were not lower than those observed during whole-thigh heating (p = 0.3392) and quadriceps heating (p = 1.0000). During all thigh heating protocols, POA blood flow remained unchanged (p = 0.0642). Whole-leg blood flow was related (second order polynomial) to increases in mean tissue temperature of the heated region during whole-thigh heating (R^2 = 0.35), quadriceps heating (R^2 = 0.19), and partial-quadriceps heating (R^2 = 0.22) (Figure 33). No relationship was observed between mean tissue temperature and diameter (Figure 33); as such, no changes in diameter were observed over time or between protocols in the CFA (p = 0.4231), SFA (p = 0.6718), and PFA (p = 0.0642). Correspondingly, relationships (second order polynomial) were observed between blood velocity and mean tissue temperature during whole-thigh heating (R^2 = 0.54), quadriceps heating (R^2 = 0.27), and partial-quadriceps heating (R^2 = 0.36) (Figure 33), which mirrored the changes in blood flow. Moreover, quadriceps skin blood flow increased ~6.6-fold (p < 0.0001) and ~5.6-fold (p < 0.0001) during whole-thigh heating and quadriceps heating, respectively, but remained unchanged during partial-quadriceps heating (p = 1.0000) as the optode was placed outside the heated area (Figure 31).

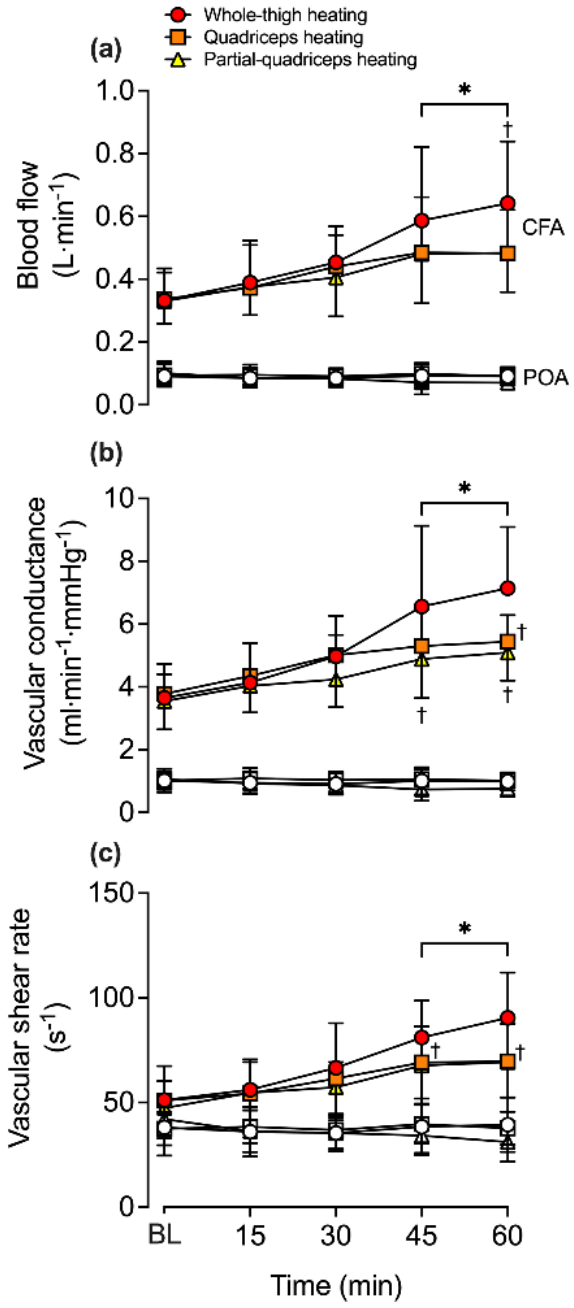

Figure 32. Blood flow (a), vascular conductance (b), and shear rate (c) during whole-thigh, quadriceps, and partial-quadriceps hyperthermia. Data represented as mean ± SD (n = 11) for the common femoral (CFA) and popliteal (POA) arteries, represented in filled and unfilled symbols, respectively. BL signifies baseline measurements. ∗Different from baseline across time within the same protocol, $p < 0.05$. † Different from whole-thigh heating.

Similar responses to blood flow were observed in vascular conductance and shear rate during the three heating protocols (Figure 33; Table 6). Following 1 h of whole-thigh heating, vascular conductance and shear rate increased ~1.7–2.1-fold and ~1.6–2-fold, respectively, in all three femoral arteries (all $p < 0.0001$). During quadriceps heating, CFA vascular conductance and shear rate increased ~1.4-fold ($p = 0.0019$ and $p = 0.0077$), respectively, and was lower than whole-thigh heating ($p = 0.0050$

and p = 0.0130). However, during quadriceps heating, no differences in shear rate across time were observed in the SFA and PFA ($p \geq 0.1401$) and as such, SFA and PFA shear rate and PFA vascular conductance were lower than whole-thigh heating (all $p < 0.05$) whilst SFA vascular conductance was not (p = 0.1328). Conversely, partial-quadriceps heating instigated ~1.4 increases in CFA vascular conductance (p = 0.0033) and shear rate (p = 0.0006), and ~1.6 in PFA vascular conductance (p = 0.0005) and shear rate ($p < 0.0001$). Vascular conductance and shear rate were lower during partial-quadriceps heating in comparison to whole-thigh for all arteries ($p <$ 0.05) apart from CFA and PFA shear rate which were not different (p = 0.2807 and p = 0.4048, respectively). No differences, however, were observed between quadriceps and partial-quadriceps heating (all p = 1.0000).

Tissue oxygen saturation responses are illustrated in Figure 32. During whole-thigh heating, quadriceps and hamstrings tissue oxygen saturation increased steadily, peaking at 92 ± 6 % units (Δ = +15 ± 8 % units, $p < 0.0001$) and 89 ± 8 % units (Δ = +18 ± 5 % units, $p < 0.0001$), respectively. Similarly, following 1 h of quadriceps heating, quadriceps tissue oxygen saturation increased to 92 ± 4 % units (Δ = +15 ± 6 % units, $p < 0.0001$) which was similar to that observed during whole-thigh heating (p = 1.000). Conversely, hamstring tissue oxygen saturation remained unchanged during quadriceps heating (p = 1.000), which differed to the responses observed during whole-thigh heating ($p < 0.0001$). No changes in quadriceps (p = 0.5270) and hamstrings (p = 0.9960) tissue oxygen saturation during partial-quadriceps heating, as both sites measured were outside the heated area. As such, quadriceps tissue oxygen saturation was lower following 1 h of partial-quadriceps heating in comparison to whole-thigh ($p < 0.0001$) and quadriceps heating ($p < 0.0001$). Similarly hamstrings tissue oxygen saturation was lower following 1 h of partial-quadriceps heating in comparison to whole-thigh heating ($p < 0.0001$) but not different from quadriceps heating (p = 1.000). Lastly, at the systemic haemodynamic level, no differences in heart rate and mean arterial pressure were observed during or between any of three heating protocols (p = 0.1689 and p = 0.7958, respectively (Table 5).

Table 6. Influence of whole-thigh, quadriceps, and partial-quadriceps heating on leg haemodynamics.

		Time (min)				
Variables	*Intervention*	0	15	30	45	60
CFA blood flow	*Whole-thigh heating*	0.33 ± 0.10	0.39 ± 0.13	0.45 ± 0.11*	0.59 ± 0.23*	0.64 ± 0.20*
(*L·min^{-1}*)	*Quadriceps heating*	0.34 ± 0.08	0.37 ± 0.09	0.44 ± 0.16	0.48 ± 0.16*	0.48 ± 0.12*†
	Partial-quadriceps heating	0.33 ± 0.09	0.38 ± 0.13	0.41 ± 0.13	0.48 ± 0.18*	0.48 ± 0.14*
SFA blood flow	*Whole-thigh heating*	0.17 ± 0.03	0.19 ± 0.06	0.22 ± 0.05	0.26 ± 0.11*	0.30 ± 0.12*
(*L·min^{-1}*)	*Quadriceps heating*	0.17 ± 0.04	0.20 ± 0.06	0.19 ± 0.06	0.23 ± 0.10	0.23 ± 0.07†
	Partial-quadriceps heating	0.18 ± 0.06	0.19 ± 0.05	0.19 ± 0.06	0.19 ± 0.6	0.21 ± 0.08†
PFA blood flow	*Whole-thigh heating*	0.12 ± 0.04	0.15 ± 0.07	0.17 ± 0.07*	0.23 ± 0.09*	0.25 ± 0.10*
(*L·min^{-1}*)	*Quadriceps heating*	0.12 ± 0.04	0.12 ± 0.04	0.14 ± 0.04	0.14 ± 0.03†	0.16 ± 0.04†
	Partial-quadriceps heating	0.11 ± 0.03	0.13 ± 0.07	0.14 ± 0.05	0.16 ± 0.05	0.18 ± 0.05*
POA blood flow	*Whole-thigh heating*	0.09 ± 0.02	0.09 ± 0.03	0.08 ± 0.03	0.09 ± 0.04	0.09 ± 0.03
(*L·min^{-1}*)	*Quadriceps heating*	0.09 ± 0.04	0.10 ± 0.03	0.09 ± 0.03	0.10 ± 0.03	0.09 ± 0.03
	Partial-quadriceps heating	0.10 ± 0.04	0.09 ± 0.03	0.08 ± 0.02	0.08 ± 0.03	0.07 ± 0.02
CFA vascular conductance	*Whole-thigh heating*	3.6 ± 1.1	4.1 ± 1.3	5.0 ± 1.3	6.6 ± 2.6*	7.1 ± 1.9*
(m*L·min^{-1}·mmHg^{-1}*)	*Quadriceps heating*	3.8 ± 1.1	4.3 ± 1.2	5.0 ± 1.6	5.3 ± 1.7*	5.4 ± 1.2*†
	Partial-quadriceps heating	3.5 ± 0.9	4.0 ± 1.4	4.2 ± 1.4	4.9 ± 1.6†	5.1 ± 1.2*†
SFA vascular conductance	*Whole-thigh heating*	1.9 ± 0.4	2.0 ± 0.6	2.5 ± 0.6	3.0 ± 1.3*	3.3 ± 1.2*
(m*L·min^{-1}·mmHg^{-1}*)	*Quadriceps heating*	1.9 ± 0.5	2.3 ± 0.7	2.2 ± 0.7	2.5 ± 1.0	2.6 ± 0.6†
	Partial-quadriceps heating	2.0 ± 0.6	2.0 ± 0.5	2.0 ± 0.5	2.0 ± 0.5†	2.2 ± 0.8†
PFA vascular conductance	*Whole-thigh heating*	1.3 ± 0.5	1.5 ± 0.7	1.9 ± 0.8*	2.5 ± 1.2*	2.7 ± 1.0*
(m*L·min^{-1}·mmHg^{-1}*)	*Quadriceps heating*	1.3 ± 0.5	1.5 ± 0.5	1.6 ± 0.5	1.6 ± 0.5†	1.8 ± 0.5†
	Partial-quadriceps heating	1.2 ± 0.3	1.4 ± 0.6	1.4 ± 0.5	1.6 ± 0.5†	1.9 ± 0.4*
POA vascular conductance	*Whole-thigh heating*	1.0 ± 0.3	0.9 ± 0.3	0.9 ± 0.3	1.0 ± 0.4	1.0 ± 0.3
(m*L·min^{-1}·mmHg^{-1}*)	*Quadriceps heating*	1.0 ± 0.4	1.1 ± 0.3	1.0 ± 0.3	1.1 ± 0.3	1.0 ± 0.2
	Partial-quadriceps heating	1.1 ± 0.3	0.9 ± 0.3	0.9 ± 0.3	0.7 ± 0.4	0.8 ± 0.3
CFA shear rate	*Whole-thigh heating*	51 ± 16	56 ± 15	67 ± 21	81 ± 18*	90 ± 22*
(*S^{-1}*)	*Quadriceps heating*	51 ± 15	54 ± 13	62 ± 17	69 ± 20*	70 ± 17*†
	Partial-quadriceps heating	48 ± 13	55 ± 15	57 ± 12	68 ± 19*	69 ± 18*
SFA shear rate	*Whole-thigh heating*	53 ± 19	56 ± 16	68 ± 20	77 ± 24*	87 ± 30*
(*S^{-1}*)	*Quadriceps heating*	56 ± 17	59 ± 13	57 ± 15	69 ± 20	68 ± 16
	Partial-quadriceps heating	55 ± 14	56 ± 13	57 ± 11	58 ± 14	62 ± 20†
PFA shear rate	*Whole-thigh heating*	63 ± 16	73 ± 22	86 ± 22	113 ± 29*	126 ± 39*
(*S^{-1}*)	*Quadriceps heating*	62 ± 17	66 ± 14	74 ± 16	77 ± 19	87 ± 29†
	Partial-quadriceps heating	60 ± 14	68 ± 16	74 ± 27	88 ± 29	102 ± 41*
POA shear rate	*Whole-thigh heating*	38 ± 13	36 ± 12	35 ± 9	39 ± 13	39 ± 13
(*S^{-1}*)	*Quadriceps heating*	37 ± 8	38 ± 8	37 ± 5	40 ± 9	38 ± 8
	Partial-quadriceps heating	42 ± 9	36 ± 10	35 ± 7	34 ± 8	31 ± 9

Values are means ± SD for 11 participants. CFA: common femoral artery; SFA: superficial femoral artery; PFA: profunda femoral artery; POA: popliteal artery. ∗Different from baseline, $p < 0.05$. † Different from whole-thigh heating, $p < 0.05$.

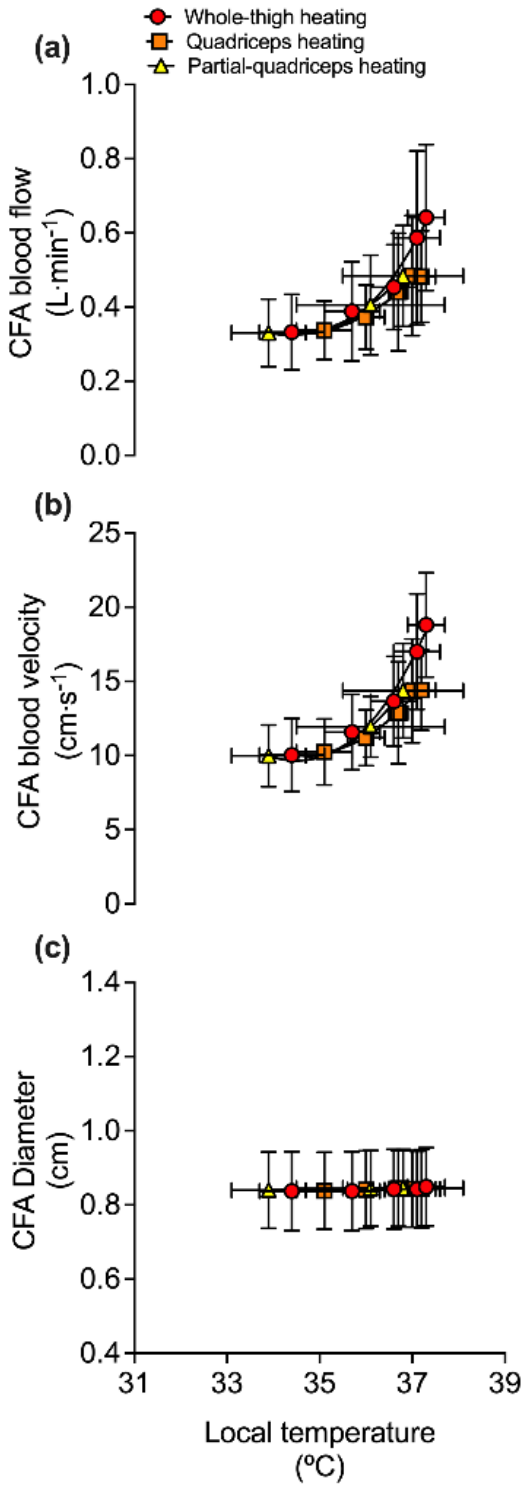

Figure 33. Relationship between the mean local tissue temperature and common femoral artery (CFA) blood flow (a), blood velocity (b), and arterial diameter (c) during whole-thigh, quadriceps, and partial-quadriceps hyperthermia. Local temperature signifies the corresponding average heated tissue temperature for the protocol, as discussed in the methods—i.e., $\overline{T}_{Whole-thigh}$, $\overline{T}_{Quadriceps}$ and $\overline{T}_{Partial-quadriceps}$. Data represented as mean ± SD (n = 11). Vertical error bars signify blood flow SD, while horizontal error bars signify local temperature SD, respectively. Lines represent the exponential fit of the data.

Discussion

The present study explored the relationship between local tissue temperature and perfusion during three levels of regional thigh heating to gain further insight into the importance of local thermosensitive mechanisms in the control of blood circulation. As per the study's primary hypothesis, all heating protocols increased CFA blood flow, with whole-thigh heating evoking a larger magnitude of hyperaemia than quadriceps heating but surprisingly, not greater than partial-quadriceps heating. Moreover, in line with the secondary hypothesis, tissue temperature, blood flow and oxygenation remained unchanged in the respective non-heated hamstrings, quadriceps, and lower-leg segment. Together, the present findings demonstrate a close coupling among tissue perfusion, oxygen saturation and temperature during regional thigh heating, which further substantiates the localised nature of the mechanisms involved in the control of blood circulation during isolated hyperthermia.

Influence of local hyperthermia on tissue perfusion

In this study, all three modalities of upper-leg heating—whole-thigh, quadriceps, and partial-quadriceps heating—evoked sustained 1.4–2-fold increases in CFA blood flow (Figure 32). In line with previous studies, local hyperthermia-induced increases in leg tissue perfusion, assessed via CFA blood flow, occurred in relation to increases in local tissue temperature (Chiesa *et al.*, 2016; Koch Esteves *et al.*, 2021; Koch Esteves *et al.*, 2023). A previous study from our laboratory found that the magnitude of hyperaemia was associated to the volume of the heated limb segment with whole-leg heating evoking the largest degree of hyperaemia, proportionally followed by upper-leg and lower-leg heating, respectively (Koch Esteves *et al.*, 2021). The present study extends those previous findings, with the observed hyperaemia during 1 h whole-thigh and quadriceps heating (+0.31, +0.15 $L \cdot min^{-1}$, respectively) being proportional to that previously reported during 1 h whole-leg heating (+0.62 $L \cdot min^{-1}$) (Koch Esteves *et al.*, 2021). These observations together substantiate the impact of volume of heated tissue on the magnitude of hyperaemia evoked by the same type of thermal intervention (whole-leg > whole-thigh > quadriceps). Furthermore, in line with the literature, whole-thigh heating stimulated a large 2-fold increase in upper-leg blood flow without affecting the unheated lower-leg or contralateral leg (Koch Esteves *et al.*, 2021). However, a novel finding was that the 1.4-fold increase in upper-leg blood flow during

quadriceps and partial-quadriceps heating was solely confined to the directly heated tissues. This is supported by the observations that hamstrings tissue oxygen saturation—a surrogate for tissue perfusion (Davis *et al.*, 2006)—in the experimental leg remained unchanged during quadriceps heating, and quadriceps skin blood flow and tissue oxygen saturation of the unheated quadriceps area (≤ 18 cm distal to the heated source) of the quadriceps during partial-quadriceps heating was unaffected (Figure 31). Collectively, these data indicate that tissue perfusion is regulated at an extremely local level, in direct response to changes in local tissue temperature, to the point that tissue perfusion can be different between two areas of the same limb segment.

Interaction between heating modalities and tissue temperature in the regulation of tissue perfusion

A pertinent finding from the present study is that the magnitude of hyperthermia-induced hyperaemia is predominantly dependent on two factors: local tissue temperature and volume of heated tissue, with the two factors strongly related. The present study used a hot water-perfused garment during whole-thigh and quadriceps heating and diathermy during partial-quadriceps heating which heated the respective limb tissue via conduction and radiation, respectively. Partial-quadriceps heating warmed the tissues homogenously via radiation at a much quicker rate than that possible through conduction (Garrett *et al.*, 2000; Draper *et al.*, 2013; Hafen *et al.*, 2018; Beninca *et al.*, 2021). The present study found that partial-quadriceps heating was able to stimulate the same magnitude of hyperaemia as quadriceps heating despite only targeting 16 % of the quadriceps' total surface area. This is likely due to the ability of diathermy to penetrate deep into the tissues and thus, uniformly heat a greater volume as ratified by its ability to increase muscle temperature at a depth of 3–3.5 cm by 4 ºC within 30 min (Hafen *et al.*, 2018). How partial-quadriceps heating was able to evoke a similar magnitude of hyperaemia to quadriceps heating is perhaps best explained mathematically. For example, a cuboid—representing the volume targeted by the heated garment during quadriceps heating—with the approximate dimensions of 42 x 30 x 1 cm for length, width, and depth, respectively can have the same volume of 1257 cm^3 as a cylinder—representing the volume targeted by the diathermy unit during partial-quadriceps heating—with a radius and height of 8 and

6.25 cm, respectively. However, the cross-sectional surface area of the cylinder is substantially smaller (16 %) than that of the cuboid. Moreover, previous studies which have employed local leg heating and leg cooling following heating or exercise provide strong evidence that tissue perfusion of the temperature-manipulated region more closely mirrors deep muscle (2–3 cm) temperature in comparison to skin temperature (Sekins *et al.*, 1984; Gregson *et al.*, 2011; Heinonen *et al.*, 2011; Pearson *et al.*, 2011; Mawhinney *et al.*, 2013; Caldwell *et al.*, 2016; Chiesa *et al.*, 2016; Mawhinney *et al.*, 2017). Most striking is a past study demonstrating that diathermy was able to increase thigh muscle temperature to ~42 ºC during simultaneous skin cooling which increased muscle blood flow from ~ 3 $mL \cdot min^{-1} \cdot 100g^{-1}$ to ~ 48 $mL \cdot min^{-1} \cdot 100g^{-1}$ (Sekins *et al.*, 1980; Sekins *et al.*, 1982; Sekins *et al.*, 1984; Giombini *et al.*, 2007). Taken together, the present findings suggest that the volume of the heated tissue may have a prominent impact on tissue perfusion, supporting the concept that deep muscle temperature is a potent stimulus for hyperthermia-induced hyperaemia.

Leg blood flow increased during all three heating protocols without affecting tissue perfusion and tissue oxygen saturation in the non-heated limb area, whether that be the adject lower-limb during whole-thigh heating or the proximal (≤ 18 cm) non-heated upper-leg areas during quadriceps and partial-quadriceps heating (Figure 31). Limb tissue perfusion during systemic hyperthermia is postulated to be mediated through local and central thermosensitive mechanisms, either working separately or in tandem (Johnson & Proppe, 1996; Chiesa *et al.*, 2019). The present finding that local tissue perfusion and temperature were unchanged in the non-heated areas alongside a maintained core temperature aligns with past studies advocating for the predominant role of local thermosensitive mechanisms in the stimulation of hyperthermia-induced hyperaemia (Chiesa *et al.*, 2015; Chiesa *et al.*, 2016; Kalsi *et al.*, 2017; Koch Esteves *et al.*, 2021). However, this finding conflicts with past studies that have demonstrated the influence of an augmentation in central haemodynamics, such as a cutaneous vasodilatory reflex drive, during whole-body and/or local-limb heating where blood flow increases not only in heated limb but also in the non-heated or cooled contralateral limb (Johnson *et al.*, 1976; Taylor *et al.*, 1984; Heinonen *et al.*, 2011; Caldwell *et al.*, 2016; Mallette *et al.*, 2016). In these studies that observed a change in central haemodynamics and/or a cutaneous vasodilatory reflex drive, core temperature is generally regarded as the primary stimulus: a 1 ºC increase in core temperature is

associated with an approximately 9-fold, 3 $L \cdot min^{-1}$, and 35 $beats \cdot min^{-1}$ increase in skin blood flow, cardiac output and heart rate, respectively (Johnson & Park, 1979; Chiesa *et al.*, 2019). In support, Heinonen *et al.* (2011) found that during unilateral calf heating where core temperature remained unchanged, blood flow increased in the heated calf but not in the control, contralateral calf; however, during whole-body heating where core temperature increased by 1 ºC, blood flow not only increased in the heated calf but also in the non-heated, contralateral calf likely due to the large reductions in vascular resistance. The observed hyperaemia in the unheated contralateral calf highlights the potency of core temperature to stimulate a cutaneous vasodilatory reflex drive (Heinonen *et al.*, 2011). Thus, when taken together, the data strongly suggest that local thermosensitive mechanisms—as opposed to an augmentation in central haemodynamic forces and reflexes—play a primary role in the regulation of peripheral tissue perfusion during local limb hyperthermia.

The strong relationship between local tissue temperature and perfusion (Figure 33) and thus, the likely predominant role of local thermosensitive mechanisms in the regulation of tissue perfusion may explain why hyperaemia was solely confined to the heated areas. However, why the heat remained confined to the area directly under the heat source and did not spread to the remainder of the limb remains of interest. Heat can be transferred between biological tissues through two modalities: intercellular conductive and vascular convective heat transfer (Khaled & Vafai, 2003; Gonzalez-Alonso, 2012). Conduction is the transfer of heat energy through a positive temperature gradient across mediums in direct contact (Incropera *et al.*, 1996), and is known to be a relatively slow process as it is reliant upon a positive temperature gradient and the thermal conductivity of the surrounding tissues (Gonzalez-Alonso, 2012). This modality is responsible for the transfer of heat from the water-perfused garment to the leg tissues during whole-thigh and quadriceps heating via a positive thermal gradient between the skin and internal tissues. On the other hand, given that the upper-leg contains a dense vascular network consisting of arterioles, post-capillary venules, capillaries, and their cellular constituents (Guven *et al.*, 2020), convection—the transfer of heat through the movement of fluid—plays a significant role in heat transfer within a limb (Incropera *et al.*, 1996; Fiala & Havenith, 2015). The resultant concomitant increase in internal limb tissue perfusion during local heating, further stimulates convective heat transfer through the various microvessels perfusing the

internal tissues, exchanging heat in the capillary beds until it reaches equilibrium with the surrounding tissues (Chato, 1980; Fiala & Havenith, 2015). Whilst the blood perfusing the heated tissues can act as a heat source to the neighbouring unheated tissues (Baish *et al.*, 1986), the large disparity between heated and non-heated tissue volume as well as the vast network vessels, mean that the heat is quickly dissipated in the surrounding unheated tissues. This is supported by past studies demonstrating a 5–10 ºC difference in tissue temperature between the heated tumour and adjacent tissues during targeted tumour hyperthermia, though the distance between the heated tumour and unheated adjacent tissue is unknown (LeVeen *et al.*, 1976; Kim *et al.*, 1977; Suit & Gerweck, 1979). Consequently, the present findings provide evidence that local heating of a limb segment does not cause a heightened tissue perfusion to the proximal non-heated tissues which quickly dissipate the heat, likely due to a combination of an insufficient thermal stimulus and the human body's ability to maintain homeostasis.

Experimental considerations

There are several methodological considerations in this study. First, during quadriceps and partial-quadriceps heating, it is not possible to directly measure which tissues are being perfused via the femoral conduit arteries. Consequently, tissue oxygen saturation measures of the heated and non-heated regions were used to assess regional tissue perfusion. Whilst there is some debate as to whether tissue oxygen saturation data solely reflect muscle perfusion or a combination of cutaneous and muscle perfusion, there is a consensus that it does indeed provide a measure of tissue perfusion (Davis *et al.*, 2006; Pearson *et al.*, 2011; Choo *et al.*, 2017; Koch Esteves *et al.*, 2021). Thus, we are confident in our interpretation of the data. Second, during partial-quadriceps heating, baseline skin and muscle temperatures were lower than the other two protocols as the leg was left exposed in ambient conditions for > 1 h whilst protocol 2 was being performed on the right leg. This introduced challenges when making between-protocol comparisons. Third, during partial-quadriceps heating, the electromagnetic waves from the diathermy unit interfered with some of the data recording equipment. Hence, we were only able to collect temperature data by pausing the diathermy unit for 1 min. While this does not directly affect the quality or validity of the present findings, it would have been of great value to have continuously measured

the increase in temperature at the onset of heating as diathermy has been shown to rapidly increase muscle temperature, as shown in previous diathermia studies (Garrett *et al.*, 2000; Draper *et al.*, 2013; Hafen *et al.*, 2018; Beninca *et al.*, 2021). Fourth, the sum of SFA and PFA blood flow do not perfectly match the measured CFA blood flow. This is likely due to difficulty faced when attempting to obtain high quality images of the PFA, in comparison to other main conduit leg arteries, as the PFA is highly influenced by the individual vessel anatomy (Hussain, 1997; Tomaszewski *et al.*, 2017; Koch Esteves & Chiesa, 2021). Nonetheless, this potential limitation does not affect the interpretation and conclusion of the present findings.

Summary

Passive whole-thigh, quadriceps, and partial-quadriceps heating evoked 1.4–2-fold increases in leg blood flow which mirrored the increases in local tissue temperature. Moreover, the quadriceps and partial-quadriceps heating protocols increased tissue perfusion to their respective heated regions without affecting perfusion and tissue oxygen saturation of the non-heated area within the same segment, likely due to an unchanged tissue temperature. These results indicate a strong relationship between local tissue temperature and perfusion. Ultimately, this further highlights the significance of local thermosensitive mechanisms in the regulation of tissue perfusion during hyperthermia.

CHAPTER 6

Study 3: Lower limb hyperthermia augments functional hyperaemia during small muscle mass exercise similarly in trained elderly and young humans

Contextual and relevant particulars for Study 3

Study 3 was published in *Experimental Physiology* in 2023. The published article can be found in the appendices.

> Koch Esteves, N., Khir, A. W., & González-Alonso, J. (2023). Lower limb hyperthermia augments functional hyperaemia during small muscle mass exercise similarly in trained elderly and young humans. *Experimental Physiology*, 00, 1–18. https://doi.org/10.1113/EP091275.

Author contributions. This study was performed at Brunel University London, Uxbridge, UK as part of the present PhD thesis with NKE as the primary researcher and author. NKE and JGA conceived and designed the research. NKE and JGA acquired the data. NKE analysed the data. NKE, AWK and JGA interpreted the data. All authors revised the manuscript and provided intellectual feedback and agree to be accountable for all aspects of the work.

Abstract

Heat and exercise therapies are recommended to improve vascular health across the lifespan. However, the haemodynamic effects of hyperthermia, exercise and their combination are inconsistent in young and elderly people. Here we investigated the acute effects of local-limb hyperthermia and exercise on limb haemodynamics in nine healthy, trained elderly (69 ± 5 years) and ten young (26 ± 7 years) adults, hypothesising that the combination of local hyperthermia and exercise interact to increase leg perfusion, albeit to a lesser extent in the elderly. Participants underwent 90 min of single whole-leg heating, with the contralateral leg remaining as control, followed by 10 min of low-intensity incremental single-leg knee-extensor exercise with both the heated and control legs. Temperature profiles and leg haemodynamics at the femoral and popliteal arteries were measured. In both groups, heating increased whole-leg skin temperature and blood flow by 9.5 ± 1.2 ºC and 0.7 ± 0.2 L·min^{-1} (>3-fold), respectively (p < 0.0001). Blood flow in the heated leg remained 0.7 ± 0.6 and 1.0 ± 0.8 L·min^{-1} higher during exercise at 6 W and 12 W, respectively (p < 0.0001). However, there were no differences in limb haemodynamics between cohorts, other than the elderly group exhibiting a 16 ± 6 % larger arterial diameter and a 51 ± 6 % lower blood velocity following heating (p < 0.0001). In conclusion, local hyperthermia-induced limb hyperperfusion and/or small muscle mass exercise hyperaemia are preserved in trained older people despite evident aged-related structural and functional alterations in their leg conduit arteries.

Keywords: Heat, exercise, blood flow, haemodynamics, ageing.

Introduction

Ageing is associated with numerous structural-functional alterations in the peripheral vasculature and the myocardium. These typically include increases in arterial diameter, wall thickness and stiffness, left ventricular hypertrophy, elevations in arterial blood pressure and reductions in maximal cardiac output (Hossack & Bruce, 1982; Rosenthal, 1987; Sonesson *et al.*, 1993; Ferrari *et al.*, 2003; Thijssen *et al.*, 2016; Nichols *et al.*, 2022). Maximal limb hyperaemia (functional hyperaemia) is also reduced in elderly populations during maximal aerobic exercise, a physical effort that engages a large muscle mass (Wahren *et al.*, 1974; Proctor *et al.*, 1998b; Beere *et al.*, 1999; Poole *et al.*, 2003; Proctor *et al.*, 2004). Findings during small muscle mass knee-extensor exercise are inconsistent, however, with studies showing a blunted hyperaemic response to low- to maximal-intensity exercise in sedentary and normally active elderly participants (Lawrenson *et al.*, 2003; Donato *et al.*, 2006; Mortensen *et al.*, 2012c) but not in lifelong endurance-trained elderly counterparts (Mortensen *et al.*, 2012c). The age-related attenuation in functional hyperaemia during low-intensity knee-extensor exercise is surprising given that a number of studies have shown no differences in lower limb blood flow between young and aged adults during matched low-intensity cycling exercise in sedentary (Beere *et al.*, 1999; Poole *et al.*, 2003), normally active (Proctor *et al.*, 2003) and endurance-trained (Wahren *et al.*, 1974) participants. To address this discrepancy in the literature and minimise the influences of different levels of aerobic fitness and cardiovascular capacity, the impact of ageing on functional hyperaemia can be investigated in exercised-trained elderly people during low-intensity exercise.

Hyperthermia—which can also instigate profound increases in limb blood flow—may provide further insight into the age-related alterations in vascular function. A wealth of literature exists on the blood flow responses to passive hyperthermia in young and aged subjects indicating that elderly adults have an attenuated hyperthermia-induced elevation in forearm perfusion (Sagawa *et al.*, 1988; Armstrong & Kenney, 1993; Rooke *et al.*, 1994; Minson *et al.*, 1998) and leg perfusion (Kenny *et al.*, 2017; Romero *et al.*, 2017). There are discrepancies in the literature, though, as two studies have reported comparable magnitudes of hyperperfusion between age cohorts in the human forearm (Kenny *et al.*, 2017) and leg (Engelland *et al.*, 2020). Moreover, the impact of superimposing local hyperthermia to exercise remains unresolved

irrespective of the population. Three studies in young individuals show no effect of localised thigh heating during knee-extensor exercise (Ferguson *et al.*, 2006), whole-body heating during prolonged submaximal single-leg and two-leg cycling (Savard *et al.*, 1988), or incremental two-leg cycling to exhaustion (Trangmar *et al.*, 2017). Conversely, two recent investigations revealed an additive effect of whole-body hyperthermia in young participants, with functional hyperaemia increasing by 0.6–0.7 $L \cdot min^{-1}$ during single-leg knee-extensor exercise in comparison to normothermic conditions (Pearson *et al.*, 2011; Chiesa *et al.*, 2015). To our knowledge, the effect of lower-limb hyperthermia on the magnitude of functional hyperaemia during small muscle mass exercise in elderly adults has never been investigated. Addressing this gap in knowledge is important in determining the potential of local heating and light exercise as a therapeutical intervention to improve health in people with reduced functional capacity.

The aim of the present study was to comprehensively examine and compare the haemodynamic responses to single-leg hyperthermia, one-legged knee-extensor exercise and combined single-leg hyperthermia and knee-extensor exercise in aged and young adults. It was hypothesised that: (a) single-leg hyperthermia and one-legged knee-extensor exercise would increase limb blood flow, which would be tightly coupled to increases in local limb temperature and/or metabolic demands; and (b) elderly participants would demonstrate an attenuated hyperaemic response to single-leg hyperthermia and knee-extensor exercise, in comparison to the young adult group.

Materials and methods

Ethical approval

The study was approved by the Brunel University London Research Ethics Committee (31692-A-Nov/2021- 34810-2) and was performed in accordance with the Declaration of Helsinki. All participants provided informed written consent prior to their participation in the present study, following a detailed written and verbal explanation of the experimental protocol.

Participants

A group of 9 healthy elderly adults (3 women) and a group of 10 healthy young (3

women) individuals participated in this study. The elderly adults had a mean ± SD age of 69 ± 5 years, a height of 172.9 ± 4.8 cm and body mass of 68.7 ± 11.7 kg, whereas the corresponding values for the young cohort were 26 ± 7 years, 171.7 ± 6.8 cm, and 71.0 ± 9.7 kg (Table 7). Participants were considered healthy and trained following the completion of a health questionnaire and a basic cardiovascular screening. All participants regularly engaged in structured sports, endurance and/or strength and conditioning training 3–7 times per week, with each session lasting 30–120 min. There were no differences in exercise frequency, duration, and modalities between age cohorts. Participants refrained from heavy exercise for 48 h, alcohol consumption for 24 h and caffeine consumption for 12 h before the commencement of the protocols. Moreover, young female participants, who had not undergone menopause, were requested to schedule their laboratory visit during the first seven days follow menses—i.e., the early follicular phase—as it is commonly associated with the lowest levels of oestrogen and progesterone.

Table 7. Participant demographic and anthropometric characteristics.

Variables	Elderly (n = 9)	Young (n = 10)
Age	69 ± 5†	26 ± 7
Sex		
Female	3 (30%)	3 (30%)
Male	6 (60%)	7 (70%)
Height (cm)	172.9 ± 4.8	171.7 ± 6.8
Mass (kg)	68.7 ± 11.7	71.0 ± 9.7
Right leg volume (litres)	10.2 ± 1.6	11.0 ± 1.9
Right leg lean volume (%)	76.3 ± 7.9	78.6 ± 6.7
Right leg non-lean volume (%)	23.7 ± 7.9	21.4 ± 6.7
Left leg volume (litres)	9.8 ± 1.9	11.3 ± 1.8
Left leg lean volume (%)	76.3 ± 8.1	78.7 ± 6.9
Left leg non-lean volume (%)	23.7 ± 8.1	21.3 ± 6.9

Values are means ± SD for 9 elderly and 10 young participants.

Experimental protocols

Participants were asked to consume their usual breakfast and report to the laboratory between 08h00 and 10h00. They were weighed in a semi-nude state and had their height (SECA 798 Scale, Germany) and leg anthropometric measurements recorded. The latter data allowed an estimate of leg composition using the method reported by Jones and Pearson (1969). Seven leg circumferences were taken at the gluteal furrow, one-third subischial (one-third of the distance between the gluteal furrow and the popliteal crease), the minimum circumference above the knee, the maximum circumference at the knee joint, the minimum circumference below the knee, the maximum circumference at the calf, and the minimum circumference at the ankle joint. Additionally, skinfold measurements were obtained at the following four sites: one-third subischial (anterior and posterior sites) and at the maximum calf circumference (lateral and medial sites) using skinfold callipers (Jones & Pearson, 1969). Subsequently, participants sat in a semi-recumbent position on the chair of a custom-built knee-extensor ergometer within the laboratory at an ambient temperature and humidity of 21 ºC and 30–40 %, respectively. Participants were then instrumented with ECG electrodes and the finometer upper-arm and middle finger cuffs to allow the assessment of central haemodynamics.

Familiarisation protocol. The familiarisation protocol commenced with a basic cardiovascular screening where participants had their ECG, cardiac output, stroke volume and leg blood flow at the common and superficial femoral arteries measured and evaluated. If participants reported abnormal values which were indicative of any underlying health issues, their participation in the study was terminated and they were recommended to schedule an appointment with their health practitioner. Following the successful completion of the health screening, participants had their left ankle and foot inserted and strapped into the boot of a modified dynamic knee-extensor exercise Monark ergometer. Participants were familiarised with the one-legged knee-extensor exercise (Andersen & Saltin, 1985), exercising on an unloaded ergometer for 5 min (Figure 1). Once the familiarisation with their left leg was completed, the participants' right ankle and foot were inserted and strapped into the boot of the ergometer, and the familiarisation protocol was repeated once more.

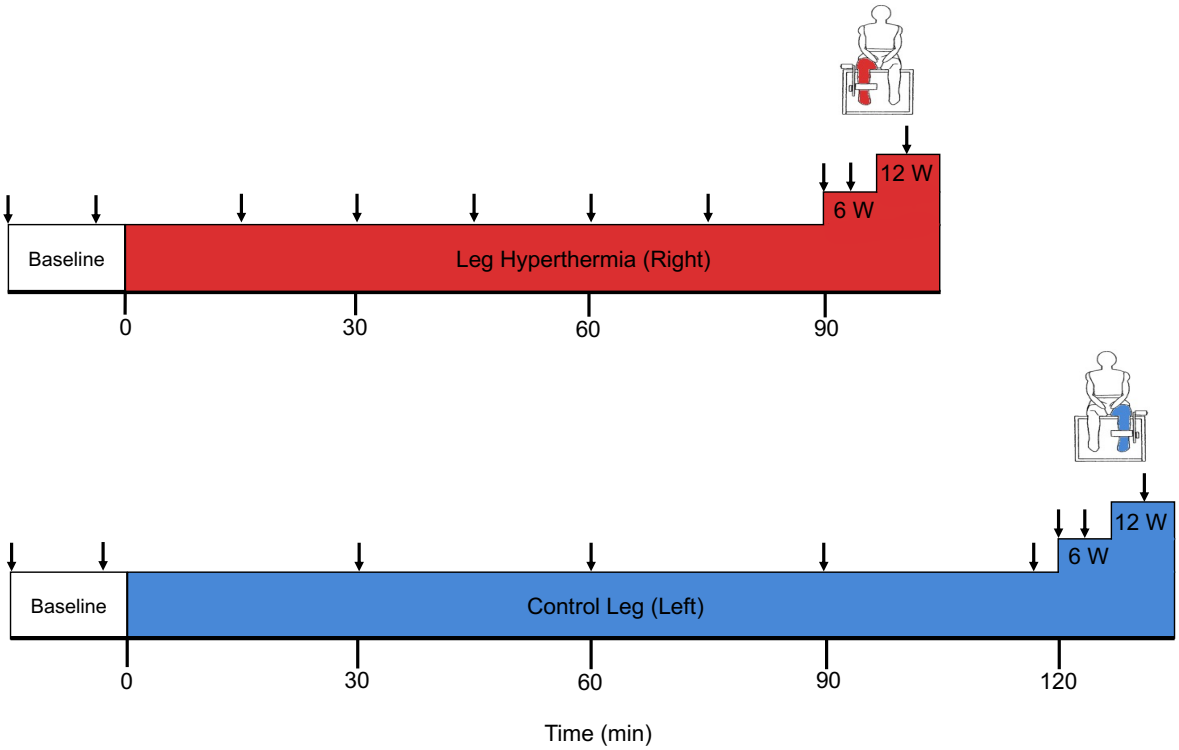

Figure 34. Schematic of experimental protocol. Downward arrows illustrate the times in which an ultrasound blood flow measurement was conducted. Blood flow was measured at the common, superficial and profunda femoral arteries and popliteal artery during rest and passive heating; however, blood flow was solely measured at the common and superficial femoral arteries during exercise. Core temperature, leg temperatures, leg tissue oxygen saturation and central haemodynamics were measured continuously throughout the protocol.

Experimental protocol. The schematic for the experimental protocol is illustrated in Figure 34. Following the successful completion of the familiarisation protocol, participants rested for the proceeding 30 min. During this time, they were instrumented with tissue oxygenation optode pads and temperature thermistors (described below). Once the participant was successfully instrumented and completed the 30 min rest period, the experimental protocol initiated with baseline measurements of the common femoral artery (CFA), superficial femoral artery (SFA), profunda (deep) femoral artery (PFA) and popliteal artery (POA) in both the right and left legs. Next, participants were fitted with a custom-made water-perfusion trouser on their right leg, which was then wrapped in a survival blanket to optimise the heating procedure by limiting heat loss from the trouser to the surrounding environment. The trouser was connected to a thermostatically controlled water circulator (Julabo F-34, Seelbach, Germany), which continuously circulated water at a temperature of 58 ºC (Figure 34). While the water temperature leaving the water circulator was substantially hotter than that commonly reported in the literature (~48–50 ºC)—including other previous studies from our

laboratory (Koch Esteves *et al.*, 2021)—the actual skin temperature was similar to those in previous studies due to the larger heat loss through the smaller diameter tubing and thus, reduced flow rate in the custom-made water-perfused trouser. During the 1.5 h right leg heating protocol, blood flow was measured every 15 min at the CFA, SFA, PFA and POA in the heated leg, and every 30 min in the control (left) leg.

Following passive whole leg heating, participants performed incremental one-legged knee-extensor exercise. The one-legged knee-extensor exercise consisted of two 5 min bouts, the first at 6 W and the second at 12 W (Figure 34). Intensity was controlled by increasing the resistance on the flywheel via metal weights. To account for the minor variations in cadence—i.e., differences in the number of knee extensions per minute—individual work rates were calculated for each stage using the following formula: $Work\ Rate = \overline{Cadence} \times Resistance$. During the exercise protocol, blood flow was measured at the CFA and SFA of the right leg during the middle of each exercise bout—i.e., at 2.5 and 7.5 min. Once the exercise protocol was completed, the heated trouser was removed; thus, concluding the protocol for the right leg. Subsequently, the control left leg was inserted and strapped into the boot of the exercise ergometer. The exercise protocol for the left leg was the same to the right leg protocol, except for the fact that the heated water-perfused trouser was not utilised. Baseline blood flow measures were taken at the CFA, SFA, PFA and POA of the left leg. This was succeeded by an incremental exercise protocol (6 W and 12 W for 5 min each) with blood flow being assessed at the same time points and vessels as in the heat leg (Figure 34).

Temperature measurements

Core temperature (T_c) was measured using a rectal probe (Ret-1 Special, Physitemp, Clifton, NJ, USA) which was self-inserted 15 cm past the sphincter muscle. Skin temperature (T_{sk}) in the quadriceps, hamstrings, and calf for both legs were measured using commercially available thermistors (IT-18, Physitemp, Clifton, NJ, USA) which were securely held in place using medical tape. T_c and T_{sk} were recorded online using a commercially available thermocouple meter (TC-2000, Sable Systems International, Las Vegas, NV, USA) connected to a data acquisition system (PowerLab 26T, ADInstruments, New Zealand). Foot T_{sk} was collected via wireless temperature loggers (DS1922L iButton Thermochron, Measurement Systems Ltd, UK). Following

the protocol, foot temperature was then exported from the wireless temperature loggers in 30 s bins using a specialist logging software (iButtons, Measurement Systems Ltd, UK). Data were then imported and analysed in Microsoft Excel software (Microsoft Corporation, Redmond, WA, USA), reported as 2 min averages. In addition, mean skin leg temperature ($\overline{T}_{Sk\_Leg}$) was calculated as an unweighted average of quadriceps, hamstrings, calf, and foot T_{sk}. Similarly, mean skin upper-leg temperature ($\overline{T}_{Sk\_Upper-Leg}$) was calculated as the unweighted average of quadriceps and hamstrings T_{sk}, and mean skin lower-leg temperature ($\overline{T}_{Sk\_Lower-Leg}$) was calculated as the unweighted average of calf and foot T_{sk}.

Haemodynamic measurements

Heart rate was continuously measured using a three-lead echocardiogram. Also, arterial blood pressure and cardiac output were measured non-invasively—at the same time points as arterial blood flow measurements—using infrared photoplethysmography (Finometer, FMS, Netherlands), through a cuff on the middle finger of the left hand. Cardiac output was calculated as heart rate × stroke volume, where stroke volume was estimated using the ModelFlow method, which incorporated corrections for age, height, and weight (Beatscope, FMS) (Wesseling *et al.*, 1993). Blood flow was measured at set time points—recording two 12 s Doppler images—throughout the protocols in the various arteries using a duplex Doppler ultrasound system (Vivid E95, GE Medical Systems, UK) with a 9 MHz linear array transducer probe (GE Medical Systems, UK) at an insonation angle of ≤ 60º, with sample volume positioned in the centre of the artery. The water-perfusion heated trouser had custom-made openings which allowed the probe to be placed on the skin with minimal heat loss. Before commencing baseline blood flow measures, arterial sites for the CFA, SFA and PFA in both legs were located and marked to ensure blood flow measures were consistently measured at the same site. SFA and PFA blood flow measurements were acquired at a distance of ≥ 2 cm from the femoral bifurcation to prevent turbulent flow disruption to the measurements, and thus improve validity of measures. Blood flow (mL·min^{-1}) was calculated using the following equation: $BF = V_{mean} \times \pi \times (\frac{D_{mean}}{2})^2 \times 60$, where V_{mean} is the average centreline blood velocity (cm·s^{-1}), and D_{mean} (cm) is the average internal diameter calculated using: $D_{mean} = \frac{1}{3}(D_{systole}) +$

$\frac{2}{3}(D_{diastole})$ (Radegran, 1997). It was not possible to directly measure PFA blood flow during the exercise protocol; therefore, during exercise, PFA blood flow was estimated using the following formula: $PFA\ blood\ flow = (CFA - SFA)\ blood\ flow$.

Shear rate (SR) was calculated using: $SR = \frac{4 \times V_{mean}}{D_{mean}}$, where V_{mean} is mean blood velocity. Additionally, vascular conductance (VC) was calculated using: $VC = BF \div MAP$, where it is represented as mL·min^{-1}·mmHg^{-1}, BF is blood flow (mL·min^{-1}) and MAP is mean arterial pressure (mmHg). Blood flow was analysed offline using a commercially available software (EchoPAC, GE Medical, Horton, Norway). Blood velocity was averaged over two 12 s Doppler images, and average diameter was determined from four 2D B-mode images. Furthermore, central haemodynamic and temperature data were collected at 1000 Hz using a commercially available data acquisition system (PowerLab 26T, ADInstruments, New Zealand) and exported in 30 s bins using a commercially available data acquisition software (LabChart 7, ADInstruments, New Zealand). Following exportation, data were imported and analysed in Microsoft Excel software (Microsoft Corporation, Redmond, WA, USA). Data are reported as 2 min averages.

Tissue oxygen saturation measures

Direct and continuous measurements of regional tissue haemoglobin oxygen saturation were obtained in the experimental and control legs using two near-infrared spectroscopy units with four optodes each (NIRS; INVOS 5100C Cerebral Oximeter; Somanetics Corp, Troy, MI, USA). The optodes were placed on the skin surrounding the quadriceps, hamstrings, calves, and feet of both legs and taped to reduce interference from external light sources.

Statistical analysis

Statistical analysis was conducted using R Studio (version 2022.07.1+554, Team (2022)). A two-way ANOVA was conducted to discover any differences in demographic and anthropometric data between the elderly and young cohorts, as well as identifying any potential anthropometric differences between legs. Moreover, three-way repeated measures ANOVA tests were performed to investigate differences in haemodynamics, flow profiles, tissue oxygenation saturation and temperature

between age cohorts, and between and within the experimental and control legs over time during the passive heating protocol and between workloads during knee-extensor exercise protocols, respectively. The repeated measures ANOVA tests were conducted following the conformation of the data's normality via Shapiro-Wilk test and Mauchly's test of sphericity. In addition, two-way repeated measures ANOVA tests were conducted to investigate differences between age cohorts and over time in systemic variables—that is, heart rate, cardiac output, mean arterial pressure, and core temperature—during the passive heating protocol. Following the two/three-way repeated measures ANOVA tests, once a significant effect was found, a Tukey's post hoc test was conducted to locate the specific time points at which those changes occurred. Results are expressed as mean ± SD. Significance is set at $p < 0.05$. Moreover, linear, exponential, and polynomial regression curve fit tests were performed using GraphPad Prism (version 8, GraphPad Software, La Jolla, California, USA) to assess the relationships among various key data. Subsequently, Akaike's Information Criterion was used to evaluate which model provides the most appropriate fit. Where an exponential curve fit was appropriate, the equation $y = y_0 \cdot e^{K \cdot x}$ was used, where y_0 is the y value (parameter investigated) when x (time) is zero and k is the rate constant. Significance of this fit is reported through 95 % confidence intervals on the estimated value of k with the null value being $k = 0$.

Results

Demographic and anthropometric characteristics

Demographic and anthropometric data for the participants are reported in Table 7. Other than age ($p < 0.0001$), no differences were observed in height, mass, leg volume and lean mass between the two cohorts. Moreover, no anthropometric differences were found between the right and left legs in either group.

Effects of passive single leg heating on thermal, haemodynamic, and tissue oxygen saturation responses in healthy aged and young participants

Regional and core temperatures. Experimental leg T_{sk} during passive heating—measured at the quadriceps, hamstrings, calf, and foot—are illustrated in Figure 35, whilst T_c and $\overline{T}_{Sk\_Leg}$ for both the experimental and control legs are reported in Table

8. As per experimental design, leg T_{sk} progressively increased at all measured sites in the heated leg following 15 mins of heating (all $p < 0.0001$), whereas $\overline{T}_{Sk\_Leg}$ for the control leg remained unchanged ($p = 0.9989$) (Table 8). Following 90 min of passive heating, both cohorts showed similar increases with $\overline{T}_{Sk\_Leg}$ for the experimental leg averaging 9.6 ± 1.3 ºC ($p < 0.0001$) in the aged cohort and 9.4 ± 1.2 ºC ($p < 0.0001$) in the young cohort, respectively (Figure 35). However, T_c remained unchanged throughout the entirety of the heating protocol ($p = 0.9359$) and was not different between cohorts ($p = 0.7400$) (Table 8).

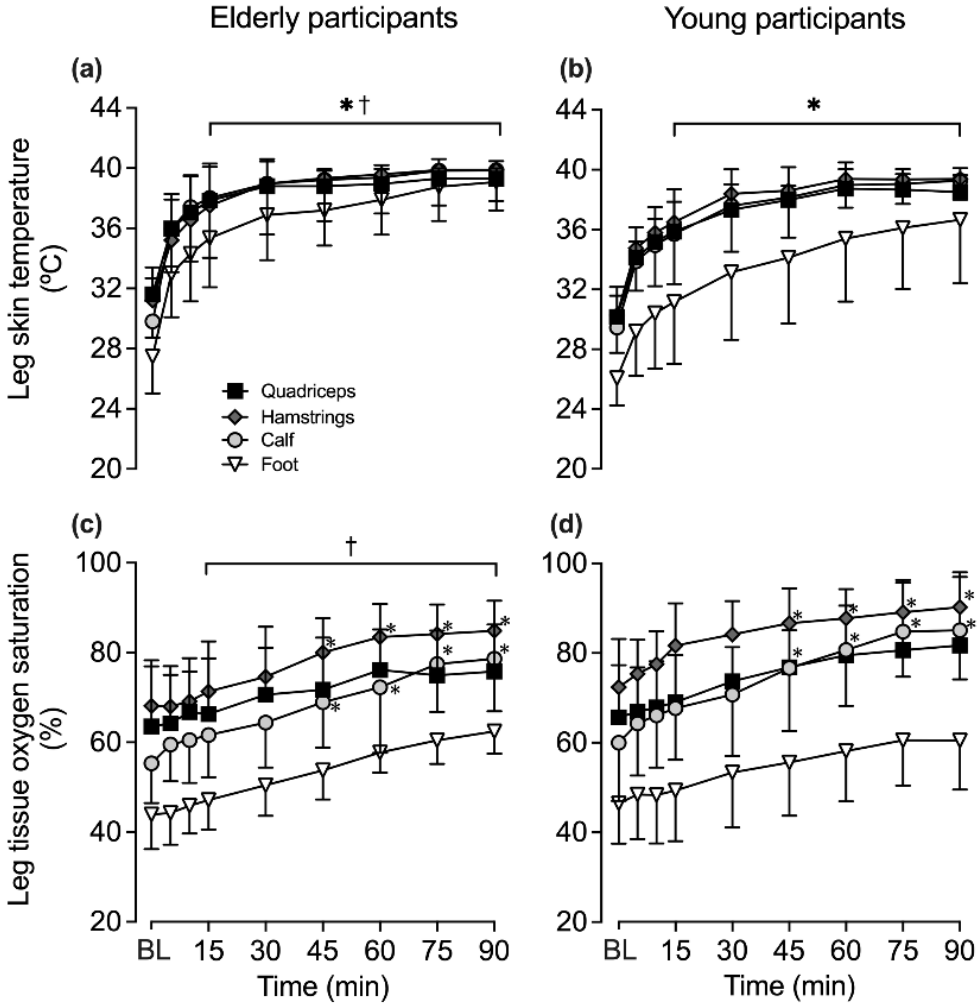

Figure 35. Heated (experimental) leg temperatures (a, b) and regional tissue oxygen saturation (c, d) during whole-leg hyperthermia, in elderly (a, c) and young participants (b, d). Data represented as mean ± SD (elderly: $n = 9$; young: $n = 10$). BL signifies baseline measurements. ∗Different from baseline, $p < 0.05$. † Different from control, young participants, $p < 0.05$.

Table 8. Influence of whole-leg heating and subsequent one-legged knee extensor exercise on body and skin temperatures and central haemodynamics.

				Passive leg heating					Control	
				Rest			One-legged knee extensor exercise		One-legged knee extensor exercise	
				Time (min)			Workload (W)		Workload (W)	
Variables	Group	Intervention	Baseline	30	60	90	6	12	6	12
T_c , ºC	Elderly		36.9 ± 0.4	36.9 ± 0.4	37.0 ± 0.4	37.2 ± 0.4	37.3 ± 0.4†‡	37.3 ± 0.4†‡	37.4 ± 0.4†	37.4 ± 0.4†
	Young		37.1 ± 0.3	36.9 ± 0.2	36.9 ± 0.2	37.0 ± 0.2	37.1 ± 0.2	37.1 ± 0.2	37.2 ± 0.1	37.2 ± 0.1
$\overline{T}_{Sk\_Leg}$, ºC	Elderly	Experimental leg	30.0 ± 1.4†	38.3 ± 2.1*†‡	38.9 ± 1.4*†‡	39.6 ± 1.2*†‡	38.2 ± 1.3‡	37.4 ± 1.0‡	33.9 ± 0.9‡	33.3 ± 0.9‡
	Young		29.1 ± 1.3	36.7 ± 1.5*	38.2 ± 1.3*	38.5 ± 1.1*	37.9 ± 1.4	37.7 ± 1.6	33.7 ± 0.7	33.3 ± 0.7
$\overline{T}_{Sk\_Leg}$, ºC	Elderly	Control leg	29.9 ± 1.5	29.6 ± 1.5	29.3 ± 1.5	29.1 ± 1.5	28.6 ± 1.2	28.6 ± 1.3	28.7 ± 1.3	29.1 ± 1.7
	Young		28.7 ± 0.9	28.2 ± 0.8	28.1 ± 0.8	28.2 ± 0.9	28.1 ± 1.2	28.0 ± 1.3	28.2 ± 1.2	28.4 ± 1.2
MAP, mmHg	Elderly		93 ± 12	91 ± 14	91 ± 8	87 ± 13	115 ± 19*	110 ± 20*	122 ± 22*	121 ± 20*
	Young		98 ± 22	94 ± 12	95 ± 13	92 ± 13	111 ± 24*	112 ± 26*	118 ± 25*	119 ± 24*
$\dot{Q}$, L·min^{-1}	Elderly		4.5 ± 1.1†	4.0 ± 1.2†	4.7 ± 2.1†	4.7 ± 1.9†	6.2 ± 1.6*†	8.0 ± 0.8*†	5.3 ± 1.2*†	6.4 ± 1.7*†
	Young		5.0 ± 1.0	5.7 ± 0.8	5.5 ± 0.7	5.8 ± 0.7	7.9 ± 1.9*	8.9 ± 2.0*	7.4 ± 1.6*	8.1 ± 1.8*
HR, beats·min^{-1}	Elderly		57 ± 10†	58 ± 12†	56 ± 10†	61 ± 12†	81 ± 24*†	79 ± 19*†	80 ± 26*†	80 ± 22*†
	Young		66 ± 9	62 ± 12	64 ± 9	69 ± 9	92 ± 22*	100 ± 24*	89 ± 26*	96 ± 32*

Values are means ± SD for 9 elderly participants and 10 young participants. Experimental leg refers to right, heated leg, whilst the control leg refers to the left, contralateral leg. $\overline{T}_{Sk\_Leg}$: mean leg skin temperature; MAP: mean arterial pressure; $\dot{Q}$: cardiac output; HR: heart rate. Values for rest, represent the responses during 90 min of passive whole-leg heating. Leg skin temperature is presented as an unweighted mean average collected at four different sites: quadriceps, hamstrings, calf, and foot. ∗Different from baseline, $p < 0.05$. † Different from respective young, control group at the same time point, $p < 0.05$. ‡ Different from respective contralateral, control leg, $p < 0.05$.

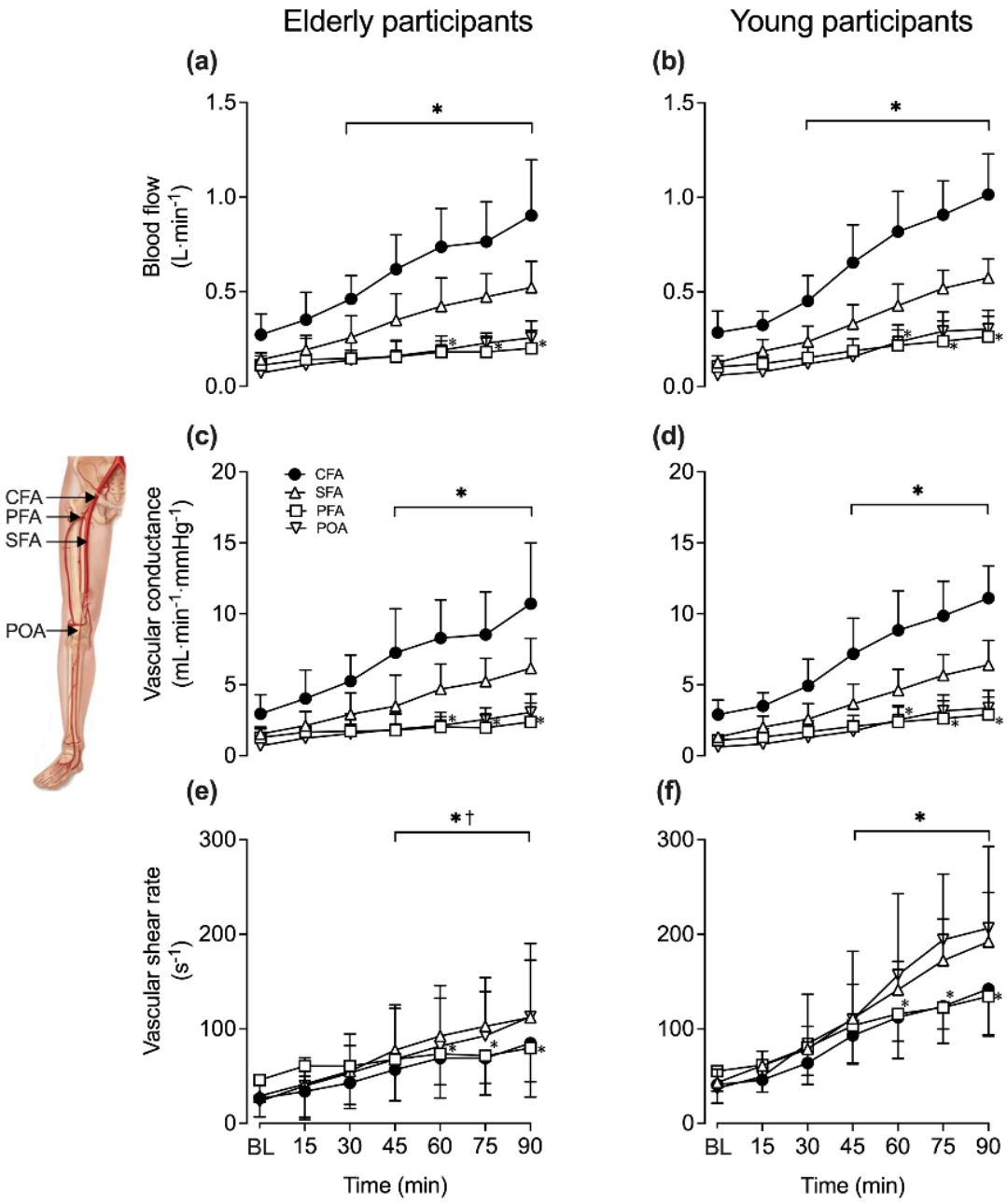

Figure 36. Blood flow (a, b), vascular conductance (c, d), and shear rate (e, f) during whole-leg hyperthermia in the common (CFA), superficial (SFA) and profunda (PFA) femoral arteries and the popliteal (POA) of the heated (experimental) leg, in elderly (a, c, e) and young participants (b, d, f). Data represented as mean ± SD (elderly: *n* = 9; young: *n* = 10). BL signifies baseline measurements. ∗Different from baseline, $p < 0.05$. † Different from control, young participants, $p < 0.05$.

Leg blood flow, tissue oxygen saturation and systemic haemodynamics. Complete haemodynamic responses during 90 min of passive whole-leg heating for the CFA, SFA, PFA and POA of the experimental leg are reported in Figure 36, whilst whole-leg blood flow for the control leg is reported in Table 9. In the heated leg, blood flow in the CFA, SFA and POA increased progressively up to values ≥ 3.5-fold above baseline (Δ = 0.68 ± 0.21, 0.42 ± 0.11, and 0.22 ± 0.10 L·min^{-1}, respectively), whilst PFA blood

flow increased ~2-fold (Δ = 0.11 ± 0.09 L·min^{-1}) following 90 min (all $p < 0.0001$). Elevations in whole-leg arterial blood velocity, blood flow and tissue oxygen saturation were exponentially related to increases in leg skin temperature—for both the aged participants (CFA blood velocity: R^2 = 0.75, k = 0.25 [0.04, 0.71]; CFA blood flow: R^2 = 0.77, k = 0.27 [0.05, 0.70]; tissue oxygen saturation: R^2 = 0.74, k = 0.03 [0.01, 0.05]) and young participants (CFA blood velocity: R^2 = 0.85, k = 0.29 [0.10, 0.64]; CFA blood flow: R^2 = 0.86, k = 0.29 [0.10, 0.62]; tissue oxygen saturation R^2 = 0.88, k = 0.03 [0.02, 0.04]) (Figure 37). No relationships were observed between leg tissue skin temperature and diameter, which remained unchanged in all measured arteries (all $p \geq 0.948$) (Figure 37). Moreover, segmental blood flow (i.e., upper-leg and lower-leg) were exponentially related to increases in local skin temperature in elderly participants (upper leg: R^2 = 0.68, k = 0.46 [0.03, 1.33]; lower leg: R^2 = 0.84, k = 0.27 [0.07, 0.56]) and young participants (upper leg: R^2 = 0.74, k = 0.27 [0.05, 0.85]; lower leg: R^2 = 0.94, k = 0.34 [0.20, 0.53]) (Figure 38). Arterial blood flow increased in parallel to blood velocity— ≥ 3.5-fold in CFA, SFA and POA and ~2-fold in PFA (all $p < 0.0001$). Despite no changes in arterial diameter over time, differences in absolute diameter were observed between the two age cohorts for all arteries, with the elderly cohort showing a 16 ± 6 % larger diameter ($p < 0.0001$)—average obtained by comparing diameters across all arteries at baseline for both legs (Table 9). Correspondingly, the elderly cohort demonstrated a 25 ± 12 % lower blood velocity at baseline conditions averaged across all arteries, with the difference between cohorts further increasing to 51 ± 6 % following 90 min of heating ($p < 0.0001$) (Table 9). Similar responses were observed in vascular conductance and shear rate in all four arteries of the heated leg (Figure 36). Vascular conductance and shear rate increased ≥ 3.4-fold in the CFA, SFA and POA and ≥ 2.1-fold in the PFA (all $p < 0.0001$). No changes in control leg blood flow and vascular conductance were observed in all arteries ($p \geq 0.9999$), nor were there any differences in arterial blood flow ($p \geq 0.0720$) or vascular conductance ($p \geq 0.5030$) between the cohorts. However, the elderly cohort showed a 51 ± 7 % lower shear rate in all arteries during baseline conditions, which was further exacerbated to 73 ± 7 % following 90 min of heating (all $p < 0.0001$), associated with a 16 ± 6 % larger diameter.

Table 9. Influence of whole-leg heating and subsequent one-legged knee extensor exercise on whole-leg skin temperature, tissue oxygen saturation and blood flow.

				Passive leg heating					Control	
				Rest			One-legged knee extensor exercise		One-legged knee extensor exercise	
				Time (min)			Workload (W)		Workload (W)	
Variables	Group	Intervention	Baseline	30	60	90	6	12	6	12
CFA blood flow, L·min^{-1}	Elderly	Experimental leg	0.27 ± 0.10	0.46 ± 0.12*‡	0.74 ± 0.20*‡	0.90 ± 0.30*‡	2.93 ± 0.48*‡	3.31 ± 0.49*‡	—	—
	Young		0.29 ± 0.11	0.45 ± 0.13*	0.82 ± 0.21*	1.02 ± 0.22*	2.42 ± 0.83*‡	2.96 ± 0.70*‡	—	—
	Elderly	Control leg	0.29 ± 0.08	0.26 ± 0.07	0.24 ± 0.08	0.24 ± 0.09	—	—	2.15 ± 0.52*‡	2.34 ± 0.39*‡
	Young		0.29 ± 0.10	0.27 ± 0.09	0.26 ± 0.07	0.27 ± 0.08	—	—	1.87 ± 0.49*	2.24 ± 0.43*
CFA blood velocity, cm·s^{-1}	Elderly	Experimental leg	6.3 ± 3.9†	10.3 ± 5.0†‡	16.5 ± 8.0*†‡	20.3 ± 11.0*†‡	63.8 ± 19.9*†‡	70.1 ± 8.9*†‡	—	—
	Young		8.5 ± 3.6	13.4 ± 4.2	23.7 ± 4.3	29.8 ± 8.3	67.4 ± 26.3*‡	83.3 ± 23.3*‡	—	—
	Elderly	Control leg	6.3 ± 2.2†	5.7 ± 1.8†	5.4 ± 2.3†	5.6 ± 3.3†	—	—	48.5 ± 16.4*†	51.5 ± 9.2*†
	Young		8.3 ± 2.9	7.7 ± 2.3	7.7 ± 2.1	7.8 ± 1.7	—	—	52.3 ± 17.1*	62.1 ± 13.4*
CFA diameter, cm	Elderly	Experimental leg	1.00 ± 0.10†	1.00 ± 0.11†	1.00 ± 0.11†	1.01 ± 0.11†	1.01 ± 0.10†	1.00 ± 0.04†	—	—
	Young		0.85 ± 0.09	0.85 ± 0.09	0.85 ± 0.09	0.86 ± 0.09	0.88 ± 0.08	0.88 ± 0.08	—	—
	Elderly	Control leg	1.00 ± 0.10†	0.99 ± 0.10†	0.99 ± 0.10†	0.99 ± 0.10†	—	—	0.99 ± 0.10†	0.98 ± 0.05†
	Young		0.86 ± 0.10	0.86 ± 0.09	0.86 ± 0.09	0.86 ± 0.10	—	—	0.88 ± 0.08	0.88 ± 0.08
Tissue oxygen saturation, %	Elderly	Experimental leg	56 ± 8†	64 ± 6*†‡	70 ± 9*†‡	73 ± 7*†‡	69 ± 6†‡	68 ± 6†‡	71 ± 9†‡	71 ± 9†‡
	Young		61 ± 9	70 ± 7*	77 ± 8*	79 ± 9*	76 ± 6	76 ± 6	79 ± 8	78 ± 8
	Elderly	Control leg	57 ± 8†	57 ± 7†	55 ± 10†	59 ± 8†	56 ± 9†	56 ± 8†	54 ± 6†	50 ± 13†
	Young		62 ± 8	64 ± 6	65 ± 5	67 ± 6	64 ± 9	64 ± 8	65 ± 7	65 ± 7

Values are means ± SD for 9 elderly participants and 10 young participants. Experimental leg refers to right, heated leg, whilst the control leg refers to the left, contralateral leg. CFA: common femoral artery. Tissue oxygen saturation reflects changes in regional tissue haemoglobin oxygen saturation. Values for rest, represent the responses during 90 min of passive whole-leg heating. ∗Different from baseline, $p < 0.05$. † Different from respective young, control group at the same time point, $p < 0.05$. ‡ Different from respective contralateral, control leg, $p < 0.05$.

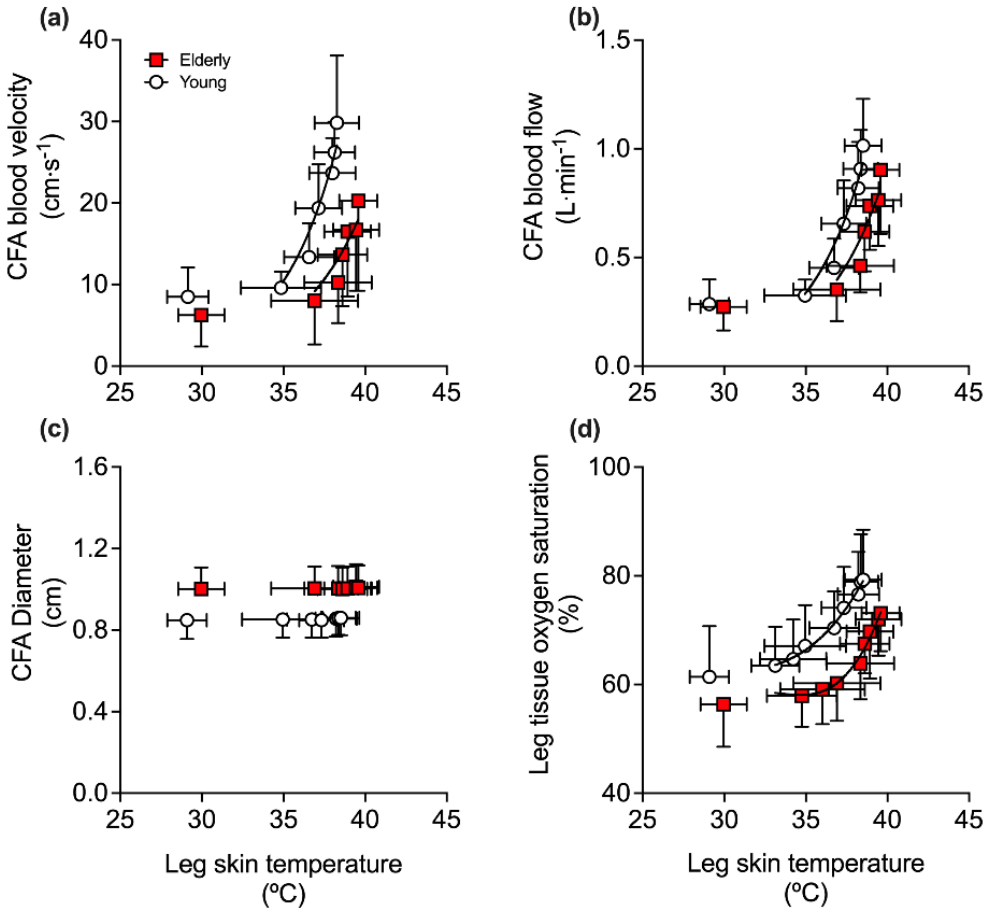

Figure 37. Relationship between the mean leg skin temperature common femoral artery (CFA) blood velocity (a), blood flow (b), diameter (c), and mean leg tissue oxygen saturation (d) during whole-leg hyperthermia in elderly and young participants. Data represented as mean ± SD (elderly: *n* = 9; young: *n* = 10). Vertical error bars signify local blood flow SD, while horizontal error bars signify local temperature SD, respectively. Lines represent the exponential fit of the data.

Tissue haemoglobin oxygen saturation increased gradually at all four sites (quadriceps, hamstrings, calf, and foot) of the heated leg, with mean leg tissue oxygen saturation increasing 17 ± 6 % units ($p < 0.0001$) in the aged cohort and 18 ± 6 % units ($p < 0.0001$) in the young cohort following 90 min of heating (Figure 35; Table 9). No differences in tissue oxygen saturation were observed in the control leg ($p = 0.9531$). However, differences between the age cohorts were observed, with the elderly cohort exhibiting a lower mean leg tissue oxygen saturation in the heated leg (73 ± 7 vs 79 ± 9 %; $p < 0.0001$) and control leg (59 ± 8 vs 67 ± 6 %; $p < 0.0001$). At the systemic haemodynamic level, no changes were observed for heart rate ($p = 0.6465$), cardiac output ($p = 0.9390$), and mean arterial pressure ($p = 0.8920$) during 90 min of heating (Table 8). However, heart rate ($p = 0.0006$) and cardiac output ($p < 0.0001$) were lower in the aged cohort in comparison to their younger counterparts (Table 8).

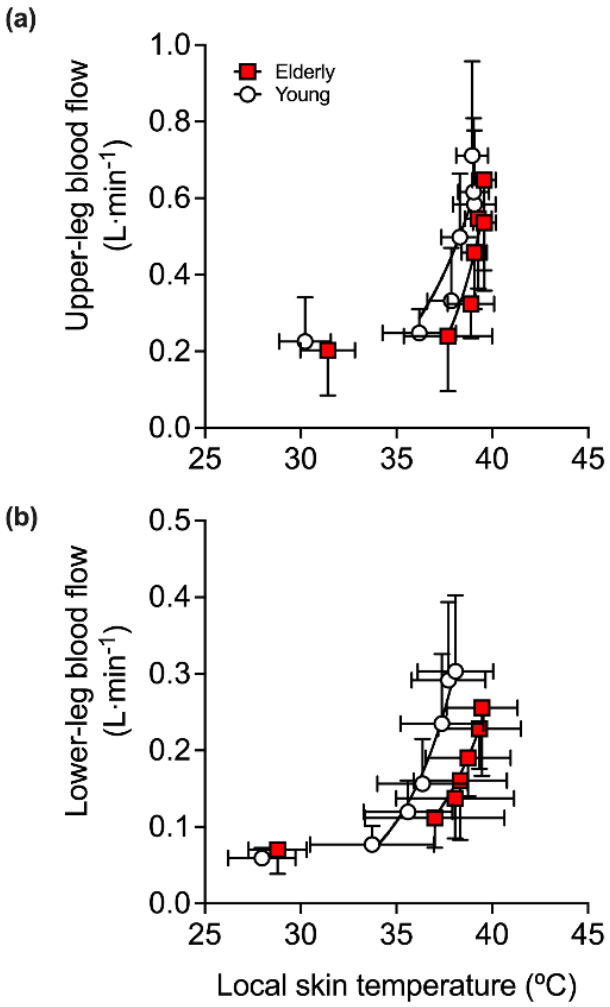

Figure 38. Relationship between the local skin temperature and local blood flow during whole-leg hyperthermia in elderly and young participants. Data represented as mean ± SD (elderly: *n* = 9; young: *n* = 10). The graphs illustrate the relationship between upper-leg blood flow and upper-leg skin temperature (a), and lower-leg blood flow and lower-leg skin temperature (b). Vertical error bars signify local blood flow SD, while horizontal error bars signify local temperature SD, respectively. Lines represent the exponential fit of the data.

Effects of incremental low-intensity knee extensor exercise and single leg heating on thermal, haemodynamic, and tissue oxygen saturation responses in healthy aged and young participants

Regional and core temperatures. As per the experimental design, heated leg T_{sk} was substantially higher at all measured sites in comparison to the control leg ($p < 0.0001$) (Figure 41; Table 9). When comparing the two legs during their respective exercise protocols, $\overline{T}_{Sk\_Leg}$ was 9.3 ± 1.5 ºC higher in the heated leg ($p < 0.0001$). Additionally, no differences were observed in $\overline{T}_{Sk\_Leg}$ between workloads ($p = 0.345$) or age cohorts ($p = 0.164$) (Figure 40; Table 9). Conversely, small but significant differences in T_c

were observed between leg exercise protocols and age cohorts: T_c was 0.1 ± 0.1 ºC lower during the heated leg exercise protocol in comparison to the control leg exercise protocol (p = 0.0441), and 0.2 ± 0.1 ºC higher in the aged cohort (p = 0.0143) (Table 8).

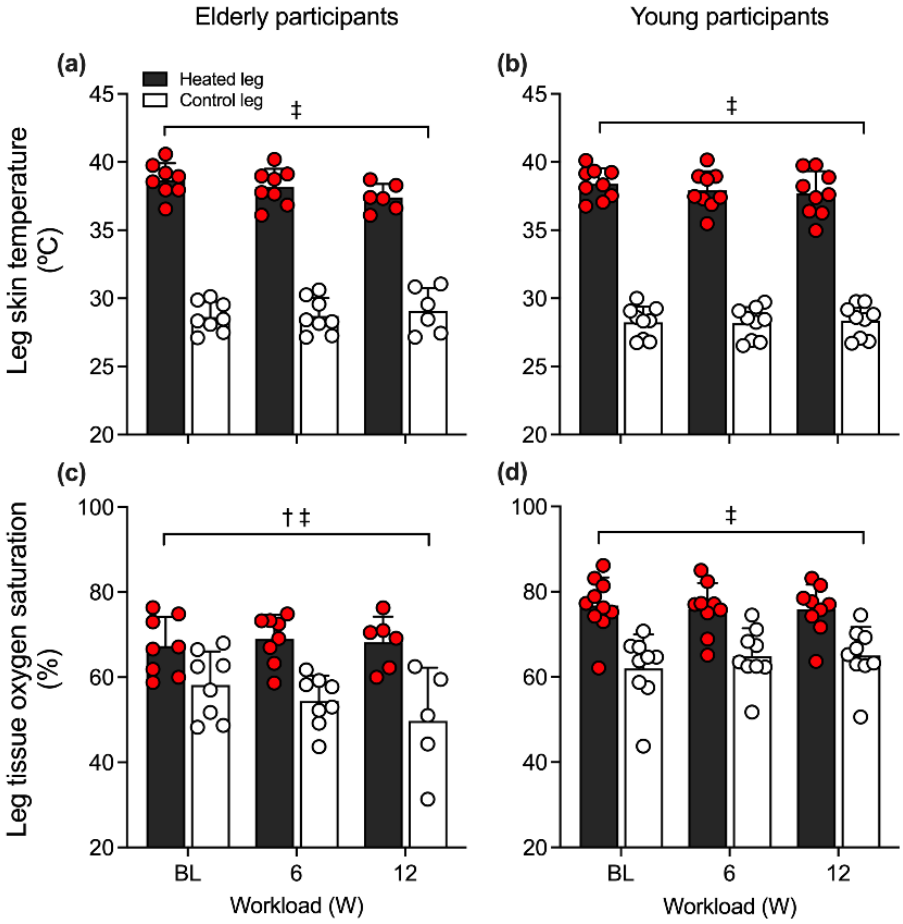

Figure 39. Heated (experimental) leg skin temperature (a, b) and tissue oxygen saturation (c, d) during one-legged knee extensor exercise with heating, in elderly (a, c) and young participants (b, d). Data represented as mean ± SD (elderly: n = 9; young: n = 9). Circles symbolise individual data points. Baseline (BL) measurements signify the measurements taken immediately prior to the start of exercise, following 90 min of whole-leg heating. Leg skin temperature and tissue oxygen saturation are presented as an unweighted mean average collected at four different sites: quadriceps, hamstrings, calf, and foot ∗Different from baseline, p < 0.05. † Different from control, young participants, p < 0.05.

Leg blood flow, tissue oxygen saturation and systemic haemodynamics. Whole-leg (CFA) haemodynamics during exercise are reported in Figure 40, and Table 9, whilst SFA and PFA blood flows are reported in Figure 41. In absolute terms, CFA blood flow in the heated-leg was 0.65 ± 0.56 and 1.04 ± 0.83 L·min^{-1} higher at 6 and 12 W, respectively, in comparison to the control exercise condition (Figure 40). Similar responses were observed in the SFA and PFA, with heated exercise inducing 0.36 ± 0.18 and 0.36 ± 0.30 L·min^{-1} higher blood flows than the control exercise for the SFA

at 6 W and 12 W, respectively and 0.28 ± 0.49 and 0.36 ± 0.53 $L \cdot min^{-1}$ for the PFA at 6 W and 12 W, respectively (all $p < 0.0001$) (Figure 41). However, no differences were observed between age cohorts ($p \geq 0.1131$). As reported earlier, arterial diameter did not change during the protocol ($p \geq 0.700$), although the average diameter was larger in the elderly cohort ($p < 0.0001$). Whole-leg vascular conductance was 7 ± 5 And 8 ± 7 $mL \cdot min^{-1} \cdot mmHg^{-1}$ higher in the heated leg compared to the control leg during 6 W and 12 W of exercise, respectively (Figure 40). No differences were observed for vascular conductance between the age cohorts ($p = 0.1540$). Likewise, CFA shear rate was 61 ± 65 and 73 ± 60 s^{-1} higher in the heated leg during 6 and 12 W of exercise, respectively (Figure 40). Lastly, differences were observed between legs ($p < 0.0001$) and between cohorts ($p = 0.0011$), with the elderly cohort reporting a reduced shear rate across both exercise workloads and legs (Figure 40).

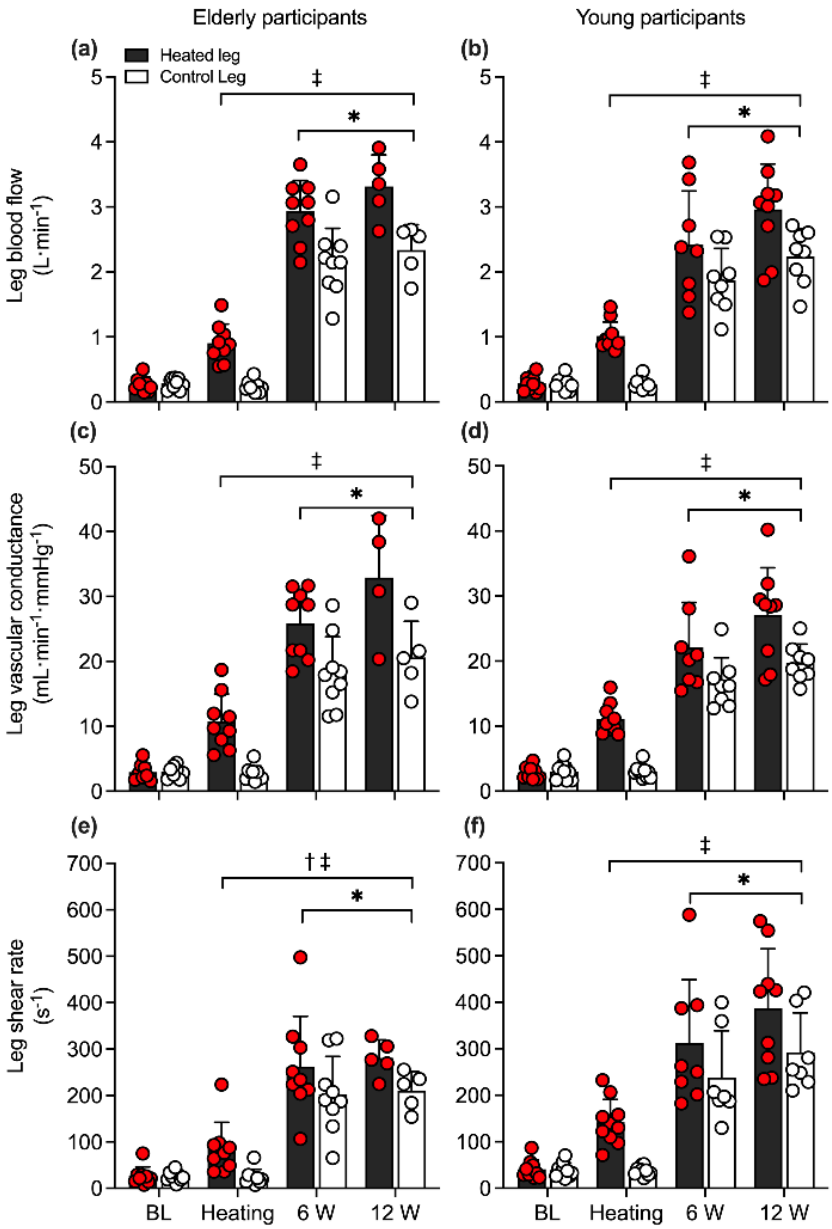

Figure 40. Blood flow (a, b), vascular conductance (c, d), and shear rate (e, f) during one-legged knee extensor exercise with and without heating, in the common femoral artery of the heated (experimental) leg and control (contralateral) leg, in elderly (a, c, e) and young (b, d, f) participants. Data represented as mean ± SD (elderly: *n* = 9; young: *n* = 9). Circles symbolise individual data points. ∗Different from baseline (BL) prior to the commencement of exercise (measurements taken following 90 min of whole-leg heating), $p < 0.05$. † Different from respective young, control group at the same time point, $p < 0.05$. ‡ Different from respective contralateral, control leg, $p < 0.05$.

No differences were observed in mean leg tissue oxygen saturation during exercise, across both workloads ($p = 0.9880$). However, differences were observed between legs and between age cohorts, with the heated leg showing a higher mean leg tissue oxygen saturation across both workloads (73 ± 7 vs. 51 ± 8 %, respectively, $p < 0.0001$) and the elderly cohort reporting a lower mean leg tissue oxygen saturation during heated-leg exercise (69 ± 6 % vs. 76 ± 6 % respectively, $p < 0.0001$) (Figure

40; Table 9). At the systemic haemodynamic level, no differences in arterial pressure or cardiac output were observed between the heated- and control-leg exercise protocols (all $p \geq 0.2160$) (Table 8). However, differences were observed between age cohorts for heart rate ($p = 0.0122$) and cardiac output ($p < 0.0001$), with the elderly cohort reporting lower values during the exercise protocols (Table 8).

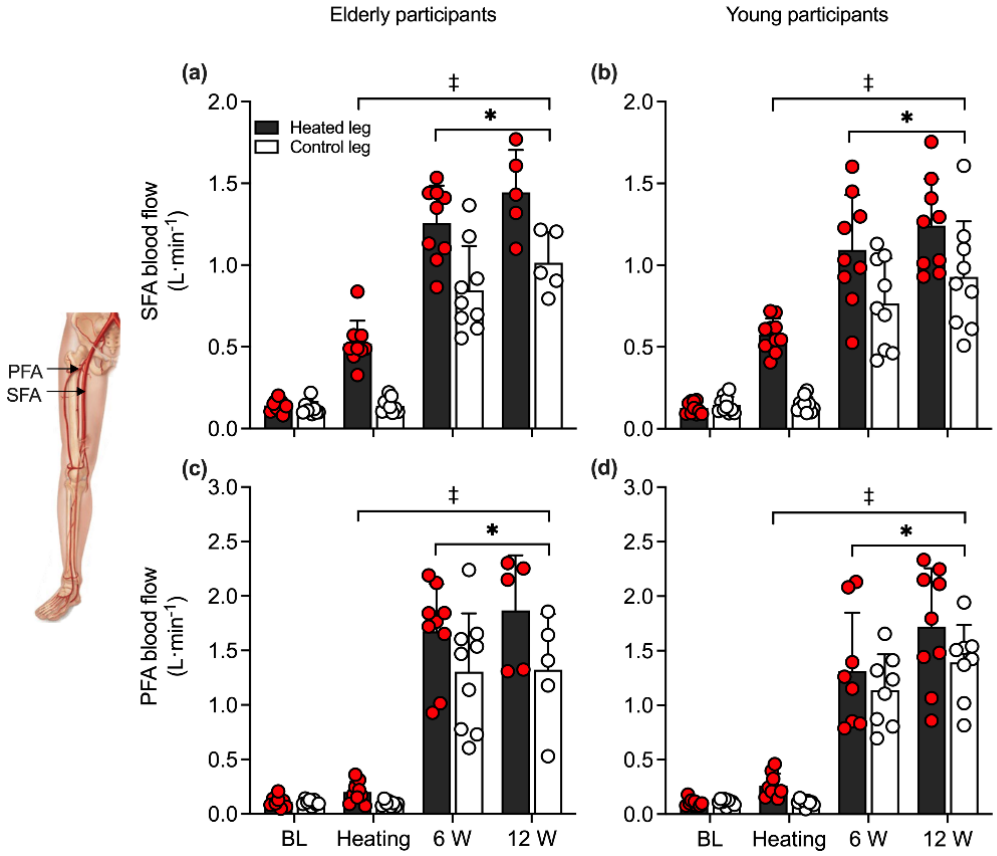

Figure 41. Blood flow during one-legged knee extensor exercise with and without heating, superficial (SFA) (a, b) and profunda (PFA) (c, d) femoral arteries of the heated (experimental) leg and control (contralateral) leg, in elderly (a, c) and young participants (b, d). Data represented as mean ± SD (elderly: $n = 9$; young: $n = 9$). Circles symbolise individual data points. Note: during exercise, PFA blood flow was calculated indirectly as the difference between CFA and SFA blood flow. ∗Different from baseline (BL) prior to the commencement of exercise (measurements taken following 90 min of whole-leg heating), $p < 0.05$. † Different from respective young, control group at the same time point, $p < 0.05$. ‡ Different from respective contralateral, control leg, $p < 0.05$.

Discussion

The present study sought to characterise the haemodynamic profiles of the major leg arteries in response to single-leg hyperthermia, single-leg knee-extensor exercise, and their combination in healthy, active elderly and young participants and in doing so, gain insight into the impact of local temperature and age on the control of limb

perfusion. In line with our primary hypothesis, blood flow was tightly coupled to the rise in local temperature and metabolic demand, with the combination of local limb hyperthermia and exercise producing a higher level of hyperaemia than exercise alone. However, contrary to our secondary hypothesis, no differences in the hyperaemic response to local limb hyperthermia, normal exercise or hyperthermic exercise were observed between the elderly and young cohort. Importantly, the equal hyperaemia happened despite the elderly group exhibiting a lower blood velocity during all three interventions. Collectively, the present findings suggest that age does indeed induce structural and functional vascular adaptations—as shown by the age-related differences in arterial diameter, blood velocity, and shear stress—but it does not alter the magnitude of hyperaemia during local limb hyperthermia, small muscle mass exercise or combined limb hyperthermia and exercise.

Effect of hyperthermia on the hyperaemic response to low-intensity knee-extensor exercise in old and young humans

An important finding of this study is that local hyperthermia augmented macro- and microcirculatory blood flow similarly at rest and during single-leg exercise, irrespective of age. Ninety minutes of passive single-leg heating increased $\overline{T}_{Sk\_Leg}$ by 9.5 ºC, inducing an average 0.6–0.7 L·min^{-1} elevation in whole-leg blood flow in the elderly and young cohorts. The relationships between the rise in local temperature (>35 °C skin temperature) and tissue perfusion for both the upper and the lower leg in both groups are consistent with previous findings from our laboratory during isolated leg heating in young participants (Figure 38) (Pearson *et al.*, 2011; Chiesa *et al.*, 2016; Koch Esteves *et al.*, 2021). Similarly, the addition of single-leg hyperthermia during knee-extensor exercise in both cohorts produced a 0.7 L·min^{-1} larger leg hyperaemia at 6 W and 1.0 L·min^{-1} at 12 W, in comparison to exercise alone. Functional hyperaemia during control-leg exercise was also independent of age, as whole-leg blood flow was comparable between the elderly and young cohorts—2.2 vs. 1.9 L·min^{-1} and 2.3 vs. 2.2 L·min^{-1} at 6 W and 12 W, respectively (Figure 40). The magnitude of the local hyperthermia-induced hyperaemia was therefore remarkedly similar at rest and during single leg knee-extensor exercise in both groups.

An age-old question is whether heat stress provides an additive stimulus for functional hyperaemia, with the premise that tissue temperature and perfusion are elevated

compared to control conditions. Evidence to date is equivocal, however, with several past studies reporting no differences in leg blood flow during two-legged cycling exercise (Savard *et al.*, 1988; Trangmar *et al.*, 2017) and single-leg knee extensor exercise (Savard *et al.*, 1988; Ferguson *et al.*, 2006), whilst others reported an increased flow of 0.6–0.7 $L \cdot min^{-1}$ between hyperthermic and control conditions (Pearson *et al.*, 2011; Chiesa *et al.*, 2015). Exercise duration, intensity, and mass of active muscle all influence the balance between heat production and heat transfer, which ultimately affect local muscle and blood temperatures (Saltin & Hermansen, 1966; Saltin *et al.*, 1968; González-Alonso *et al.*, 2000; Gonzalez-Alonso *et al.*, 2015). Since local tissue perfusion is strongly related to local skin and muscle temperature (Koch Esteves *et al.*, 2021), it is plausible that some of the aforementioned studies did not find an effect of environmental heat stress or external heating on functional hyperaemia because these interventions did not sufficiently increase deep tissue temperature above the control exercise condition (Savard *et al.*, 1988; Ferguson *et al.*, 2006; Trangmar *et al.*, 2017). In this light, it is important to consider that whole-leg exercise—e.g., cycling—increases tissue and blood temperatures in the whole leg whereas knee-extensor exercise solely increases temperature in the quadriceps muscle (González-Alonso *et al.*, 2000). Localised thigh heating, on the other hand, increases only temperature and perfusion in the upper-leg segment (Koch Esteves et al. 2021). Thus, the larger differences in tissue temperature across the whole leg between the present heated and control exercise protocols likely rationalise the additive effect of leg hyperthermia on functional hyperaemia.

While the influence of combined local-leg heating and exercise on whole-leg hyperaemia was consistent in both groups, the precise distribution of the additional exertional hyperaemia remains uncertain. Assuming that lower-leg perfusion—supplied by the popliteal artery—remained stable during knee-extensor exercise, which following passive leg heating increased by ~0.2 $L \cdot min^{-1}$ (Figure 36), it is estimated that upper-leg hyperaemia was 0.5–0.8 $L \cdot min^{-1}$ higher during combined leg hyperthermia and exercise at the two workloads examined, in comparison to control exercise. Additionally, if the hyperthermia-induced hyperaemia is equally distributed among the thigh tissues, we estimated that 0.2–0.4 $L \cdot min^{-1}$ would be perfusing the anterior thigh tissues, with a significant portion added to the normal exercise hyperaemia perfusing the active quadriceps muscle, which would amount to a 9–18

% elevation in exertional hyperaemia. Evidence in the literature further supports this argument. On one hand, muscle perfusion and tissue oxygen saturation increase and leg arteriovenous oxygen difference declines in response to passive local hyperthermia (Keller *et al.*, 2010; Heinonen *et al.*, 2011; Pearson *et al.*, 2011; Chiesa *et al.*, 2015). Moreover, leg (quadriceps) $\dot{V}O_2$ is maintained during knee-extensor exercise under hyperthermic conditions in association with compensatory reductions in arteriovenous oxygen differences (Pearson *et al.*, 2011; Chiesa *et al.*, 2015). The higher tissue oxygen saturation seen in this study during leg heating and knee-extensor exercise suggests that a higher muscle hyperaemia and corresponding increased oxygen delivery were met by a lower oxygen extraction from the circulation in both the elderly and young cohorts (Figure 38). Together, these findings reveal that hyperthermia induces increases in muscle perfusion during knee-extensor exercise despite an unchanged metabolic demand. Furthermore, these observations lend support to the notion that rises in local temperature are a putative stimulus for augmenting muscle tissue perfusion during low-intensity small muscle mass exercise in both young and elderly individuals.

Effect of age on the thermal mechanisms enhancing skeletal muscle perfusion during low-intensity knee-extensor exercise

The literature surrounding the effect of age on functional hyperaemia in response to hyperthermia and exercise is riddled with inconsistencies, yet the prevailing view is that age attenuates functional hyperaemia. According to Darcy's law of flow—the hydraulic equivalent of Ohm's law—skeletal muscle perfusion is determined by vascular conductance and perfusion pressure gradient. Using mean arterial pressure as a surrogate, no differences in the perfusion pressure gradient were observed during single-leg heating and between the elderly and young cohorts (Table 8). Hence, hyperperfusion during single-leg heating was solely associated with increases in vascular conductance (Figure 36). A blunted vascular conductance is commonly reported in elderly individuals during hyperthermia and exercise and postulated as a primary mediator of the age-associated attenuation in tissue perfusion (Martin *et al.*, 1995; Kenney, 2001; Hearon & Dinenno, 2016). Reduced bioavailability of endothelium-derived vasoactive substances (i.e., nitric oxide) and compromised functional sympatholysis—i.e., a reduced ability to counteract vasoconstriction and

maintain adequate blood flow in the presence of elevated sympathetic nerve activity—have all been implicated in this phenomenon (Hearon & Dinenno, 2016). From a global haemodynamic perspective, an age-related attenuation in hyperaemia during local lower-leg hyperthermia (Romero *et al.*, 2017) and knee-extensor exercise (Lawrenson *et al.*, 2003; Donato *et al.*, 2006; Mortensen *et al.*, 2012c) has been associated with a lower vascular conductance in elderly sedentary and normally active subjects, which in some cases is largely the result of an elevated mean arterial pressure (Lawrenson *et al.*, 2003; Donato *et al.*, 2006; Mortensen *et al.*, 2012c). In contrast, the present elderly cohort demonstrated a comparable vascular conductance, mean arterial pressure and blood flow to their younger counterparts (Figure 40) in line with recent studies during local two-leg hyperthermia (Engelland *et al.*, 2020), and submaximal knee-extensor exercise in endurance-trained age cohorts (Mortensen *et al.*, 2012c). The discrepancies among studies may be explained, at least in part, by regular participation in physical activity which has been shown to preserve arterial responsiveness to vasodilator infusion and functional sympatholysis with age (Mortensen *et al.*, 2012c; Kruse *et al.*, 2018; Piil *et al.*, 2018). Alternatively, functional sympatholysis may not impose a limitation on the hyperaemic response in the present study because—unlike two-leg heating (Engelland *et al.*, 2020), whole-body hyperthermia (Crandall *et al.*, 1999; Cui *et al.*, 2004) and two-legged cycling (Katayama & Saito, 2019)—single-leg heating and moderate-intensity dynamic knee-extensor exercise (30 W) consistently or transiently reduce muscle sympathetic nerve activity compared to baseline values (Takahashi *et al.*, 2011; Katayama & Saito, 2019). Moreover, circulating noradrenaline levels remain low (~1–2 $nmol \cdot L^{-1}$) when core temperature is unchanged during heating and moderate intensity single-leg knee-extensor exercise (Powers *et al.*, 1982; Pearson *et al.*, 2011; Chiesa *et al.*, 2019). It, therefore, appears that lifelong regular engagement in physical activity and the low vasoconstrictor activity during single-leg hyperthermia and exercise contribute to the preservation of functional hyperaemia during local limb heating and small muscle mass exercise.

Increases in leg vascular conductance were approximately 50 % higher during heated-leg exercise than control-leg exercise; however, no changes in conduit arterial diameter were observed (Figure 40). According to the Hagen-Poiseuille's law, vascular conductance (the inverse of resistance) is predominantly determined by

vessel diameter and blood viscosity. It is possible that vasodilation occurred downstream in the small arteries and resistance arterioles (i.e., microcirculation), thereby contributing to the increases in blood velocity and flow in both age groups (Koch Esteves *et al.*, 2021). During passive hyperthermia in young adults, a myriad of potential thermosensitive mechanisms has been shown to positively impact blood velocity and thus, blood flow. Thermal stimuli could activate intravascular signalling-transduction mechanisms (Kellogg *et al.*, 1999; Minson *et al.*, 2001; Paniagua *et al.*, 2001; Laughlin *et al.*, 2008; Gifford *et al.*, 2014) and/or stimulate the release of vasoactive molecules from erythrocytes such as ATP (Pearson *et al.*, 2011; Kalsi & Gonzalez-Alonso, 2012; Kalsi *et al.*, 2017), which in turn induce vasodilatation of the resistance vessels. Additionally, reductions in blood viscosity and frictional resistance (Snyder, 1971; Cinar *et al.*, 2001; Shin *et al.*, 2004; Lim *et al.*, 2010) in conjunction with increases in red blood cell deformability and dispersion (Manteuffel-Szoege, 1960; Manteuffel-Szoege, 1969; Cinar *et al.*, 2001; Pinho *et al.*, 2016) may also play a role in the observed hyperthermia-induced hyperaemia. However, ageing has been found to negatively impact these thermosensitive and rheological mechanisms, evoking increases in blood viscosity (Carallo *et al.*, 2011; Simmonds *et al.*, 2013), reductions in red blood cell deformability (Simmonds *et al.*, 2013), and an impaired response to various vasodilators (Holowatz & Kenney, 2010; Mortensen *et al.*, 2012c; Wray & Richardson, 2015; Hearon & Dinenno, 2016). Regular engagement in physical activity has been shown to preserve these mechanisms (Ernst, 1987; Mortensen *et al.*, 2012c; Simmonds *et al.*, 2013; Groot *et al.*, 2016) and thus, may explain why the present elderly and young cohorts showed comparable magnitudes of hyperaemia. Nonetheless, if these thermosensitive vascular mechanisms and blood rheological properties do indeed worsen with age, they could at least in part elucidate why the present elderly cohort exhibited a lower blood velocity following heating, knee-extensor exercise, and its combination.

The present elderly cohort also displayed a larger diameter across all measured conduit arteries which was sufficient to compensate for the lower blood velocity (Figure 37). An age-related increase in arterial diameter is well established in the literature (Kawasaki *et al.*, 1987; Sandgren *et al.*, 1999; Hirata *et al.*, 2006; Gonzales *et al.*, 2009), with the aortic diameter increasing by as much as 24 % between the ages of 25 and 70 years (Sonesson *et al.*, 1993). These increases are generally associated

with an increase in arterial stiffness (Kawasaki *et al.*, 1987; Sonesson *et al.*, 1993) and therefore, indicative of an impaired endothelial-dependent vasodilator function (Anderson, 2006). However, Hickson *et al.* (2010) postulated that an increase in (aorta) diameter without a proportional increase in arterial wall thickness may be an adaptation to offset the age-related increase in arterial stiffness. In this construct, one could speculate that the increased femoral artery diameter in the elderly cohort is an adaptive response to maintian flow rate as blood velocity is attenuated, a hypothesis that warrants further investigation.

Experimental considerations

There are several methodological considerations in this study. *First*, the present experimental design included two fixed absolute exercise workloads—6 W and 12 W—for all participants. Absolute, low-intensity workloads were selected as the approach allowed for the direct comparison of hyperaemia between the control and the hyperthermic conditions, and between old and young cohorts. Whilst utilising relative workloads would have merit, it is important to acknowledge that the overtime power output variability per kick is large during single leg knee-extensor exercise (González-Alonso *et al.*, 2000). Thus, using relative workloads—especially if differences in workloads were less than 6 W—would have introduced additionally variability between protocols and cohorts. Although comparing absolute workloads can pose some limitations (Donato *et al.*, 2006), the present observation that exercise hyperaemia was not different in the elderly and young cohorts suggests that a potentially higher relative intensity in the elderly did not affect the outcomes of the study. *Secondly*, arterial leg blood velocity, flow and tissue oxygen saturation did not initially increase as rapidly, and to the same magnitude, as local skin temperature. Figure 37 and Figure 38 clearly show that whole-leg hyperaemia lagged in comparison to the rapid increase in mean leg skin temperature. This initial uncoupling of temperature and haemodynamics was likely due to internal tissue temperature not being measured; thus, it could not be included in the calculation of mean leg temperature and help establish the impact of whole-leg tissue hyperthermia on limb haemodynamics. When using hot water-perfused garments, as in this study, deep tissue temperature takes longer to increase because conductive heat transfer relies on a positive temperature gradient between the heated skin to the cooler deep tissues.

This likely explains why the present correlations were not as strong as those reported in our previous study (*Chapter 4*), which had estimates of regional leg hyperthermia (Koch Esteves *et al.*, 2021). Notwithstanding this limitation, our data lend further support to the notion that internal temperature and concomitant increases in deep tissue blood flow contribute to the observed hyperthermia-induced limb hyperaemia (Heinonen *et al.*, 2011; Pearson *et al.*, 2011; Chiesa *et al.*, 2016; Koch Esteves *et al.*, 2021). *Thirdly*, participants were considered *trained* due to their regular participation in structured exercise which was assessed via a self-completed questionnaire as opposed to an objective quantification of exercise capacity (e.g., a maximal oxygen consumption test or accelerometery data). Although this modality of training status assessment is liable to self-desirability bias (Nederhof, 1985), we are confident that our participants are indeed trained—particularly our elderly cohort—as their leg muscle mass was comparable to the young cohort. This differs from non-trained elderly individuals where leg muscle mass is decreased by ~16% when compared to young 25 year old adults (Naruse *et al.*, 2023). Consequently, the present elderly cohort were classified as *trained* as they did not present characteristics of normal age-related sarcopenia. Whilst the present study attempted to isolate the effect of age on the haemodynamic responses to single-limb hyperthermia and exercise, rather than the commonly associated age-related reductions in physical activity, the present findings should not be extrapolated to aged sedentary individuals who will exhibit a higher level of sarcopenia. *Lastly*, although lower limb hyperthermia increased blood flow in all major vessels in both the elderly and young groups, the magnitude of increase in blood flow was greater in SFA than PFA. This could be due to the thermal intervention inducing a smaller increase in deep tissue temperature, which is typically warmer than the superficial tissues (Savard *et al.*, 1988); thus, producing a weaker thermal stimulus for vasodilation and/ or hemorheological changes. Moreover, measurements of PFA blood flow are influenced by the individual vessel anatomy (Tomaszewski *et al.*, 2017), making it more difficult to obtain high quality images compared to the other main leg conduit arteries (Hussain, 1997; Koch Esteves & Chiesa, 2021). Based on PFA blood flow estimates from the measurements of CFA and SFA blood flow, it appears that the magnitude of increase in PFA blood flow was underestimated. Therefore, this limitation does not have a bearing on the conclusions of the study.

Summary

Passive lower limb heating increased leg blood flow and vascular conductance over three-fold—alongside increases in regional tissue haemoglobin oxygen saturation—which occurred in parallel to increases in local temperature. Moreover, passive leg heating had an additive effect on blood flow during knee-extensor exercise with the combination of hyperthermia and exercise exhibiting the largest magnitude of functional hyperaemia. Notably, no differences in the functional hyperaemic responses were observed between the healthy, active elderly and the young cohorts despite the larger femoral artery diameter, and lower central haemodynamics and blood velocity in the elderly. These findings reject the idea that age *per se* compromises local hyperthermia-induced limb hyperperfusion or small muscle group functional hyperaemia, notwithstanding structural age-related differences in the vasculature. Further research is warranted to investigate the hyperaemic responses to local limb heating and larger muscle mass exercise—such as walking—in elderly active and sedentary participants to establish the safety and effectiveness of combined local heating and exercise in enhancing circulatory function and vascular health.

CHAPTER 7: General Discussion and Conclusions

Introduction

The present thesis aimed to (a) comprehensively characterise the mechanisms that govern hyperaemia during various areas of local leg hyperthermia—ranging from partial-quadriceps heating to whole-leg heating—in young adults, and (b) explore the effect of age on the hyperaemic response to whole-leg heating, small muscle mass exercise and their combination between healthy, active elderly and young adults. *Chapters 3* and *4* explored the blood flow responses in the major arteries of the leg during whole-leg, upper-leg, lower-leg, quadriceps, and partial-quadriceps heating—reporting strong positive relationships between local tissue temperature and tissue perfusion, with the magnitude of hyperaemia dependent on the temperature and area of heated tissues. *Chapter 6* studied the haemodynamic responses to whole-leg heating, small muscle mass exercise and their combination in healthy, trained elderly individuals, comparing their responses to the young adult control group. This study demonstrated that age *per se* does not attenuate the functional hyperaemic response to local limb hyperthermia, small muscle mass exercise and their combination, and that local limb hyperthermia had an additive effect on functional hyperaemia during small muscle mass exercise. Collectively, these findings add further evidence to the growing body of literature in favour of utilising exercise and heat to improve and maintain vascular health in elderly adults.

The present chapter will syndicate the main conclusions from the experimental chapters to provide an overview of the novel findings; discussing their impact on the current literature, avenues for future research, and any limitations which should be considered.

Main findings

Local haemodynamic responses during passive whole-leg and segmental-leg hyperthermia

It is well established that hyperthermia instigates increases in tissue perfusion in attempt to dissipate heat and thus, minimise thermal homeostatic disturbances (Crandall & Wilson, 2015). Current literature demonstrates a clear effect of whole-leg and leg-segment heating on local and systemic haemodynamics (Heinonen *et al.*,

2011; Pearson *et al.*, 2011; Chiesa *et al.*, 2016; Kuhlenhoelter *et al.*, 2016; Romero *et al.*, 2017; Thomas *et al.*, 2017; Walsh *et al.*, 2019). However, direct, comprehensive assessments of blood flow at the major conduit arteries supplying the heated area were lacking; thus, the extent to which local limb hyperthermia increased tissue perfusion within a limb was obscure. The present thesis provides direct and indirect measures of local tissue perfusion during five leg heating protocols: whole-leg, upper-leg, lower-leg, quadriceps, and partial-quadriceps heating. All protocols increased tissue temperature to a similar degree for their respective heated regions. Consequently, they produced increases in whole-leg blood flow, ranging from as low as 1.4-fold during quadriceps and partial-quadriceps heating to as high as 3.5-fold during whole-leg heating. Classic studies argue that this increase in tissue perfusion is predominately directed towards the skin for heat dissipation purposes (Roddie *et al.*, 1956; Edholm *et al.*, 1957; Blair *et al.*, 1960; Detry *et al.*, 1972; Johnson & Proppe, 1996). However, the presently observed ~2-fold increases in profunda femoral artery blood (which supplies the deep tissues of the upper-leg) and increases in tissue oxygen saturation (an index of microcirculatory tissue perfusion) during whole-leg and upper-leg heating, indicate that at least some portion of the observed hyperaemia perfuses the muscle. As such, the present findings align with contemporary research challenging this classic viewpoint, and provide further evidence that muscle blood flow is augmented during hyperthermia (Heinonen *et al.*, 2011; Pearson *et al.*, 2011; Chiesa *et al.*, 2015; Chiesa *et al.*, 2016; Kalsi *et al.*, 2017).

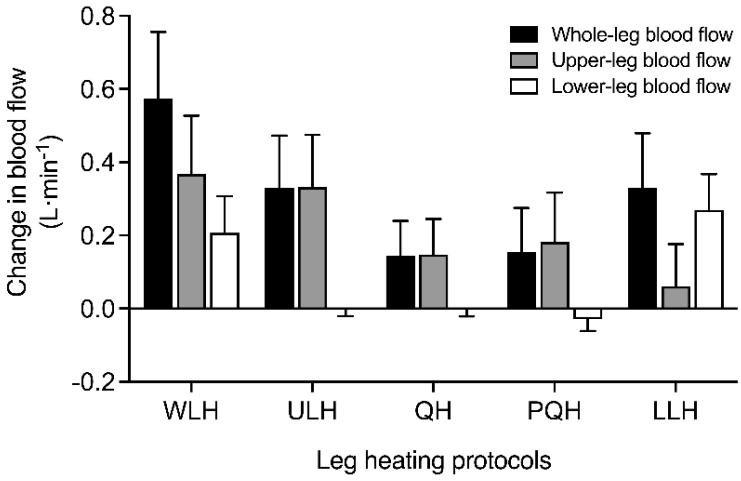

Figure 42. Changes in regional blood flow during 1 h whole leg and segmental leg heating. Data represented as mean ± SD. Whole-leg heating (WLH) data are collated from *Chapters 3 and 5* from young adults (*n* = 19), upper-leg heating data (*n* = 19) are collated from *Chapters 3 and 4*, quadriceps

heating (QH) and partial-quadriceps heating (PQH) data were obtained from *Chapter 5* (n = 11), whilst lower-leg heating (LLH) data were obtained from *Chapter 4* (n = 8). Whole-leg and lower-leg blood flow are reported from direct measures of common femoral and popliteal blood flow, respectfully, whilst upper-leg blood flow is calculated as the difference between the two. A MANOVA test, incorporating a Bonferroni's post hoc test, was conducted using SPSS (Version 26; IBM) to compare the difference in blood flow between the three heating protocols—results are presented in text.

Figure 42 illustrates the change in whole-leg, upper-leg and lower-leg blood flow following 1 h of whole-leg, upper-leg, lower-leg, quadriceps, and partial-quadriceps heating. As expected, whole-leg heating stimulated the largest magnitude of whole-leg hyperaemia (p < 0.001 for all comparisons), amounting to a 3-fold increase in whole-leg blood flow, whilst both upper-leg and lower-leg heating protocols produced a similar magnitude of hyperaemia (p = 1.000). Interestingly, upper-leg hyperaemia during upper-leg heating was comparable to upper-leg blood flow during whole-leg heating (p = 1.000) and similarly, lower-leg hyperaemia during lower-leg heating was comparable to lower-leg blood flow during whole-leg heating (p = 0.248) (Figure 42). These increases in segmental leg blood flow during segmental-leg heating occurred without affecting blood flow in the adjacent, non-heated leg segment. Furthermore, quadriceps heating—which involved heating the anterior half of the upper-leg—produced half of the upper-leg hyperaemia observed during upper-leg heating (0.15 vs 0.33 $L \cdot min^{-1}$, p = 0.008, respectively). Collectively, these findings demonstrate that heating a leg segment in isolation produces a comparable magnitude of hyperaemia for that same segment during whole-leg heating, without altering temperature and blood flow in the adjacent or proximal unheated regions.

It should be noted that the hyperaemia observed during partial-quadriceps heating does not conform to this relationship as it was of similar magnitude as that during upper-leg hyperaemia (p = 1.000). This is likely explained by the modality in which partial-quadriceps heating was conducted. Despite only heating 16 % of the quadriceps' total surface area, partial-quadriceps heating utilised diathermy which can heat the various tissue layers uniformly through radiation, as opposed to the conductive methods used in the other protocols which is reliant on a positive temperature gradient. Therefore, it is possible that while the surface area heated during partial-quadriceps heating was > 6 times smaller than that during quadriceps heating, the actual volume of heated tissue may have been similar because it reached the deeper tissues. Additionally, partial-quadriceps heating increased muscle temperature from 34 °C to 37 °C. Although a similar peak quadriceps muscle

temperature of ~37 ºC was observed in the whole-thigh and quadriceps heating protocols (*Chapter 5*), the magnitude of increase was different with whole-thigh, quadriceps and partial-quadriceps heating increasing quadriceps muscle temperature by 1.8 ºC, 1.5 ºC and 2.7 ºC, respectively. The thermal stimulus was therefore potentially greater during partial-quadriceps heating, which could also explain the significant hyperaemia despite the smaller heated area. Nonetheless, the present results provide strong evidence for the role of local thermosensitive mechanisms as the primary regulators of limb tissue perfusion during local hyperthermia.

Local haemodynamic responses during whole-leg heating and small muscle mass exercise

Individually, hyperthermia and exercise are well known to evoke hyperaemia (Chiesa *et al.*, 2019) and therefore, it is plausible to hypothesise that the addition of thermal stress would produce an additive effect on functional hyperaemia during exercise. The findings from experimental *Chapter 6* support this hypothesis, with local leg hyperthermia stimulating a 0.7–1.0 $L \cdot min^{-1}$ increase in functional hyperaemia. However, the literature is inconclusive with studies reporting no additive effect of heating on functional hyperaemia during two-legged cycling (Savard *et al.*, 1988; Trangmar *et al.*, 2017) and single-leg knee-extensor exercise (Savard *et al.*, 1988; Ferguson *et al.*, 2006), with others reporting a 0.6–0.7 $L \cdot min^{-1}$ increase in functional hyperaemia during single-leg knee extensor exercise (Pearson *et al.*, 2011; Chiesa *et al.*, 2015). Despite these discrepancies, it appears that any possibility of an additive effect of hyperthermia on functional hyperaemia is lost during large muscle mass exercise (Savard *et al.*, 1988; Nielsen *et al.*, 1990; Nielsen *et al.*, 1993; Nielsen *et al.*, 1997; Trangmar *et al.*, 2017). This may be explained by the higher metabolic and thermoregulatory demands of whole-body exercise, which likely result in a reduced cutaneous circulation, an earlier onset of sweating, and an augmented ability to dissipate heat from the active muscles (Chiesa *et al.*, 2019). Notwithstanding, the question as to why some past studies investigating the hyperaemic responses to hyperthermia and small muscle mass exercise did not find an additive effect of heating whilst others did remains. As demonstrated in *Chapters 3 and 4*, local tissue perfusion is highly sensitive to changes in local tissue temperature. Since blood and muscle temperature can increase substantially during exercise (Saltin & Hermansen, 1966;

Saltin *et al.*, 1968; González-Alonso *et al.*, 2000; Gonzalez-Alonso *et al.*, 2015), it appears possible that some studies did not report an additive effect of hyperthermia on functional hyperaemia due to an insufficient difference in tissue temperature between their control and hyperthermic exercise conditions (Savard *et al.*, 1988; Ferguson *et al.*, 2006).

Moreover, if there is indeed an additive effect of hyperthermia on functional hyperaemia during small muscle mass exercise, where does this additional blood flow go? A logical assumption, particularly if employing the classic perspective that increases in hyperaemia during hyperthermia are confined to the cutaneous circulation (Johnson & Proppe, 1996), is that the additional perfusion is distributed to the skin to aid heat dissipation. However, findings from *Chapter 6* counter this viewpoint, arguing that the heighted blood flow is perfusing the active muscle. Past studies employing passive leg hyperthermia have reported increases in muscle blood flow and tissue oxygen saturation, whilst arteriovenous oxygen difference decreases (Keller *et al.*, 2010; Heinonen *et al.*, 2011; Pearson *et al.*, 2011; Chiesa *et al.*, 2015). During the combination of local hyperthermia and knee-extensor exercise, leg $\dot{V}O_2$ of the engaging quadriceps remains stable (Pearson *et al.*, 2011; Chiesa *et al.*, 2015). Thus, when taken together, alongside the present findings that tissue oxygen saturation increased during the combination of hyperthermia and exercise which is indicative of a heightened perfusion in the microcirculation, it appears that hyperthermia induces increases in muscle perfusion during knee-extensor exercise despite an unchanged metabolic demand.

Evidence of local thermosensitive mechanisms as chief regulators of hyperaemia during leg hyperthermia

A key objective of the present thesis was to gain further insight into the mechanisms responsible for stimulating large-fold increases in limb tissue perfusion during local hyperthermia. During whole-body heating, increases in temperature, particularly core temperature, cause an augmentation in central haemodynamics and thermal reflexes (Johnson & Proppe, 1996; Crandall & Gonzalez-Alonso, 2010; Chiesa *et al.*, 2019). Collectively, this results in an increased cardiac output, heart rate and cutaneous and muscle perfusion, alongside a reduction in splanchnic, renal, and cerebral organ blood flow as the body attempts to dissipate heat (Johnson & Proppe, 1996; Crandall &

Gonzalez-Alonso, 2010; Chiesa *et al.*, 2019). Whilst local thermosensitive mechanisms—such as a heightened vasodilator activity in conjunction with functional sympatholysis—also play a role to instigate hyperaemia, these mechanisms are typically overshadowed in the literature with the prevailing view favouring the primary role of central haemodynamics and thermal reflexes (Edholm *et al.*, 1957; Roddie *et al.*, 1957; Blair *et al.*, 1960; Rowell, 1974; Johnson & Rowell, 1975; Johnson & Proppe, 1996). In this model, core temperature is regarded as the primary stimulus as a 1 ºC increase in core temperature is associated with an approximately 9-fold, 2.6 $L \cdot min^{-1}$, 31 $beats \cdot min^{-1}$ increase in skin blood flow (Chiesa *et al.*, 2019), cardiac output (Figure 2), and heart rate (Figure 3), respectively. In contrast, more recent studies have begun to challenge this traditional stance, demonstrating that local limb hyperthermia instigates large increases in tissue perfusion whilst core temperature and thus, central haemodynamics remain relatively stable (Heinonen *et al.*, 2011; Pearson *et al.*, 2011; Chiesa *et al.*, 2015; Chiesa *et al.*, 2016; Kalsi *et al.*, 2017). Findings from the present thesis align with these studies, reporting ≥1- to 3-fold increases in whole-leg blood flow. During prolonged whole-leg heating, cardiac output increased in proportion to the increase in whole-leg blood flow; although it remained unchanged during leg segmental heating, likely due to the smaller magnitude of change and measurement error associated with the ModelFlow method (Shibasaki *et al.*, 2011). However, no changes in cardiac stroke volume, left ventricular contractility and late systolic flow deceleration (as demonstrated through wave-intensity derived variables) were observed during all levels of leg heating. Similarly, in a sister study from our laboratory, prolonged whole-leg heating did not stimulate changes in whole body oxygen consumption or minute ventilation (Gibson *et al.*, 2023); therefore, ruling out the role of respiratory and metabolic-related mechanisms. Collectively, these findings are indicative of the primary role of local thermosensitive mechanisms in regulating peripheral tissue perfusion during local hyperthermia, as opposed to central haemodynamic forces.

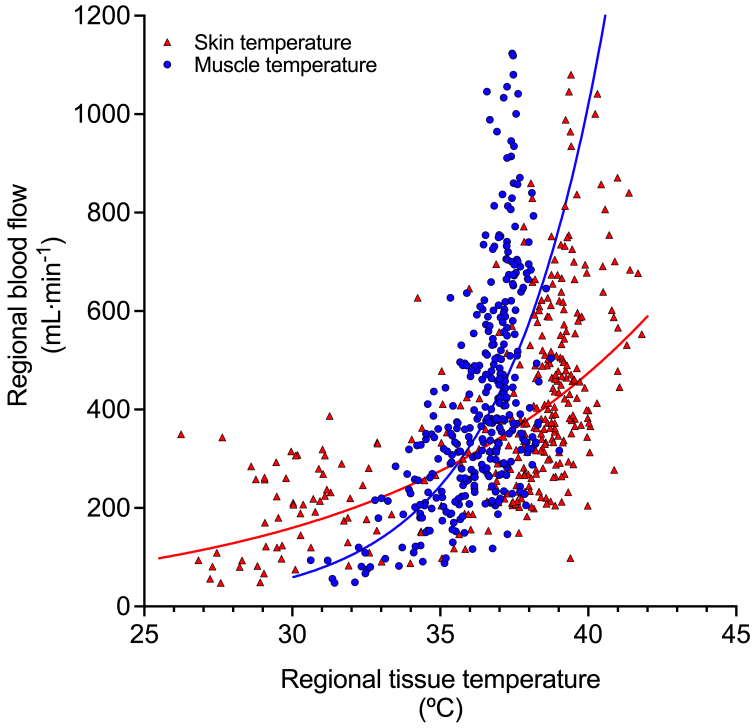

Figure 43. Relationship between regional tissue temperatures and blood flow during various protocols of local leg hyperthermia. Linear regression between temperature and the natural logarithm-transformed blood flow data, conducted on R Studio (version 2022.07.1+554, Team (2022)). Data are obtained from experimental *Chapters 4 and 5* during whole-leg, upper-leg (thigh), lower-leg, quadriceps, and partial-quadriceps heating. Data from *Chapter 6* was omitted as muscle temperature was not measured. Regional blood flow was reported as either the heated upper-leg or lower-leg blood flow. Upper-leg blood flow was calculated as the difference between common femoral artery and popliteal artery blood flow, whilst lower-leg blood flow was simply represented by popliteal artery blood flow. Tissue temperatures are reported as those measured in the heated regions. Regression results: skin temperature: $\boldsymbol{y = e^{0.11x+0.11}}$; $\boldsymbol{R^2 = 0.44}$ $\boldsymbol{(p < 0.0001)}$; muscle temperature: $\boldsymbol{y = e^{0.28x+0.28}}$; $\boldsymbol{R^2 = 0.46}$ $\boldsymbol{(p < 0.0001)}$.

Given that core temperature, considered the primary driving stimulus of thermoregulatory mechanisms, remained unchanged during all leg heating protocols, the question arises: what driving stimulus and mechanisms are responsible for peripheral tissue hyperaemia during local heating? During the present protocols, tissue perfusion was tightly coupled with local tissue temperature. This is particularly evident since tissue perfusion—represented by direct conduit artery blood flow and tissue oxygen saturation—solely increased in the heated segments of the leg whilst the perfusion in the adjacent and proximal unheated leg segments remained stable. Apart from the partial-quadriceps heating protocol which utilised diathermy, the leg heating protocols increased tissue temperature through conduction between the heated garment and the skin; as such, skin temperature increased substantially

quicker than muscle temperature. However, blood flow did not increase in the same rapid fashion as skin temperature, almost lagging until internal muscle temperature also increased. Figure 43 illustrates the relationship between regional blood flow (represented as upper- and lower-leg blood flow) and regional tissue temperature (expressed as skin and muscle temperature). Linear regression analysis between regional temperature and the natural logarithm-transformed regional blood flow data, reveals that there is an exponential relationship between regional blood flow and temperature, and that approximately 44–46 % of the variability in blood flow data can be explained by changes in skin and muscle tissue temperature (Figure 43). Whilst the predicting potential of regional mean tissue temperature on regional blood flow is lower than hypothesised, it may be explained by certain limitations of the model. For example, regional tissue temperature was estimated using relatively few data sites and was simply an estimation, utilising a weighted average based on the volume ratios of different tissue compartments (Wang *et al.*, 1999). Arguably, the predominant limiting factor of the present regression model is that it does not account for the volume of heated tissue. If one assumes that the present heating protocols increased tissue temperature to a similar degree, then the magnitude of hyperaemia is also largely dependent on the volume of heated tissue (Figure 44). Though, it should be noted that quantifying the volume of heated tissue poses some challenges, particularly given that deep (≥ 5 cm) muscle temperature—where temperature is ~37 ºC—likely did not significantly increase following the present heating protocols. Nonetheless, representing blood flow as an estimation of upper- or lower-leg blood flow during the quadriceps- and partial-quadriceps heating protocols—particularly when tissue temperature solely increased in a small area—likely explains why temperature in the present regression model, does not account for a larger proportion of the variability in blood flow data.

In addition, the regression model employed in Figure 43 provides further insights into the effect of skin and muscle temperature on regional tissue perfusion. Whilst regional skin and muscle temperature account for a similar amount of variability in the blood flow data (44 % and 46 %, respectively), muscle temperature had a substantially larger coefficient than skin temperature, (0.28 vs 0.11, respectively). Consequently, it appears that the effect of muscle temperature on regional blood flow might be more than double of that of the skin temperature. This is in line with previous studies which

demonstrate that tissue perfusion is more sensitive to changes in muscle temperature than skin temperature during both local leg heating and leg cooling protocols (Sekins *et al.*, 1984; Gregson *et al.*, 2011; Heinonen *et al.*, 2011; Pearson *et al.*, 2011; Mawhinney *et al.*, 2013; Caldwell *et al.*, 2016; Chiesa *et al.*, 2016; Mawhinney *et al.*, 2017).

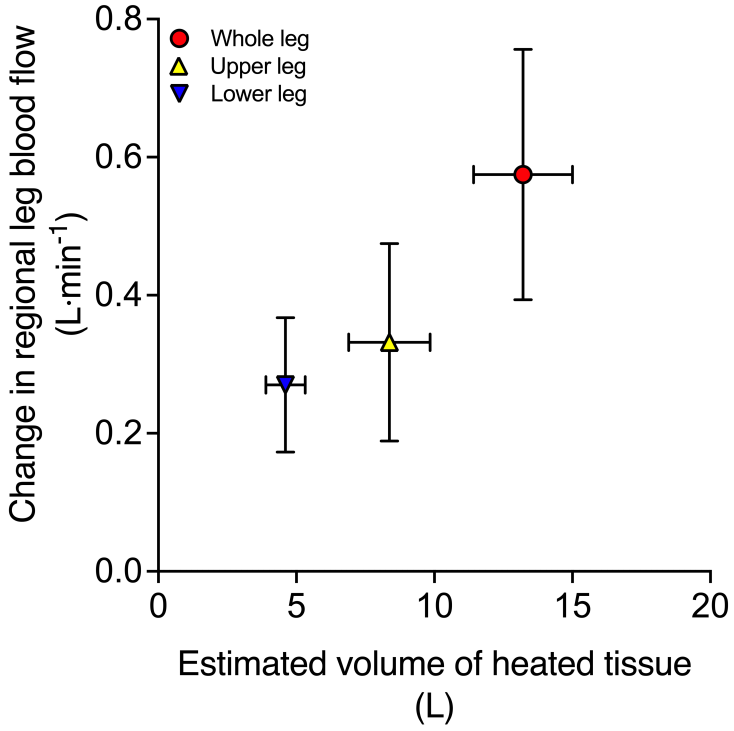

Figure 44. Changes in regional blood flow vs estimated volume of heated tissue during 1 h whole leg and segmental leg heating. Data represented as mean ± SD for each heating protocol. Whole-leg heating data are collated from Chapters 4 and 6 from young adults (*n* = 19), upper-leg heating data (*n* = 19) are collated from Chapters 4 and 5, whilst lower-leg heating data were obtained from Chapter 4 (*n* = 8). Whole-leg (common femoral artery) blood flow is reported in whole-leg heating data point, upper-leg (difference between common femoral and popliteal artery) blood flow is reported in the upper-leg heating data point, and lower-leg (popliteal artery) blood flow is reported in the lower-leg heating data point. Volumes were calculated using the participants' body mass and previously reported segment mass to body mass ratios (Clauser et al., 1969). Note: this analysis assumes that all protocols increased tissue temperature to a similar magnitude. The volumes utilised are likely an overestimation of the actual volume of heated tissue as deep muscle temperature (≥ 5 cm) probably did not significantly increase above its baseline (~ 37 ºC).

Whole-leg blood flow increased 1.4–3.5-fold during the various heating protocols; however, whilst 'vasodilation' is frequently and colloquially coined as the primary mechanism inducing hyperthermia-induced hyperaemia, no changes in conduit artery diameter were observed at the macrocirculatory level. Consequently, the large magnitude of hyperaemia observed must be the result of a heightened blood velocity.

Utilising Darcy's law of flow as a conceptual framework, whereby tissue perfusion is the product of vascular conductance and perfusion pressure gradient, one can further explore the potential mechanisms governing the observed hyperaemia (Figure 45). No differences in perfusion pressure were observed during any of the presently used leg heating protocols, from whole-leg heating to partial-quadriceps heating, as shown through a maintained mean arterial pressure. Therefore, an increased vascular conductance appears to be the primary factor variable responsible for the hyperthermia-induced hyperaemia. Vascular conductance, as per Hagen-Poiseuille's law, is predominantly determined by vessel diameter and blood viscosity (Figure 45). Since no changes in conduit artery diameter were observed, vasodilation likely occurred futher downstream in the microcirculation. *In vitro* research exploring the effect of temperature on vessel smooth muscle contractility revealed that temperature does not have a direct impact (Vanhoutte & Shepherd, 1970; Ives *et al.*, 2011). Thus, vasodilation of the smaller arterioles, resistance vessels and capillaries, and the resultant increases in blood velocity and flow, are likely stimulated by a variety of thermosensitive mechanisms such as the activation of intravascular signalling-transduction pathways (Kellogg *et al.*, 1999; Minson *et al.*, 2001; Paniagua *et al.*, 2001; Laughlin *et al.*, 2008; Gifford *et al.*, 2014) and/or the release of vasoactive molecules from erythrocytes such as ATP (Pearson *et al.*, 2011; Kalsi & Gonzalez-Alonso, 2012; Kalsi *et al.*, 2017). Whilst these intravascular vasodilatory mechanisms plausibly play an important role in increasing tissue perfusion, heat-modulated changes in blood rheology likely account for at least part of the increase. These potential rheological responses include a decreased blood viscosity and frictional resistance (Snyder, 1971; Cinar *et al.*, 2001; Shin *et al.*, 2004; Lim *et al.*, 2010) in conjunction with an increased red blood cell deformability and dispersion (Manteuffel-Szoege, 1960; Manteuffel-Szoege, 1969; Cinar *et al.*, 2001; Pinho *et al.*, 2016).

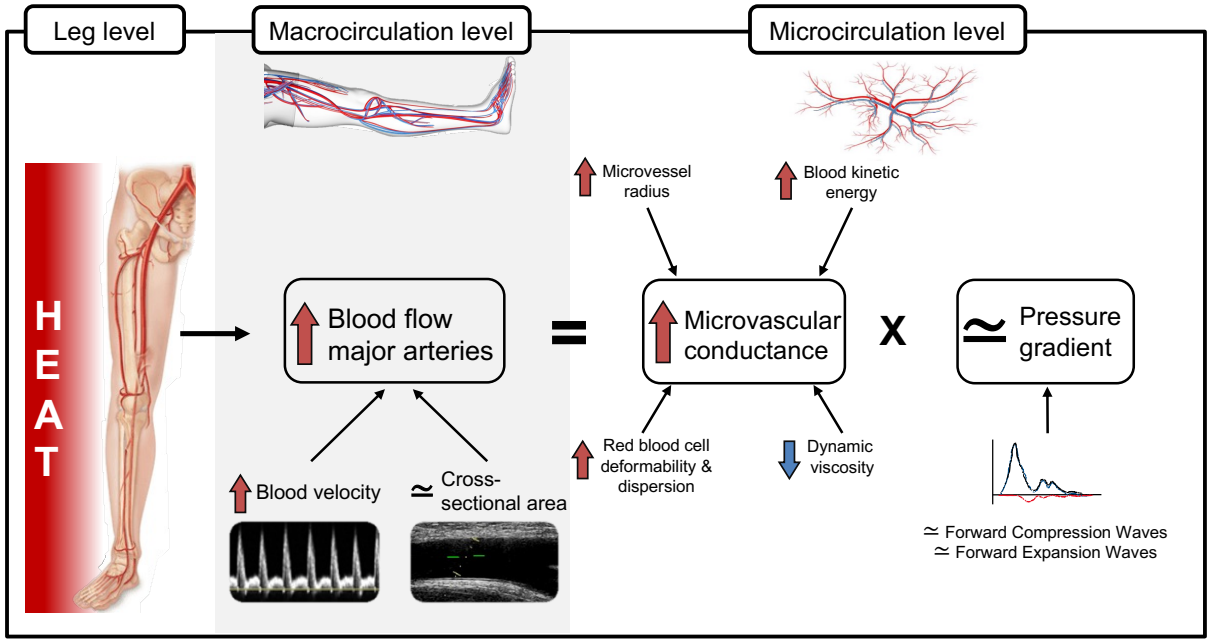

Figure 45. Schematic illustration of the haemodynamic effects of hyperthermia and the likely thermosensitive mechanisms involved in the thermal control of regional leg hyperaemia. Leg level: passive leg heating. Macrocirculation level: hyperthermic effect on the macrocirculation: passive heating increases blood velocity and thus, conduit artery blood flow without affecting arterial diameter. Microcirculation level: hyperthermic effect on the microcirculation: passive leg heating increases microcirculatory blood flow. Pressure gradient or perfusion pressure remains unchanged during passive leg heating, further supported by the unchanged forward compression and forward expansion waves which represent left ventricular contractility and late systolic flow deceleration, respectively. Consequently, the increase in microvascular circulation is the result of an increase in microvascular conductance. In turn, the elevation in microvascular conductance could be the result of an increase in microvessel diameter, red blood cell deformability and dispersion and/or blood kinetic energy, and/or a decrease in blood viscosity.

Effect of age on the functional hyperaemic response to leg hyperthermia and small muscle mass exercise

A major aim of the thesis was to explore the effect of age on the hyperaemic response to passive limb hyperthermia, small muscle mass exercise, and their combination. Data from *Chapter 6* revealed that age did not impact the hyperaemic response to single-leg heating, knee-extensor exercise, and their combination with whole-leg blood flow increasing ~3-fold, ~9-fold and ~12-fold, respectively. This challenges the consensus that ageing reduces vascular function and consequently, attenuates functional hyperaemia (Wray & Richardson, 2015; Hearon & Dinenno, 2016; Kenney *et al.*, 2021). Whilst this age-related attenuation is apparent during high-intensity, large muscle mass exercise (Beere *et al.*, 1999; Poole *et al.*, 2003) and severe heat stress (Sagawa *et al.*, 1988; Minson *et al.*, 1998), there are also studies reporting a reduction in the hyperaemic response to local hyperthermia (Romero *et al.*, 2017), low-intensity

large muscle mass exercise (Proctor *et al.*, 1998b; Proctor *et al.*, 2004) and small muscle mass exercise (Lawrenson *et al.*, 2003; Donato *et al.*, 2006). However, numerous studies—alongside the present findings—challenge this concept, reporting comparable hyperaemic responses between elderly and young adults (Beere *et al.*, 1999; Poole *et al.*, 2003; Proctor *et al.*, 2003; Mortensen *et al.*, 2012c; Piil *et al.*, 2018; Engelland *et al.*, 2020).

Commonly postulated key factors contributing to an age-related attenuation in functional hyperaemia include reductions in maximal cardiac output and nitric oxide availability, increases in blood pressure and vascular resistance, as well as impairments in functional sympatholysis, thermosensitive mechanisms, and rheological mechanisms (Hossack & Bruce, 1982; Ferrari *et al.*, 2003; Holowatz & Kenney, 2010; Simmonds *et al.*, 2013; Wray & Richardson, 2015; Hearon & Dinenno, 2016). The lack of age-related decline in functional hyperaemia in the present elderly cohort can be broadly explained by two overarching themes: (1) the low metabolic and thermoregulatory demand of the present protocols, and (2) the regular participation in physical activity. Firstly, the low metabolic and thermoregulatory demands of single-leg heating, low-intensity knee-extensor exercise and their combination—which do not evoke increases in sympathetic activity (Takahashi *et al.*, 2011; Katayama & Saito, 2019)—indicate that an age-related attenuation in maximal cardiac output and functional sympatholysis likely do not act as a limiting factor. Additionally, age-related increases in vascular resistance and thus, decreases in vascular conductance, likely play a role in the age-related attenuation in functional hyperaemia; however, the present cohort showed a comparable vascular conductance to their younger cohort. According to Darcy's law and Hagen-Poiseuille's law, vascular conductance is highly dependent on arterial blood pressure, changes in arterial diameter linked to bioavailability of vasodilators and functional sympatholysis, and certain rheological factors such as blood viscosity and erythrocyte deformability (Figure 45). Whilst age can impair the aforementioned factors (Pinto, 2007; Simmonds *et al.*, 2013; Wray & Richardson, 2015; Hearon & Dinenno, 2016), regular participation in physical activity has been shown to negate the drastic effects of age on these factors (Ernst, 1987; Mortensen *et al.*, 2012c; Simmonds *et al.*, 2013; Groot *et al.*, 2016; Kruse *et al.*, 2018; Piil *et al.*, 2018). However, despite the preventative potential of regular physical activity on the aforementioned factors and mechanisms, the elderly cohort reported a lower

blood velocity across all conditions in comparison to their younger counterparts. Interestingly, this attenuation in blood velocity was offset by a larger arterial diameter. Whilst increases in arterial diameter with age are well documented (Kawasaki *et al.*, 1987; Sandgren *et al.*, 1999; Hirata *et al.*, 2006; Gonzales *et al.*, 2009) and commonly associated with an impaired vascular function (Kawasaki *et al.*, 1987; Sonesson *et al.*, 1993; Anderson, 2006), the present findings of a large arterial diameter may be indicative of an adaptation to offset an attenuated blood velocity and thus, produce a hyperaemic response of a comparable magnitude to young adults. Nonetheless, further research is warranted to accept or refute this hypothesis.

Importance of findings

The present thesis provides evidence suggesting that limb tissue blood flow during varied regional hyperthermia—from whole-leg heating to partial-quadriceps heating—is regulated by local thermoregulatory mechanisms. This challenges past literature favouring central haemodynamic mechanisms and/or thermal reflexes in response to a heightened core temperature as the primary regulators of peripheral tissue perfusion (Edholm *et al.*, 1957; Roddie *et al.*, 1957; Blair *et al.*, 1960; Rowell, 1974; Johnson & Rowell, 1975; Johnson & Proppe, 1996). Collectively, the present experimental chapters demonstrate that hyperthermia-induced hyperaemia is highly localised as conduit artery blood flow supplying the heated area, alongside tissue oxygen saturation and skin blood flow, increased several-fold in the heated area whilst tissue perfusion of the non-heated area—whether that be the control contralateral leg, adjacent leg segment, or proximal area of the same leg segment—remained unchanged. Consequently, in this paradigm, the local thermosensitive mechanisms appear to play a primary role in the regulation of hyperthermia-induced hyperaemia, whilst the heart undertakes a secondary role by increasing cardiac output to accommodate the hyperaemia.

Findings from the present thesis further challenge traditional concepts. It is generally assumed that the hyperaemia during hyperthermia is directed towards the cutaneous circulation in attempt to dissipate heat (Roddie *et al.*, 1956; Edholm *et al.*, 1957; Blair *et al.*, 1960; Detry *et al.*, 1972; Johnson & Proppe, 1996). Whilst it is apparent that a greater magnitude of blood flow is indeed direct towards the skin, the present results alongside contemporary literature from the previous decade provides compelling

evidence that muscle blood flow does indeed increase during hyperthermia (Heinonen *et al.*, 2011; Pearson *et al.*, 2011; Chiesa *et al.*, 2015; Chiesa *et al.*, 2016; Kalsi *et al.*, 2017). Past studies have reported whole-body skin blood flow during hyperthermia as the difference between cardiac output and visceral blood flow (Koroxenidis *et al.*, 1961; Rowell *et al.*, 1969; Minson *et al.*, 1998), or as the difference between pre- and post-heating limb blood flow (Taylor *et al.*, 1984). Since both these skin blood flow estimations heavily rely on the assumption that muscle blood flow is unchanged during hyperthermia, it is likely that past studies utilising this method have overestimated cutaneous blood flow. Therefore, as supported by the present findings, caution is advised when interpreting systemic skin blood flow estimations using the aforementioned methods.

Age is commonly associated to declines in vascular health and function (Taddei *et al.*, 1995; Taddei *et al.*, 2000; Richards *et al.*, 2014; Trinity *et al.*, 2015; Groot *et al.*, 2016). Since shear stress, which increases ≥ 3-fold during whole-leg heating, is generally accepted as an important stimulus for vascular remodelling (Zarins *et al.*, 1987; Girerd *et al.*, 1996; Vita *et al.*, 2008; Green *et al.*, 2017), a great number of research studies have investigated the therapeutic efficacy of passive hyperthermia to improve and maintain cardiovascular health (Imamura *et al.*, 2001; Kihara *et al.*, 2002; Naylor *et al.*, 2011; Carter *et al.*, 2014; Brunt *et al.*, 2016; Romero *et al.*, 2017; Thomas *et al.*, 2017; Cullen *et al.*, 2020; Engelland *et al.*, 2020). Experimental *Chapter 6* established that age—in exercise-trained individuals—does not drastically attenuate one's hyperaemic response to single-leg heating, small muscle mass exercise, and their combination. During passive single-leg heating, whole-leg blood flow and shear stress increased > 3-fold in the elderly participants. This has important implications as it demonstrates that whole-leg heating can produce similar magnitudes of hyperaemia and shear stress as moderate whole-body hyperthermia where core temperature increased by ≥ 1 ºC (Chiesa *et al.*, 2016). Thus, providing an avenue whereby strong haemodynamic stimuli can be applied over prolonged periods without significant systemic physiological strain or thermal discomfort. Additionally, a main finding was that local heating has an additive effect on functional hyperaemia during small muscle mass exercise in elderly participants. This suggests that local limb hyperthermia has the potential to be utilised as a simple means to enhance the haemodynamic stimuli during low-intensity exercise. This could greatly benefit populations—particularly sedentary

elderly adults and clinical patients—that have a diminished vascular function and consequently, struggle to complete day-to-day physical tasks and physical activity.

Methodological considerations

All three experimental chapters included a detailed *experimental considerations* section which highlights the specific limitations of chapters. The following section will flag and discuss the implications of limitations which are shared between the experimental chapters.

There are several methods to assess blood flow such as ultrasound Doppler, positron-emission tomography, microdialysis, thermal dilution, and plethysmography. The present thesis utilised ultrasound Doppler to directly measure arterial blood velocity and diameter and thus, blood flow at the femoral and popliteal arteries. Ultrasound Doppler is an advantageous method as it allows for a direct, non-invasive, real-time assessment of conduit artery blood flow; however, it is sensitive to movement artifacts and highly dependent on the skill of the sonographer and as such, the coefficient of variation for blood flow ranges between 3–12 % (Shoemaker *et al.*, 1996; Rådegran, 1999; Pearson *et al.*, 2011). During preliminary testing for the present thesis, a coefficient of variation of 7.9 % was obtained for blood flow at the common femoral artery at rest. Though this may appear to be a somewhat large degree of variation, it is noteworthy that all the heating and exercise protocols induced increases in common femoral artery blood flow of 100–1200%; therefore, providing confidence in the measurements.

Additionally, the present thesis aimed to provide further insight into the distribution of blood between the tissues of the leg. During whole-leg heating, this was largely achieved by assessing the ratio of blood between the superficial and profunda femoral arteries, as the latter is known to perfuse the deep (muscle) tissues of the upper leg. The common femoral artery feeds the bifurcation of the superficial and profunda femoral arteries; therefore, theoretically, the sum of superficial and profunda femoral artery blood flow should be equal to the blood flow measured at the common femoral artery. However, this association between the three femoral arteries was not consistently observed in the present studies. This is likely due to the large variability in the anatomy of the profunda femoral artery (Tomaszewski *et al.*, 2017), which

makes it more challenging to obtain high quality blood flow measurements when compared to the other femoral arteries (Hussain, 1997; Koch Esteves & Chiesa, 2021). Consequently, it is likely that the magnitude of increase in profunda femoral artery blood flow was underestimated which has little effect on the conclusions of the thesis.

Directions for future research

The current thesis presents evidence that local thermosensitive mechanisms regulate limb perfusion during local hyperthermia. This opposes the more traditional cardio-centric perspective whereby hyperthermia-induced hyperaemia is controlled through an augmentation in central haemodynamics and/or thermal reflexes in response to increases in core temperature (Edholm *et al.*, 1957; Roddie *et al.*, 1957; Blair *et al.*, 1960; Rowell, 1974; Johnson & Rowell, 1975; Johnson & Proppe, 1996). Whilst the evidence presented in this thesis are robust, future studies should aim to simultaneously investigate both vascular and cardiac function data during passive whole-body and local limb heating—a method that has previously been employed during exercise (Watanabe *et al.*, 2020). These data would undoubtably shed further light on the chief regulators of tissue perfusion during passive hyperaemia.

Furthermore, the presently observed large increases in hyperaemia during acute local hyperthermia, in both young and elderly individuals, highlight the potential of local limb heating as a therapeutic intervention for improving and maintaining vascular health. Since increases in shear stress, through increases in blood flow, are known to promote positive vascular adaptation (Zarins *et al.*, 1987; Girerd *et al.*, 1996; Vita *et al.*, 2008; Green *et al.*, 2017), it is plausible to hypothesise that repeated single-leg hyperthermia would improve vascular function in the heated leg. However, during single-leg heating, no reflexive hyperaemic responses were observed in the contralateral leg—which is supportive of local, as opposed to central mechanisms in the regulation of peripheral limb tissue perfusion. Therefore, future studies could aim to conduct an interventional single-leg heating study, whilst using the contralateral leg as a control, to assess the effects of single-leg heating on vascular function. Utilising the combination of wave intensity analysis, as employed in *Chapter 4*, and passive leg movement would be of great benefit—with the former providing a direct measure of arterial distensibility and insight into central haemodynamics (Feng & Khir, 2010; Borlotti *et al.*, 2012; Pomella *et al.*, 2018) and the latter an assessment of whole-leg vascular function (Mortensen

et al., 2012a; Trinity *et al.*, 2012; Rossman *et al.*, 2016; Willmott *et al.*, 2017). In doing so, not only would this provide further evidence into the potential of heat therapy, but also shed further light into the mechanisms—whether they be local or central—regulating vascular function.

Additionally, while the present thesis provides equivocal evidence supporting local thermosensitive mechanisms as the primary mechanisms governing hyperaemia during local hyperthermia, the precise mechanisms were not herein directly investigated. As such, it would be of great benefit to conduct combined pharmacological and hyperthermic interventions to further deduct the mechanisms responsible for the regulation of hyperthermia-induced hyperaemia. In past attempts, α1-adrenoreceptor agonist were infused during single-leg heating, thereby pharmacologically blocking a thermosensitive afferent pathway (Keller *et al.*, 2010). It was found that the α1-adrenoreceptor blockade attenuated the hyperthermia-induced hyperaemic response, though heating-induced vasodilation did mitigate the magnitude of vasoconstriction in comparison to the normothermic conditions. However, a pharmacological intervention, as the aforementioned, may not necessarily reflect the mechanisms underpinning heat-induced hyperaemia, as muscle sympathetic nerve activity has been shown to decline during leg heating (Takahashi *et al.*, 2011), unlike whole-body hyperthermia (Powers *et al.*, 1982; Niimi *et al.*, 1997; Cui *et al.*, 2002; Cui *et al.*, 2004). Consequently, an alternative could be to systematically block various well-documented vasodilators and receptors—e.g., nitric oxide, cyclooxygenase, prostaglandins, adenosine, P_1 and P2Y receptors, ATP and an endothelium derived hyperpolarizing factor block—in isolation and conjunction, whilst measuring blood plasma concentrations of these vasodilators during local limb hyperthermia (Hasséssian *et al.*, 1993; Mortensen *et al.*, 2007; Mortensen *et al.*, 2009a; Hellsten *et al.*, 2012; Kalsi & Gonzalez-Alonso, 2012; Wang *et al.*, 2016). Past studies utilising this approach have at most reduced blood flow by ~30 % during exercise or vasodilatory interventions; this is likely due to redundancy mechanisms whereby the regulation of blood flow and oxygen delivery is remarkably sophisticated that if one mechanism fails, another mechanism engages (Boushel & Kjaer, 2004; Hellsten *et al.*, 2012). Thus, whilst it is unlikely that the combination of these pharmacological interventions alongside local-limb heating will provide a definitive answer as to which local thermoregulatory mechanisms are responsible for the mediation of local

hyperthermia-induced hyperaemia, it certainly would shed further light on the mechanisms at play.

Lastly, a significant finding was that local hyperthermia had an additive effect on functional hyperaemia during small muscle mass exercise in both elderly and young adults. This could have important ramifications for populations who are unable to perform moderate to strenuous physical activity, as local heating may provide an enhanced haemodynamic stimulus during low intensity exercise or daily physical tasks. As such, an intervention study comparing the effect of passive heating, exercise, and their combination on vascular function in certain populations would be of great interest. However, current literature suggests that the additive effect of hyperthermia on functional hyperaemia is diminished during large muscle mass exercise (Nielsen *et al.*, 1990; Nielsen *et al.*, 1993; Nielsen *et al.*, 1997; Trangmar *et al.*, 2017), likely due to the higher metabolic and thermoregulatory demands of whole-body exercise resulting in a reduced cutaneous circulation, an earlier onset of sweating, and an increased heat dissipation from the active muscles (Chiesa *et al.*, 2019). Since it is unplausible to imagine individuals regularly performing single-leg heating and knee-extensor exercise in real world scenarios (as performed in *Chapter 6*), further research investigating the acute effects of hyperthermia during low-intensity large muscle mass exercise, such as walking, is warranted.

Summary of findings

The present thesis provides a comprehensive synopsis on the effects of local hyperthermia and exercise on tissue perfusion and in doing so, offers a novel insight into the regulation of hyperaemia in young and elderly participants. Data from all three chapters demonstrate that local leg heating, from whole-leg heating to partial-quadriceps heating, stimulate large increases in leg hyperaemia in a temperature- and volume-dependent manner. Upper-leg and lower-leg heating produced hyperaemia to a similar magnitude of its corresponding leg segment during whole-leg heating, and quadriceps heating produced hyperaemia that was approximately half of that observed during upper-leg heating. Moreover, no changes in temperature and tissue perfusion were observed in the control contralateral leg during whole-leg heating, unheated adjacent leg segment during upper-leg and lower-leg heating, or the unheated proximal area of the same leg segment during quadriceps and partial-quadriceps

heating. When taken together, alongside the findings that core temperature and central haemodynamics remained relatively unchanged, the present findings strongly advocate for the primary role of local thermosensitive mechanisms in the regulation of limb tissue perfusion during local hyperthermia.

Findings from *Chapter 6* revealed that hyperthermia has an additive effect on functional hyperaemia during low-intensity, small muscle mass exercise in young and elderly individuals. Furthermore, despite age-related functional and structural changes in vasculature, elderly participants reported a comparable magnitude of hyperaemia to young adults. This suggests that age *per se* does not attenuate the functional hyperaemic response to local heating and small muscle mass exercise.

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APPENDICES

Ethical Approval Letters

Study 1.

University Research Ethics Committee
Brunel University London
Kingston Lane
Uxbridge
UB8 3PH
United Kingdom

www.brunel.ac.uk

24 January 2018

LETTER OF APPROVAL

Applicant: Dr Oliver Gibson

Project Title: Heat Shock Protein (HSP) responses to passive heating

Reference: 7692-TISS-Jan/2018- 10865-2

Dear Dr Oliver Gibson,

The Research Ethics Committee has considered the above application recently submitted by you.

The Chair, acting under delegated authority has agreed that there is no objection on ethical grounds to the proposed study. Approval is given on the understanding that the conditions of approval set out below are followed:

- The agreed protocol must be followed. Any changes to the protocol will require prior approval from the Committee by way of an application for an amendment.

Please note that:

- Research Participant Information Sheets and (where relevant) flyers, posters, and consent forms should include a clear statement that research ethics approval has been obtained from the relevant Research Ethics Committee.
- The Research Participant Information Sheets should include a clear statement that queries should be directed, in the first instance, to the researcher. Complaints, on the other hand, should be directed, in the first instance, to the Chair of the relevant Research Ethics Committee.
- Approval to proceed with the study is granted subject to receipt by the Committee of satisfactory responses to any conditions that may appear above, in addition to any subsequent changes to the protocol.
- The Research Ethics Committee reserves the right to sample and review documentation, including raw data, relevant to the study.

Kind regards,

Professor Peter Hobson

Chair, University Research Ethics Committee

Brunel University London

University Research Ethics Committee
Brunel University London
Kingston Lane
Uxbridge
UB8 3PH
United Kingdom

www.brunel.ac.uk

14 March 2018

LETTER OF APPROVAL

Applicant: Dr Oliver Gibson

Project Title: Heat Shock Protein (HSP) responses to passive heating

Reference: 7692-A-Feb/2018- 11768-1

Dear Dr Oliver Gibson,

The Research Ethics Committee has considered the above amendment application recently submitted by you.

The Chair, acting under delegated authority has agreed that there is no objection on ethical grounds to the proposed amendment to the study. Approval is given on the understanding that the conditions of approval set out below are followed:

- The agreed protocol must be followed. Any changes to the protocol will require prior approval from the Committee by way of a new application for an amendment.

Please note that:

- Research Participant Information Sheets and (where relevant) flyers, posters, and consent forms should include a clear statement that research ethics approval has been obtained from the relevant Research Ethics Committee.
- The Research Participant Information Sheets should include a clear statement that queries should be directed, in the first instance, to the Supervisor (where relevant), or the researcher. Complaints, on the other hand, should be directed, in the first instance, to the Chair of the relevant Research Ethics Committee.
- Approval to proceed with the study is granted subject to receipt by the Committee of satisfactory responses to any conditions that may appear above, in addition to any subsequent changes to the protocol.
- The Research Ethics Committee reserves the right to sample and review documentation, including raw data, relevant to the study.

Kind regards,

Professor Peter Hobson

Chair, University Research Ethics Committee

Brunel University London

University Research Ethics Committee
Brunel University London
Kingston Lane
Uxbridge
UB8 3PH
United Kingdom

www.brunel.ac.uk

5 November 2019

LETTER OF APPROVAL

APPROVAL HAS BEEN GRANTED FOR THIS STUDY TO BE CARRIED OUT BETWEEN 05/11/2019 AND 01/03/2020

Applicant (s): Dr Oliver Gibson

Project Title: Heat Shock Protein (HSP) responses to passive heating

Reference: 7692-A-Oct/2019- 20622-1

Dear Dr Oliver Gibson

The Research Ethics Committee has considered the above application recently submitted by you.

The Chair, acting under delegated authority has agreed that there is no objection on ethical grounds to the proposed study. Approval is given on the understanding that the conditions of approval set out below are followed:

- On your poster, please add that the study has been reviewed and approved by the University Research Ethics Committee.
- The agreed protocol must be followed. Any changes to the protocol will require prior approval from the Committee by way of an application for an amendment.

Please note that:

- Research Participant Information Sheets and (where relevant) flyers, posters, and consent forms should include a clear statement that research ethics approval has been obtained from the relevant Research Ethics Committee.
- The Research Participant Information Sheets should include a clear statement that queries should be directed, in the first instance, to the Supervisor (where relevant), or the researcher. Complaints, on the other hand, should be directed, in the first instance, to the Chair of the relevant Research Ethics Committee.
- Approval to proceed with the study is granted subject to receipt by the Committee of satisfactory responses to any conditions that may appear above, in addition to any subsequent changes to the protocol.
- The Research Ethics Committee reserves the right to sample and review documentation, including raw data, relevant to the study.
- You may not undertake any research activity if you are not a registered student or employee of Brunel University or if you cease to become registered, including abeyance or temporary withdrawal. As a deregistered student you would not be insured to undertake research activity. Research activity includes the recruitment of participants, undertaking consent procedures and collection of data. Breach of this requirement constitutes research misconduct and is a disciplinary offence.

Dr Derek Healy

Chair of the University Research Ethics Committee

Brunel University London

University Research Ethics Committee
Brunel University London
Kingston Lane
Uxbridge
UB8 3PH
United Kingdom

www.brunel.ac.uk

20 November 2020

LETTER OF CONDITIONAL APPROVAL

Applicant (s): Dr Oliver Gibson

Project Title: Heat Shock Protein (HSP) responses to passive heating

Reference: 7692-A-Oct/2020- 28065-1

Dear Dr Oliver Gibson,

The Research Ethics Committee has considered the above amendment application recently submitted by you.

The Chair, acting under delegated authority has agreed that there is no objection on ethical grounds to the proposed study. Approval is given on the understanding that the conditions of approval set out below are followed:

- **The project is approved for recommencement after 2 December 2020 providing the current national lockdown measures are then lifted.**
- **The participants must be recruited from Brunel staff already in a workplace bubble as set out in the application.**
- **Please contact Dr Derek Healy to discuss your Risk Assessments prior to commencement of the study.**
- **The agreed protocol must be followed. Any changes to the protocol will require prior approval from the Committee by way of an application for an amendment.**
- **In addition to the above, please ensure that you monitor and adhere to all up-to-date local and national Government health advice for the duration of your project.**

Please note that:

- Research Participant Information Sheets and (where relevant) flyers, posters, and consent forms should include a clear statement that research ethics approval has been obtained from the relevant Research Ethics Committee.
- The Research Participant Information Sheets should include a clear statement that queries should be directed, in the first instance, to the Supervisor (where relevant), or the researcher. Complaints, on the other hand, should be directed, in the first instance, to the Chair of the relevant Research Ethics Committee.
- Approval to proceed with the study is granted subject to receipt by the Committee of satisfactory responses to any conditions that may appear above, in addition to any subsequent changes to the protocol.
- The Research Ethics Committee reserves the right to sample and review documentation, including raw data, relevant to the study.

Kind regards,

Dr Derek Healy

Chair of the University Research Ethics Committee

Brunel University London

College of Health and Life Sciences Research Ethics Committee (DLS)
Brunel University London
Kingston Lane
Uxbridge
UB8 3PH
United Kingdom

www.brunel.ac.uk

2 October 2018

LETTER OF APPROVAL

Applicant: Mr Nuno Abel Koch Esteves

Project Title: Haemodynamic responses to thigh heating and cooling

Reference: 12388-MHR-Oct/2018- 14276-2

Dear Mr Nuno Abel Koch Esteves

The Research Ethics Committee has considered the above application recently submitted by you.

The Chair, acting under delegated authority has agreed that there is no objection on ethical grounds to the proposed study. Approval is given on the understanding that the conditions of approval set out below are followed:

- The agreed protocol must be followed. Any changes to the protocol will require prior approval from the Committee by way of an application for an amendment.

Please note that:

- Research Participant Information Sheets and (where relevant) flyers, posters, and consent forms should include a clear statement that research ethics approval has been obtained from the relevant Research Ethics Committee.
- The Research Participant Information Sheets should include a clear statement that queries should be directed, in the first instance, to the Supervisor (where relevant), or the researcher. Complaints, on the other hand, should be directed, in the first instance, to the Chair of the relevant Research Ethics Committee.
- The Research Ethics Committee reserves the right to sample and review documentation, including raw data, relevant to the study.
- You may not undertake any research activity if you are not a registered student of Brunel University or if you cease to become registered, including abeyance or temporary withdrawal. As a deregistered student you would not be insured to undertake research activity. Research activity includes the recruitment of participants, undertaking consent procedures and collection of data. Breach of this requirement constitutes research misconduct and is a disciplinary offence.

Professor Christina Victor

Chair

College of Health and Life Sciences Research Ethics Committee (DLS)
Brunel University London

College of Health and Life Sciences Research Ethics Committee (DLS)
Brunel University London
Kingston Lane
Uxbridge
UB8 3PH
United Kingdom

www.brunel.ac.uk

10 May 2019

LETTER OF APPROVAL

Applicant: Professor José González-Alonso

Project Title: Haemodynamic responses to lower leg heating

Reference: 16795-MHR-Apr/2019- 18896-1

Dear Professor José González-Alonso

The Research Ethics Committee has considered the above application recently submitted by you.

The Chair, acting under delegated authority has agreed that there is no objection on ethical grounds to the proposed study. Approval is given on the understanding that the conditions of approval set out below are followed:

- Advert - Please add both the date of approval and the expiry date (your end date) to the section where you mention the study has been approved.
- PIS - Please change the section on 'what are the indemnity arrangements' to the text within the PIS guidance on the College Research Ethics IntraBrunel page at https://intra.brunel.ac.uk/chls/research/Pages/default.aspx# removing the current text.
- PIS - Please look at the college templates on the research pages, please amend the contact for further information and complaints to Professor Christina Victor, Chair College of Health and Life Sciences Research Ethics Committee, Christina.victor@brunel.ac.uk
- The agreed protocol must be followed. Any changes to the protocol will require prior approval from the Committee by way of an application for an amendment.

Please note that:

- Research Participant Information Sheets and (where relevant) flyers, posters, and consent forms should include a clear statement that research ethics approval has been obtained from the relevant Research Ethics Committee.
- The Research Participant Information Sheets should include a clear statement that queries should be directed, in the first instance, to the Supervisor (where relevant), or the researcher. Complaints, on the other hand, should be directed, in the first instance, to the Chair of the relevant Research Ethics Committee.
- The Research Ethics Committee reserves the right to sample and review documentation, including raw data, relevant to the study.

Professor Christina Victor

Chair

College of Health and Life Sciences Research Ethics Committee (DLS)
Brunel University London

Study 2.

College of Health, Medicine and Life Sciences Research Ethics Committee (DLS)
Brunel University London
Kingston Lane
Uxbridge
UB8 3PH
United Kingdom

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27 August 2021

LETTER OF APPROVAL

APPROVAL HAS BEEN GRANTED FOR THIS STUDY TO BE CARRIED OUT BETWEEN 01/09/2021 AND 31/12/2022

Applicant (s): Mr Nuno Abel Koch Esteves Prof. José González-Alonso

Project Title: Exploring the acute blood flow effects of knee-extensor (leg-kicking) exercise and local leg heating in healthy young and aged participants

Reference: 31692-MHR-Aug/2021- 33803-2

Dear Mr Nuno Abel Koch Esteves

The Research Ethics Committee has considered the above application recently submitted by you.

The Chair, acting under delegated authority has agreed that there is no objection on ethical grounds to the proposed study. Approval is given on the understanding that the conditions of approval set out below are followed:

- D23 - PIS: After completing all changes to the PIS please proof read and spell check your final version to remove typographical errors, correct pasting errors (e.g. in the "When exercising" paragraph), etc. When completed please have your supervisor check this over.
- D23 - PIS: You have, as requested, included Professor Louise Mansfield as the Chair of the College of Health, Medicine and Life Sciences Research Ethics Committee, but have not edited correctly – you need to remove Derek Healy from these sections.
- D23 - PIS: Please simplify your PIS where possible – it is improved but you still use some technical terms such as 'endothelial function' and 'anthropometric measures'. The previous review noted: "The PIS is too technical and needs updating. In particular, scientific terminology should be replace by lay language, e.g. 'haemodynamic' (in the title) could be replace by 'blood flow', 'leg extensor' by 'leg-kicking', 'endothelial function' by 'function of the inner lining of the blood vessels', 'acute' by 'immediate', 'superficial and profunda femoral artery', by 'the main blood vessels supplying the lower legs', 'anthropometric measures' by 'body measures', 'core temperature' by 'body temperature', 'aborted' by 'stopped', etc."
- D23 - PIS: You mention insurance available "providing the insurance broker and underwriter are notified in advance of the work and agree to provide cover." – you need to contact Linda Hazell prior to commencing the study so the broker and underwriter are notified and amend the PIS so participants are aware of the agreed arrangements (rather than potentially agreed).
- D23 - PIS: Please consider whether opening the windows during testing not going to affect experimental conditions? (You state in your application that the experimental conditions will be controlled at ~21 C with 30-40% relative humidity).

- **The agreed protocol must be followed. Any changes to the protocol will require prior approval from the Committee by way of an application for an amendment.**
- In addition to the above, please ensure that you monitor and adhere to all up-to-date local and national Government health advice for the duration of your project.
-

Please note that:

- Research Participant Information Sheets and (where relevant) flyers, posters, and consent forms should include a clear statement that research ethics approval has been obtained from the relevant Research Ethics Committee.
- The Research Participant Information Sheets should include a clear statement that queries should be directed, in the first instance, to the Supervisor
  (where relevant), or the researcher. Complaints, on the other hand, should be directed, in the first instance, to the Chair of the relevant
- Research Ethics Committee.
- The Research Ethics Committee reserves the right to sample and review documentation, including raw data, relevant to the study.

You may not undertake any research activity if you are not a registered student of Brunel University or if you cease to become registered, including abeyance or temporary withdrawal. As a deregistered student you would not be insured to undertake research activity. Research activity includes the recruitment of participants, undertaking consent procedures and collection of data. Breach of this requirement constitutes research misconduct and is a disciplinary offence.

Professor Louise Mansfield

Chair of the College of Health, Medicine and Life Sciences Research Ethics Committee (DLS)

Brunel University London

College of Health, Medicine and Life Sciences Research Ethics Committee (DLS)
Brunel University London
Kingston Lane
Uxbridge
UB8 3PH
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www.brunel.ac.uk

6 September 2021

LETTER OF APPROVAL

APPROVAL HAS BEEN GRANTED FOR THIS STUDY TO BE CARRIED OUT BETWEEN 01/09/2021 AND 31/12/2022

Applicant (s): Mr Nuno Abel Koch Esteves

Project Title: Exploring the acute blood flow effects of knee-extensor (leg-kicking) exercise and local leg heating in healthy young and aged participants

Reference: 31692-A-Sep/2021- 34092-1

Dear Mr Nuno Abel Koch Esteves

The Research Ethics Committee has considered the above application recently submitted by you.

The Chair, acting under delegated authority has agreed that there is no objection on ethical grounds to the proposed study. Approval is given on the understanding that the conditions of approval set out below are followed:

- Please consider the following comment from the reviewer: "I have no issue with the proposed amendment. I would however suggest that the researchers consider using a different picture for skinfold calipers in the PIS, as the current one is somewhat misleading (i.e. suggesting that abdominal skinfold thickness will be measured, when the researchers are now proposing to solely focus on legs measurements)."
- **The agreed protocol must be followed. Any changes to the protocol will require prior approval from the Committee by way of an application for an amendment.**
- In addition to the above, please ensure that you monitor and adhere to all up-to-date local and national Government health advice for the duration of your project.

Please note that:

- Research Participant Information Sheets and (where relevant) flyers, posters, and consent forms should include a clear statement that research ethics approval has been obtained from the relevant Research Ethics Committee.
- The Research Participant Information Sheets should include a clear statement that queries should be directed, in the first instance, to the Supervisor (where relevant), or the researcher. Complaints, on the other hand, should be directed, in the first instance, to the Chair of the relevant Research Ethics Committee.
- The Research Ethics Committee reserves the right to sample and review documentation, including raw data, relevant to the study.
- You may not undertake any research activity if you are not a registered student of Brunel University or if you cease to become registered, including abeyance or temporary withdrawal. As a deregistered student you would not be insured to undertake research activity. Research activity includes the recruitment of participants, undertaking consent procedures and collection of data. Breach of this requirement constitutes research misconduct and is a disciplinary offence.

Professor Louise Mansfield

Chair of the College of Health, Medicine and Life Sciences Research Ethics Committee (DLS)

Brunel University London

College of Health, Medicine and Life Sciences Research Ethics Committee (DLS)
Brunel University London
Kingston Lane
Uxbridge
UB8 3PH
United Kingdom

www.brunel.ac.uk

27 September 2021

LETTER OF APPROVAL

APPROVAL HAS BEEN GRANTED FOR THIS STUDY TO BE CARRIED OUT BETWEEN 01/09/2021AND 31/12/2022

Applicant (s): Mr Nuno Abel Koch Esteves

Project Title: Exploring the acute blood flow effects of knee-extensor (leg-kicking) exercise and local leg heating in healthy young and aged participants

Reference: 31692-A-Sep/2021- 34357-1

Dear Mr Nuno Abel Koch Esteves

The Research Ethics Committee has considered the above application recently submitted by you.

The Chair, acting under delegated authority has agreed that there is no objection on ethical grounds to the proposed study. Approval is given on the understanding that the conditions of approval set out below are followed:

- **The agreed protocol must be followed. Any changes to the protocol will require prior approval from the Committee by way of an application for an amendment.**
- In addition to the above, please ensure that you monitor and adhere to all up-to-date local and national Government health advice for the duration of your project.

Please note that:

- Research Participant Information Sheets and (where relevant) flyers, posters, and consent forms should include a clear statement that research ethics approval has been obtained from the relevant Research Ethics Committee.
- The Research Participant Information Sheets should include a clear statement that queries should be directed, in the first instance, to the Supervisor (where relevant), or the researcher. Complaints, on the other hand, should be directed, in the first instance, to the Chair of the relevant Research Ethics Committee.
- The Research Ethics Committee reserves the right to sample and review documentation, including raw data, relevant to the study.
- You may not undertake any research activity if you are not a registered student of Brunel University or if you cease to become registered, including abeyance or temporary withdrawal. As a deregistered student you would not be insured to undertake research activity. Research activity includes the recruitment of participants, undertaking consent procedures and collection of data. Breach of this requirement constitutes research misconduct and is a disciplinary offence.

Professor Louise Mansfield

Chair of the College of Health, Medicine and Life Sciences Research Ethics Committee (DLS)

Brunel University London

College of Health, Medicine and Life Sciences Research Ethics Committee (DLS)
Brunel University London
Kingston Lane
Uxbridge
UB8 3PH
United Kingdom

www.brunel.ac.u

8 November 2021

LETTER OF APPROVAL

APPROVAL HAS BEEN GRANTED FOR THIS STUDY TO BE CARRIED OUT until 31/12/2022

Applicant (s): Mr Nuno Abel Koch Esteves

Project Title: Exploring the acute blood flow effects of knee-extensor (leg-kicking) exercise and local leg heating in healthy young and aged participants

Reference: 31692-A-Nov/2021- 34810-2

Dear Mr Nuno Abel Koch Esteves

The Research Ethics Committee has considered the above application recently submitted by you.

The Chair, acting under delegated authority has agreed that there is no objection on ethical grounds to the proposed study. Approval is given on the understanding that the conditions of approval set out below are followed:

- **The agreed protocol must be followed. Any changes to the protocol will require prior approval from the Committee by way of an application for an amendment.**
- In addition to the above, please ensure that you monitor and adhere to all up-to-date local and national Government health advice for the duration of your project.
-

Please note that:

- Research Participant Information Sheets and (where relevant) flyers, posters, and consent forms should include a clear statement that research ethics approval has been obtained from the relevant Research Ethics Committee.
- The Research Participant Information Sheets should include a clear statement that queries should be directed, in the first instance, to the Supervisor (where relevant), or the researcher. Complaints, on the other hand, should be directed, in the first instance, to the Chair of the relevant research Ethics Committee.
- The Research Ethics Committee reserves the right to sample and review documentation, including raw data, relevant to the study.
- You may not undertake any research activity if you are not a registered student of Brunel University or if you cease to become registered, including abeyance or temporary withdrawal. As a deregistered student you would not be insured to undertake research activity. Research activity includes the recruitment of participants, undertaking consent procedures and collection of data. Breach of this requirement constitutes research misconduct and is a disciplinary offence.

Professor Louise Mansfield

Chair of the College of Health, Medicine and Life Sciences Research Ethics Committee (DLS)

Brunel University London

Study 3.

College of Health, Medicine and Life Sciences Research Ethics Committee (DLS)
Brunel University London
Kingston Lane
Uxbridge
UB8 3PH
United Kingdom

www.brunel.ac.uk

5 October 2022

LETTER OF APPROVAL

APPROVAL HAS BEEN GRANTED FOR THIS STUDY TO BE CARRIED OUT BETWEEN 11/10/2022 AND 31/07/2023

Applicant (s): Miss Jeneil Toni-Ann McDonald Mr Nuno Koch Esteves

Project Title: Effect of regional upper leg heating on blood flow and tissue oxygenation responses

Reference: 38109-MHR-Oct/2022- 41690-2

Dear Miss Jeneil Toni-Ann McDonald

The Research Ethics Committee has considered the above application recently submitted by you.

The Chair, acting under delegated authority has agreed that there is no objection on ethical grounds to the proposed study. Approval is given on the understanding that the conditions of approval set out below are followed:

- **PIS- In your PIS you mention breaking confidentiality. You need a plan as to how to proceed if you need to break confidentiality. The usual course of action would be to alert your supervisor and then follow their advice. If it were possible that you might encounter a participant at risk of immediate harm, you would need to agree a suitable course of action with your supervisor before the study starts, and the supervisor needs to ensure he or she is available during the interview phase. You will have support information to hand in the debrief form.**

- **The agreed protocol must be followed. Any changes to the protocol will require prior approval from the Committee by way of an application for an amendment.**
- **Please ensure that you monitor and adhere to all up-to-date local and national Government health advice for the duration of your project.**

Please note that:

- Research Participant Information Sheets and (where relevant) flyers, posters, and consent forms should include a clear statement that research ethics approval has been obtained from the relevant Research Ethics Committee.
- The Research Participant Information Sheets should include a clear statement that queries should be directed, in the first instance, to the Supervisor (where relevant), or the researcher. Complaints, on the other hand, should be directed, in the first instance, to the Chair of the relevant Research Ethics Committee.
- Approval to proceed with the study is granted subject to receipt by the Committee of satisfactory responses to any conditions that may appear above, in addition to any subsequent changes to the protocol.
- The Research Ethics Committee reserves the right to sample and review documentation, including raw data, relevant to the study.
- If your project has been approved to run for a duration longer than 12 months, you will be required to submit an annual progress report to the Research Ethics Committee. You will be contacted about submission of this report before it becomes due.
- You may not undertake any research activity if you are not a registered student of Brunel University or if you cease to become registered, including abeyance or temporary withdrawal. As a deregistered student you would not be insured to undertake research activity. Research activity includes the recruitment of participants, undertaking consent procedures and collection of data. Breach of this requirement constitutes research misconduct and is a disciplinary offence.

Professor Louise Mansfield

Chair of the College of Health, Medicine and Life Sciences Research Ethics Committee (DLS)

Health Questionnaire and Consent Form Templates

Study 1.

College of Health and Life Sciences
Department of Life Sciences
Centre for Human Performance, Exercise and Rehabilitation

Brunel University London

CONSENT FORM

The participant should complete the whole of this sheet		
	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 agree that your anonymised data will be used not only in this study but also in future research decisions?		
Do you understand that your information will be given to your GP if deemed necessary?		
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:		

Researcher name:	Signature:

Brunel's Standard Health Assessment & Consent Form
PRE-PARTICIPATION HEALTH CHECK QUESTIONNAIRE

Health and safety 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: …… DOB: ……/……/……
Doctors Surgery Address: ……...
Emergency Contact Name & Number: ……

Please answer the following questions:	**YES**	**NO**
1. Has your doctor ever diagnosed a heart condition or recommend only medically supervised exercise?	☐	☐
2. Do you suffer from chest pains, heart palpitations or tightness of the chest?	☐	☐
3. Do you have known high blood pressure? If yes, please give details (i.e. medication)	☐	☐
4. Do you have low blood pressure or often feel faint or have dizzy spells?	☐	☐
5. Do you have known hypercholesteremia?	☐	☐
6. Have you ever had any bone or joint problems, which could be aggravated by physical activity?	☐	☐
7. Do you suffer from diabetes? If yes, are you insulin dependent?	☐	☐
8. Do you suffer from any lung/chest problem, i.e. Asthma, bronchitis, emphysema?	☐	☐
9. Do you suffer from epilepsy? If yes, when was the last incident?	☐	☐
10. Do you have any history of infectious diseases (e.g.HIV, Hep B)	☐	☐
11. Are you taking any medication?	☐	☐
12. Have you had any injuries in the last year? E.g., back problems, muscle strains etc.…	☐	☐
13. Are you currently enrolled in any other studies?	☐	☐
14. **I have recently participated in a blood donation program**	☐	☐
15. Are you a smoker?		
16. Do you exercise on a regular basis (at least 60 min a week)?	☐	☐
17. 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:

Study 2.

College of Health, Medicine, and Life Sciences
Department of Life Sciences
Division of Sport, Health and Exercise Sciences

Brunel University London

CONSENT FORM

The participant should complete the whole of this sheet		
	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? State:		
Do you understand that you will not be referred to by name in any report concerning the study?		
Do you agree that your anonymised data will be used not only in this study but also in future research decisions?		
Do you understand that your information will be given to your GP if deemed necessary?		
Do you understand that you are free to withdraw from the study:		
• at any time?		
• without having to give a reason for withdrawing?		
• that you have a 2-week period following the completion of the protocol to withdraw your data from the study?		
Do you agree to take part in this study?		
Signature of Research Participant:		
Date:		
Name in capitals:		

Researcher name:	Signature:
Researcher name:	Signature:

Brunel's Standard Health Assessment & Consent Form
PRE-PARTICIPATION HEALTH CHECK QUESTIONNAIRE

Health and safety 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: …… DOB: ……/……/……
Doctors Surgery Address: ……...
Emergency Contact Name & Number: ……

Please answer the following questions:	**YES**	**NO**
18. Has your doctor ever diagnosed a heart condition or recommend only medically supervised exercise?	☐	☐
19. Do you suffer from chest pains, heart palpitations or tightness of the chest?	☐	☐
20. Do you have known high blood pressure? If yes, please give details (i.e., medication)	☐	☐
21. Do you have low blood pressure or often feel faint or have dizzy spells?	☐	☐
22. Do you have known hypercholesteremia?	☐	☐
23. Have you ever had any bone or joint problems, which could be aggravated by physical activity?	☐	☐
24. Do you suffer from diabetes? If yes, are you insulin dependent?	☐	☐
25. Do you suffer from any lung/chest problem, i.e., Asthma, bronchitis, emphysema?	☐	☐
26. Do you suffer from epilepsy? If yes, when was the last incident?	☐	☐
27. Do you have any history of infectious diseases (e.g.HIV, Hep B)	☐	☐
28. Are you taking any medication?	☐	☐
29. Have you had any injuries in the last year? E.g., back problems, muscle strains etc.…	☐	☐
30. Are you currently enrolled in any other studies?	☐	☐
31. **I have recently participated in a blood donation program**	☐	☐
32. Are you a smoker?	☐	☐
33. Do you exercise on a regular basis (at least 60 min a week)?	☐	☐
34. 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:

Study 3.

College of Health, Medicine and Life Sciences
Department of Life Sciences
Centre for Human Performance, Exercise and Rehabilitation

Brunel University London

CONSENT FORM

The participant should complete the whole of this sheet		
	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? State:		
Do you understand that you will not be referred to by name in any report concerning the study?		
Do you agree that your anonymised data will be used not only in this study but also in future research decisions?		
Do you understand that your information will be given to your GP if deemed necessary?		
Do you understand that you are free to withdraw from the study:		
• at any time?		
• without having to give a reason for withdrawing?		
• that you have a 2-week period following the completion of the protocol to withdraw your data from the study?		
Do you agree to take part in this study?		
Do you agree to undergo a COVID-19 test on the day of your scheduled laboratory visit?		
Signature of Research Participant:		
Date:		
Name in capitals:		

Researcher name:	Signature:

Brunel's Standard Health Assessment & Consent Form
PRE-PARTICIPATION HEALTH CHECK QUESTIONNAIRE

Health and safety 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 investigator's ability, an individual must know his/her limitations.

Participant Name: …… DOB: ……/……/……
Doctors Surgery Address: ……...
Emergency Contact Name & Number: ……

Please answer the following questions:	**YES**	**NO**
35. Has your doctor ever diagnosed a heart condition or recommend only medically supervised exercise?	☐	☐
36. Do you suffer from chest pains, heart palpitations or tightness of the chest?	☐	☐
37. Do you have known high blood pressure? If yes, please give details (i.e., medication)	☐	☐
38. Do you have low blood pressure or often feel faint or have dizzy spells?	☐	☐
39. Do you have known hypercholesteremia?	☐	☐
40. Have you ever had any bone or joint problems, which could be aggravated by physical activity?	☐	☐
41. Do you suffer from diabetes? If yes, are you insulin dependent?	☐	☐
42. Do you suffer from any lung/chest problem, i.e. Asthma, bronchitis, emphysema?	☐	☐
43. Do you suffer from epilepsy? If yes, when was the last incident?	☐	☐
44. Do you have any history of infectious diseases (e.g.HIV, Hep B)	☐	☐
45. Are you taking any medication?	☐	☐
46. Have you had any injuries in the last year? E.g., back problems, muscle strains etc…	☐	☐
47. Are you currently enrolled in any other studies?	☐	☐
48. I have recently participated in a blood donation programme	☐	☐
49. Are you a smoker?	☐	☐
50. Do you exercise on a regular basis (at least 60 min a week)?	☐	☐
51. If you are female and applicable, how many days have passed since your last menses? ……...	☐	
52. Describe your weekly exercise routines (mode, frequency, intensity):		

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:

COVID-19 PRE-SCREENING HEALTH QUESTIONNAIRE

Participant Name: …… DOB: ……/……/……

Doctors Surgery Address: ……...
Emergency Contact Name & Number: ……

Please answer the following questions:	**YES**	**NO**
1. Have you obtained a negative result following a COVID-19 test within the last 24 hours?	☐	☐
2. Are you fully vaccinated against COVID-19? If yes, please state the dates you received your vaccinations (DD/MM/YY): ……/……/…… & ……/……/……	☐	☐
3. Within the last 14 days, have you experienced a new cough that you cannot attribute to another health condition?	☐	☐
4. Within the last 14 days, have you experienced a shortness of breath that you cannot attribute to another health condition?	☐	☐
5. Within the last 14 days, have you experienced a sore throat, loss of smell or taste that you cannot attribute to another health condition?	☐	☐
6. Within the last 14 days, have you measured a temperature/fever of ≥ 37.8ºC or experienced the sensation of a fever?	☐	☐
7. Within the last 14 days, have you had close contact with someone who is or was sick with suspected or confirmed COVID-19? *(please note that contact is defined as within 2m for ≥10 consecutive mins)*	☐	☐
8. Within the last 14 days, have you or a household member been isolating?	☐	☐

If you've answered yes to questions 3–8, please contact us immediately via email or phone call, as you may not be able to participate in the present study.

For further guidance on COVID-19, please visit: https://www.nhs.uk/conditions/coronavirus-covid-19/

If you are experiencing any symptoms including: a continuous cough, shortness of breath, a fever of equal to or above 37.8ºC, and/or loss of taste or smell, please stay at home and contact 111 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:_____

Publications

Study 1. Regional thermal hyperaemia in the human leg: evidence of the importance of thermosensitive mechanisms in the control of the peripheral circulation

Received: 11 May 2021 | Revised: 13 June 2021 | Accepted: 15 June 2021

DOI: 10.14814/phy2.14953

ORIGINAL ARTICLE

Physiological Reports

Regional thermal hyperemia in the human leg: Evidence of the importance of thermosensitive mechanisms in the control of the peripheral circulation

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Funding information
This study was completed without external funding.

Abstract

Hyperthermia is thought to increase limb blood flow through the activation of thermosensitive mechanisms within the limb vasculature, but the precise vascular locus in which hyperthermia modulates perfusion remains elusive. We tested the hypothesis that local temperature-sensitive mechanisms alter limb hemodynamics by regulating microvascular blood flow. Temperature and oxygenation profiles and leg hemodynamics of the common (CFA), superficial (SFA) and profunda (PFA) femoral arteries, and popliteal artery (POA) of the experimental and control legs were measured in healthy participants during: (1) 3 h of whole leg heating (WLH) followed by 3 h of recovery ($n = 9$); (2) 1 h of upper leg heating (ULH) followed by 30 min of cooling and 1 h ULH bout ($n = 8$); and (3) 1 h of lower leg heating (LLH) ($n = 8$). WLH increased experimental leg temperature by 4.2 ± 1.2ºC and blood flow in CFA, SFA, PFA, and POA by ≥3-fold, while the core temperature essentially remained stable. Upper and lower leg blood flow increased exponentially in response to leg temperature and then declined during recovery. ULH and LLH similarly increased the corresponding segmental leg temperature, blood flow, and tissue oxygenation without affecting these responses in the non-heated leg segment, or perfusion pressure and conduit artery diameter across all vessels. Findings demonstrate that whole leg hyperthermia induces profound and sustained elevations in upper and lower limb blood flow and that segmental hyperthermia matches the regional thermal hyperemia without causing thermal or hemodynamic alterations in the non-heated limb segment. These observations support the notion that heat-activated thermosensitive mechanisms in microcirculation regulate limb tissue perfusion during hyperthermia.

KEYWORDS

blood flow, heat, hemodynamics, thermal mechanisms

1 | INTRODUCTION

Passive whole and segmental limb hyperthermia increase local tissue perfusion in association with elevations in calculated limb vascular conductance (Kalsi et al., 2017; Keller et al., 2010; Pearson et al., 2011; Romero et al., 2017). Nevertheless, the exact vascular locus in which hyperthermia increases blood flow and tissue perfusion within the limb vascular tree remains elusive. Classic whole body hyperthermia studies suggest that central hemodynamic factors—that is, changes in mean arterial pressure and cardiac output—modulate the regulation of peripheral blood flow during hyperthermia (Blair et al., 1960; Edholm et al., 1957). Recent evidence, however, reveals that single leg heating elicits similar leg blood flow responses to moderate whole body hyperthermia (T_c +1°C), despite large differences in systemic hemodynamics and temperature responses (Chiesa et al., 2016; Pearson et al., 2011). During local hyperthermia, limb blood flow is elevated in a local tissue temperature-dependent manner, suggesting that local thermosensitive mechanisms in the limb vasculature rather than central hemodynamic factors play a crucial role in limb tissue blood flow regulation during hyperthermia (Chiesa et al., 2016; Heinonen et al., 2011; Pearson et al., 2011). Nonetheless, it remains unknown whether heating a limb segment—that is, the upper or lower leg—solely increases temperature and blood flow in the heated region or whether it would also evoke responses in the adjacent non-heated limb segment.

Conduit artery blood flow is the product of vascular conductance and perfusion pressure gradient (Laughlin, 1999). In local limb hyperthermic conditions where perfusion pressure remains stable, an increase in vascular conductance—and thus an increase in arterial diameter and/or blood velocity—could explain the hyperthermia-induced hyperemia. Nevertheless, whether local hyperthermia increases conduit artery diameter remains equivocal, with studies reporting increases (Kalsi et al., 2017), decreases (Thomas et al., 2017), or no changes in arterial diameter (Chiesa et al., 2016; Coombs et al., 2021; Pearson et al., 2011; Teixeira et al., 2017). Moreover, hyperthermia may directly act on the conduit artery supplying blood to the heated region by increasing arterial stiffness/decreasing arterial distensibility rather than altering diameter, as seen during incremental exercise (Pomella et al., 2018). The current literature suggests that central and/or regional arterial stiffness is unchanged during acute hyperthermia (Ganio et al., 2011; Moyen et al., 2016; Schlader et al., 2019) and declines following the cessation of heating (Lee et al., 2018; Sugawara & Tomoto, 2021; Thomas et al., 2017). To our knowledge, however, no study has explored the effects of hyperthermia on arterial distensibility using local techniques—such as the *PU*-loop or ln(*D*)*U*-loop (Feng & Khir, 2010; Khir et al., 2001). This could provide further evidence to support the therapeutic potential of local hyperthermia for the treatment of circulatory diseases and/or rehabilitation (Brunt et al., 2016; Coombs et al., 2019; Thomas et al., 2017), as arterial stiffness—which might rise during long-term bed rest, leg immobilization, and sedentary behavior (Bleeker et al., 2005; Bohn et al., 2017; van Duijnhoven et al., 2010; Mortensen et al., 2012)—is commonly associated with increases in cardiovascular mortality (Vlachopoulos et al., 2010).

The aim of the present study was threefold. First, to comprehensively investigate the tissue temperature and oxygenation profiles and the hemodynamic responses in the major arteries of the human leg during prolonged whole leg heating and the subsequent recovery, and during isolated upper leg and lower leg heating. Second, to establish the relationships among conduit artery hyperemia, local tissue oxygenation, and local hyperthermia. And third, to determine the impact of local hyperthermia on local arterial stiffness and distensibility. It was hypothesized that: (1) local hyperthermia would result in profound and sustained increases in blood flow profiles in the arteries supplying the heated leg/leg segment; (2) no changes in the hemodynamic and temperature profiles would be observed in the control leg/adjacent leg segment; (3) local hyperemia and tissue oxygenation are positively related to regional temperature; and (4) local arterial distensibility would largely remain unchanged during whole leg hyperthermia.

2 | MATERIALS AND METHODS

2.1 | Participants

This study consisted of three protocols: (1) whole leg heating; (2) upper leg heating; and (3) lower leg and foot heating. In total, eight healthy men and one healthy woman (mean ± SD: age 28 ± 11 years; height 177 ± 8 cm; mass 79.7 ± 9.1 kg) participated in protocol 1, five healthy men and three healthy women (age 32 ± 14 years; height 174 ± 10 cm; mass 72.7 ± 13.9 kg) in protocol 2, and five healthy men and three healthy women (age 29 ± 11 years; height 176 ± 9 cm; mass 72.3 ± 11.2 kg) in protocol 3. Three participants completed all three protocols whereas four completed two. Prior to the start of the study, informed written consent was obtained from all participants following a detailed written and verbal explanation of the experimental protocol. Participants were considered healthy following the completion of a health questionnaire. All procedures were approved by the Brunel University London Research Ethics Committee and are in agreement with the ethical principles stated in the Declaration of Helsinki (2013). Participants refrained from heavy exercise for 48 h, alcohol consumption for 24 h, and caffeine consumption for 12 h before the commencement of the protocols.

2.2 | Experimental protocols

For all three protocols, participants were asked to consume their usual breakfast and report to the laboratory between 08h00 and 09h00, whereby they fasted until the completion of the protocol. They were weighed in a semi-nude state and had their height measured (SECA 798 Scale) and then asked to rest in a supine position on a custom-built bed within a climate chamber set at 21ºC, where they remained for the entire duration of the study.

Protocol 1: Effects of prolonged whole leg heating on thermal, hemodynamic, and tissue oxygenation responses

Protocol 1 consisted of 3 h of whole leg heating, followed by 3 h of passive recovery. Following instrumentation—ECG electrodes, intravenous cannulation at the antecubital vein, and temperature thermistors (described below)—participants were fitted with a custom-made water-perfusion trouser on their right leg, which was then wrapped in a survival blanket to limit heat loss. The trouser was connected to a thermostatically controlled water circulator (Julabo F-34), which continuously circulated 50ºC water for the first 1.5 h of heating. The water was later reduced to 48ºC to prevent a large increase in core temperature. Blood flow was measured every 30 min in the common (CFA), superficial (SFA) and profunda (PFA) femoral arteries, and popliteal artery (POA) of the experimental and control legs (Figure 1). A 5 ml of venous blood sample and subjective perceptual measures were also collected at the same time points. Hemoglobin and hematocrit concentrations were measured using a commercially available Hb analyzer (Hb 201^{+} system, HemoCue AB), and a microscope and a ruled apparatus were used after centrifuging blood samples for 5 min (Billett, 1990), respectively. Hemoglobin and hematocrit values were then used to calculate blood volume, red blood cell volume, and plasma volume changes and estimate their absolute values using previously described methods (Dill & Costill, 1974; Sawka et al., 1992). Subjective perceptual measures were obtained with a 5-point thermal comfort scale (T_{comf}) (1 representing comfortable and 5 very uncomfortable, respectively) (Willmott et al., 2017) and an 8-point thermal sensation (T_{sens}) scale (0 representing unbearably cold and 8 unbearably hot, respectively) (Toner et al., 1986).

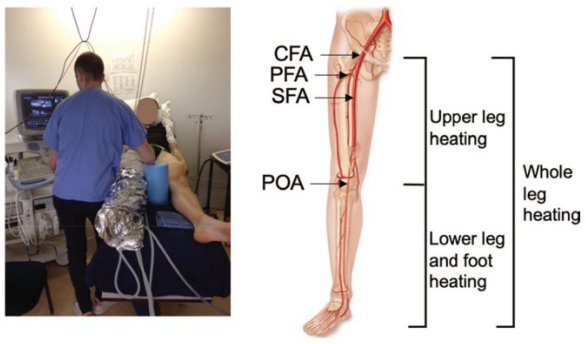

FIGURE 1 Example of experimental set up. Illustration of the experimental set up—participant was undergoing whole leg heating in the present example. The diagram shows the segments where blood flow was measured for the CFA, SFA, PFA, and POA, respectively, and which segments were heated for the various protocols

Protocol 2: Effects of upper leg heating on thermal, hemodynamic, and tissue oxygenation responses. Following the instrumentation of ECG electrodes and temperature thermistors, much like protocol 1, participants were fitted with a custom-made water-perfusion trouser on their right upper leg, which was then wrapped in a survival blanket to prevent heat loss (Figure 1). The trouser was connected to a thermostatically controlled water circulator, which continuously circulated 50ºC water for the first 1 h of heating, before circulating 20ºC water for the 30 min of cooling, and lastly circulated 50ºC water for the last 1 h of heating. Blood flow was measured every 10 min in the CFA and POA of the experimental leg, and in the control leg at baseline and 150 min of the protocol.

Protocol 3: Effects of lower leg and foot heating on thermal, hemodynamic, and tissue oxygenation responses. Following the instrumentation of ECG electrodes and temperature thermistors, much like the previous protocols, participants were fitted with a custom-made water-perfusion trouser on their right lower leg and foot, which were then wrapped in a survival blanket to limit heat loss. The trouser was connected to a thermostatically controlled water circulator, which continuously circulated 50ºC water for 1 h of heating. Blood flow was measured every 20 min in the CFA, SFA, PFA, and POA of the experimental leg (Figure 1), and in the control leg at baseline and 60 min of the protocol.

2.3 | Temperature measurements

Core temperature (T_c) was measured using a rectal probe (Ret-1 Special, Physitemp) which was self-inserted 15 cm past the sphincter muscle. Skin temperature (T_{sk}) in the thigh and calf for both the experimental and control legs was measured using commercially available thermistors (IT-18, Physitemp) which were securely held in place using medical tape. Muscle temperature (T_m) in the vastus lateralis muscle and in the gastrocnemius muscle of the experimental and control legs in protocol 1, and only of the experimental leg in protocols 2 and 3, was measured using thermistors (T-204f, Physitemp) inserted via an 18G catheter ~3 cm into the mid-portion of the muscle. T_c, T_m, and T_{sk} were recorded online using a commercially available thermocouple meter (TC-2000, Sable Systems International) connected to a data acquisition system (PowerLab 26T, ADInstruments). Additionally, mean leg temperature ($\overline{T}_{Leg}$) was calculated as a weighted average:

$\overline{T}_{\text{Leg}} = (T_{\text{m thigh}} \times 0.66) + \left(\frac{T_{\text{sk thigh}} + T_{\text{sk hamstring}}}{2} \times 0.06\right) + (T_{\text{m calf}} \times 0.25) + (T_{\text{sk calf}} \times 0.03)$; mean upper leg temperature ($\overline{T}_{\text{Upper leg}}$) was calculated as: $\overline{T}_{\text{Upper leg}} = (T_{\text{m thigh}} \times 0.92) + \left(\frac{T_{\text{sk thigh}} + T_{\text{sk hamstring}}}{2} \times 0.08\right)$; and mean lower leg temperature ($\overline{T}_{\text{Lower leg}}$) was calculated as: $\overline{T}_{\text{Lower leg}} = (T_{\text{m calf}} \times 0.88) + (T_{\text{sk calf}} \times 0.12)$. Mean leg and segmental leg temperature formulas were created using previously reported volume ratios of the different tissue compartments in the leg (Wang et al., 1999).

2.4 | Hemodynamic measurements

Heart rate was continuously measured using a three-lead echocardiogram. Moreover, arterial blood pressure, stroke volume, and cardiac output were measured non-invasively—at the same time points as the arterial blood flow measurements—using infrared photoplethysmography (Finometer, FMS, Netherlands), through a cuff on the middle finger of the right hand. Cardiac output was calculated as heart rate × stroke volume, where stroke volume was directly estimated using the Modelflow method, which incorporated corrections for age, height, and weight (BeatScope, FMS) (Wesseling et al., 1993). Blood flow was measured at set time points—recording two 12 s Doppler scans—throughout the protocols in the various arteries using a duplex Doppler ultrasound system (Vivid 7 Dimension, 198 GE Medical) with a 10 MHz linear array transducer probe (GE Medical Systems) at an insonation angle of ≤60°, with sample volume positioned in the center of the artery. The water-perfusion heated trouser had custom-made openings which allowed the probe to be placed on the skin with minimal heat loss. Before commencing baseline blood flow measures, arterial sites for the CFA, SFA, PFA, and POA in both legs were located and marked to ensure that blood flow measures were consistently measured at the same site. SFA and PFA blood flow measurements were acquired at a distance of ≥2 cm from the femoral bifurcation to prevent turbulent flow disruption to the measurements, and thus improve the validity of measures. Blood flow (ml min^{-1}) was calculated using the following equation: $BF = V_{\text{mean}} \times \pi \times \left(\frac{D_{\text{mean}}}{2}\right)^2 \times 60$, where V_{mean} is the average blood velocity (cm s^{-1}) and D_{mean} (cm) is the average diameter calculated using: $D_{\text{mean}} = \frac{1}{3}(D_{\text{systole}}) + \frac{2}{3}(D_{\text{diastole}})$ (Rådegran, 1997). Moreover, the arterial diameter was determined using CAROLAB (Zahnd et al., 2013) which was a prerequisite for the assessment of arterial distensibility and wave intensity parameters—outlined in the subsequent section. CAROLAB (Zahnd et al., 2013) uses block matching to provide an accurate measurement of the vessel diameter at each frame. A comparison of arterial diameter between the two methods was performed to ensure validity and reliability. On average, the continuous arterial wall tracking software revealed diameter values 1.9 ± 2.0% ($p < 0.0001$) higher than those obtained via the previously described weighted average of peak systolic and diastolic diameter. Additionally, blood flow was expressed in terms relative to tissue mass—that is, ml min^{-1} 100 g^{-1}—and was calculated using the participants' body mass and previously reported segment mass to body mass ratios (Clauser et al., 1969).

Shear rate (SR) was calculated using: $\text{SR} = \frac{4 \times V_{\text{mean}}}{D_{\text{mean}}}$, where V_{mean} is the mean blood velocity. Additionally, vascular conductance (VC) was calculated using the following formula: VC = BF÷MAP, where it is represented as ml min^{-1} mmHg^{-1}, BF is blood flow (ml min^{-1}), and MAP is the mean arterial pressure (mmHg). Blood flow was analyzed offline using commercially available software (EchoPAC, GE Medical). Blood velocity was averaged over two 12 s Doppler scans and average diameter was determined from four 2D B-mode images. Furthermore, blood pressure and temperature data were collected at 1000 Hz using a commercially available data acquisition system (PowerLab 26T, ADInstruments) and exported in 1 min bins using a commercially available data acquisition software (LabChart 7, ADInstruments). Following exportation, data were imported and analyzed in Microsoft Excel software (Microsoft Corporation). Data are reported as 2 min averages throughout the three protocols. Additionally, quadriceps skin blood flow was measured in all three leg heating protocols via laser-Doppler flowmetry (PeriFlux Flowmetry System), reported in perfusion units (PU). The probe was attached to the skin of the thigh, specifically on the vastus lateralis.

2.5 | Wave intensity and local arterial distensibility

Following the obtainment of the ultrasound B-mode scans, as described above, images were exported as DICOM files for offline analysis. Wave speed calculation and wave intensity analysis were only performed on the CFA of both the experimental and control legs as the DICOM image quality for the other arteries, particularly that of the PFA and POA, was not sufficient for diameter block matching. Diameter waveform extraction was performed using CAROLAB (Zahnd et al., 2013), which uses block matching to provide an accurate measurement of the vessel diameter at each frame. Extracted diameter waveforms were saved as Excel files (Microsoft Corporation) for later analysis. Doppler ultrasound DICOM files were analyzed in MATLAB (version R2019b, The MathWorks, Inc.) to extract the flow velocity waveforms, using custom-designed algorithms as previously reported (Negoita et al., 2018). Diameter waveform data were obtained for the CFA every 30 min.

These diameter and flow velocity waveforms were then used to calculate wave speed (c) using the ln(D)U-loop method (Feng & Khir, 2010); the following equation was used: $c = \pm \frac{1}{2} \frac{\mathrm{d}U_{\pm}}{\mathrm{d}(\ln D)_{\pm}}$ where dU and d(ln$D$) are the incremental differences between adjacent data of velocity (U) and diameter (D). Moreover, forward compression waves (FCW)

and forward expansion waves (FEW), which reflect left ventricular performance in early and late systole, respectively, were calculated using previously documented techniques (Pomella et al., 2018). Data outputs were averaged over two scans for the same time point, with three waveforms analyzed per scan. Subsequently, with the determination of c, distensibility (D_s) was calculated using the following Bramwell and Hill (1922) equation: $D_s = p^{-1} \times c^{-2}$, where p represents blood density which was assumed equal to 1050 kg m^{-3} (Pomella et al., 2018).

2.6 | Tissue oxygenation measures

Direct and continuous measures of regional tissue hemoglobin (venous) oxygen saturation (% rSO_2) were obtained in the experimental and control legs using two near-infrared spectroscopy units with four optode pads each (NIRS; INVOS 5100C Cerebral Oximeter; Somanetics Corp). The optode pads were placed on the skin surrounding the quadriceps, hamstrings, calf, and foot of both the control and experimental legs and taped to reduce interference from external light sources.

2.7 | Statistical analysis

Statistical analysis was conducted using R (version 3.5.1, Team [2013]). For protocol 1, linear mixed-effects models (two-way) were performed to investigate differences in hemodynamics, flow profiles, and temperature between and within the experimental and control legs over time. In addition, linear mixed-effects models (one-way) were conducted to investigate differences over time in systemic variables—that is, heart rate, cardiac output, stroke volume, blood pressure, perceptual measures, and non-leg temperatures. For protocols 2 and 3, linear mixed-effects models (one-way) were conducted to investigate differences over time in all variables—that is, hemodynamics, flow profiles, and temperature. Following the linear mixed-effects models, once a significant effect was found, a Tukey's *post hoc* test was conducted to locate the specific time points at which those changes occurred. Additionally, linear, exponential, and polynomial regression curve fit tests were performed using GraphPad Prism (version 8, GraphPad Software) to assess the relationship between various key data. Subsequently, Akaike's Information Criterion was used to evaluate which model provides the most appropriate fit. Where an exponential curve fit was appropriate, the equation $y = y_0 \cdot e^{-K \cdot x}$ was used, where y_0 is the y value when x (time) is zero and k is the rate constant. Significance of this fit is reported through 95% confidence intervals on the estimated value of k with the null value being $k = 0$. Moreover, a MANOVA test was conducted using SPSS (Version 26; IBM) to compare the difference in blood flow between the three heating protocols. After, if a significant effect was found, a Bonferroni's *post hoc* test was conducted to locate the specific time points at which those changes occurred.

3 | RESULTS

3.1 | Protocol 1: Effects of prolonged whole leg heating on thermal, hemodynamic, and tissue oxygenation responses

Regional and core temperatures, and thermal perception

Full leg and core temperature responses are illustrated in Figure 2. As per the design, quadriceps, hamstring, calf and foot T_{sk}, and quadriceps and calf T_m, and thus, $\overline{T}_{Leg}$ of the experimental leg increased progressively during the 3 h whole leg heating protocol, whereas it remained unchanged or declined in the control leg (Figure 2). Specifically, experimental $\overline{T}_{Leg}$ increased by 3.6 ± 0.3°C ($p < 0.0001$) following 3 h of

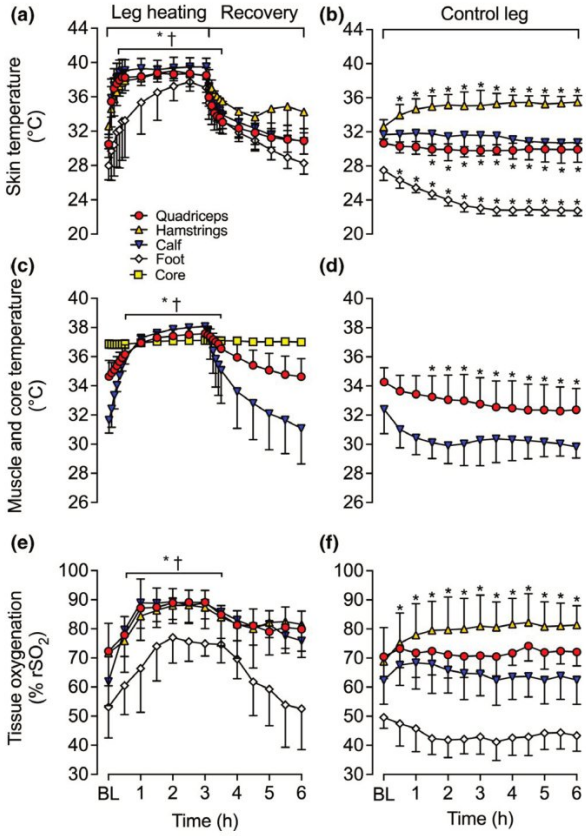

FIGURE 2 Core and leg temperatures (a, b, c, d) and regional tissue oxygenation (e, f) during whole leg hyperthermia and recovery in the experimental (heated; a, c, e) and control (contralateral; b, d, f) legs. Data represented as mean ± SD ($n = 9$). BL signifies baseline measurements. ∗ Different from baseline, $p < 0.05$. † Different from respective control (contralateral) leg, $p < 0.05$. All variables below the half-tick down line reported significant differences

heating, while the control $\overline{T}_{Leg}$ steadily declined throughout the protocol ($\Delta = -2.2 \pm 1.5$°C, $p < 0.0001$). Experimental $\overline{T}_{Leg}$ remained elevated during the first 1 h of recovery ($p = 0.008$), before declining toward baseline. However, T_c was only significantly elevated at 3 h (0.2 ± 0.2°C; $p < 0.0001$), reaching a peak temperature of 37.1 ± 0.2°C (Figure 2). Perceptual responses—thermal comfort (T_{comf}) and thermal sensation (T_{sens})—remained low and stable throughout the 6 h protocol. T_{comf} averaged 1.2 ± 0.8 units ($p = 0.214$), while T_{sens} averaged 4.4 ± 0.7 units ($p = 0.041$) increasing by 1 unit between 1.5 and 2.5 h of whole leg heating ($p = 0.029$).

Leg blood flow, tissue oxygenation, and systemic hemodynamics

Complete blood flow responses for the CFA, SFA, PFA, and POA are illustrated in Figure 3a. In the experimental leg, CFA, SFA, and POA blood flow increased ≥3-fold and PFA blood flow increased ~2-fold within the first hour of whole leg heating ($p < 0.0001$) (Figure 3a). Thereafter, arterial blood flow remained elevated and plateaued around 1.5–2 h until the cessation of heating (Figure 3a). During the subsequent 3 h recovery period, blood flow in the CFA, SFA, and POA remained elevated for the first 30 min (CFA and SFA: $p < 0.0001$; POA: $p = 0.010$) and then steadily decreased toward baseline values (Figure 3a). No changes in blood flow were observed in control leg for all four arteries throughout the entirety of the 6 h protocol ($p > 0.05$) (Figure 3b).

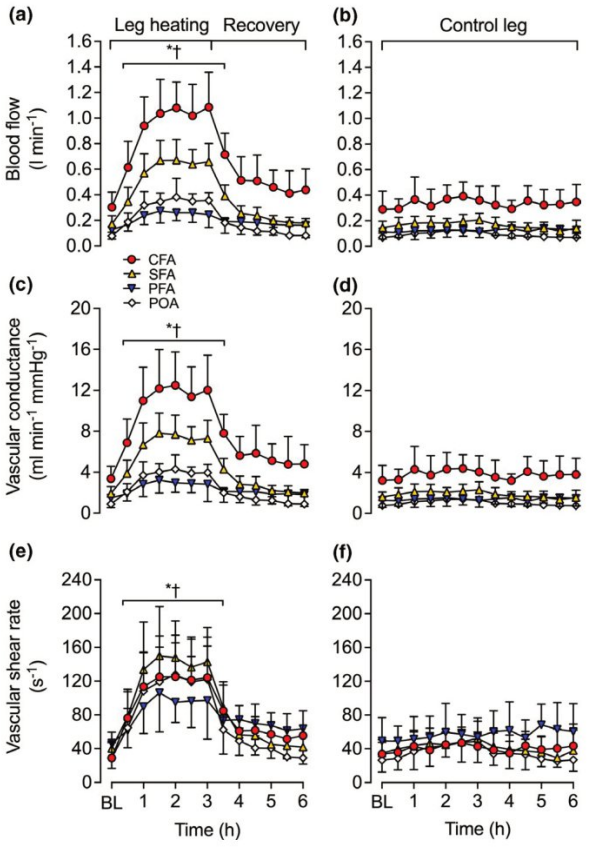

FIGURE 3 Blood flow (a, b), vascular conductance (c, d), and shear rate (e, f) during whole leg hyperthermia and recovery in CFA, SFA, PFA, and POA of the experimental (heated; a, c, e) and control (contralateral; b, d, f) legs. Data represented as mean ± SD ($n = 9$). BL signifies baseline measurements. ∗ Different from baseline, $p < 0.05$. † Different from respective control (contralateral) leg, $p < 0.05$. All variables below the half-tick down line reported significant differences

In line with the previously mentioned arterial responses, average upper and lower leg blood flow were ~3-fold higher in the experimental leg than the control leg following the whole leg heating protocol ($p < 0.0001$). Additionally, upper leg blood flow was higher than lower leg blood flow at all times ($p < 0.0001$). On average, experimental upper leg blood flow was 305 ± 249 ml min^{-1} higher than experimental lower leg blood flow during heating. However, when accounted for estimated tissue mass, upper and lower leg blood flow were similar following 3 h of whole leg heating (9.3 ± 2.8 vs. 10.5 ± 2.0 ml min^{-1} 100 g^{-1}, $p = 0.369$). Similar responses were observed in shear rate and vascular conductance in all four arteries of the experimental leg (Figure 3c and 3e). Experimental leg CFA, SFA, PFA, and POA shear rate and vascular conductance increased during the whole leg heating protocol, with ~4-fold increase being observed in the CFA, SFA, and POA at 3 h ($p < 0.0001$), while a ~ 2-fold increase was observed in the PFA ($p < 0.0001$). In contrast, no changes were observed in the control leg (Figure 3d and 3f). Moreover, whole leg heating increased upper leg tissue oxygenation by $16 \pm 9\%$ rSO_2 units ($p < 0.0001$), and lower leg tissue oxygenation by $24 \pm 9\%$ rSO_2 units ($p < 0.0001$) of the experimental leg (Figure 2e). Furthermore, tissue oxygenation remained unchanged in the control leg ($p > 0.05$, respectively) with the exception of hamstring tissue oxygenation which increased by $12 \pm 7\%$ rSO_2 units at 6 h ($p < 0.0001$; Figure 2f). However, when all the control leg sites were evaluated together, a close linear relationship was observed between the changes in local temperature and tissue oxygenation ($R^2 = 0.88$, $p < 0.0001$).

Increases in arterial blood flow were exponentially related to increases in local temperature (upper leg: $R^2 = 0.98$, $k = 0.46$ [0.37,0.57]; lower leg: $R^2 = 0.98$; $k = 0.26$ [0.20,0.35]) (Figure 4b) and were attributed to an increased blood velocity (all $p \leq 0.012$) (Figure 4a), as arterial diameter remained constant throughout the heating protocol ($p > 0.1$) (Figure 4c). In agreement with the global blood flow dynamic responses, increases in regional tissue oxygenation were exponentially related to increases in local temperature (upper leg: $R^2 = 0.96$, $k = 0.08$ [0.06,0.10]; lower leg: $R^2 = 0.98$, $k = 0.06$ [0.05,0.07]) (Figure 4d). Experimental leg quadriceps skin blood flow increased during whole leg heating from 3 ± 2 to 78 ± 37 PU ($p = 0.006$) before slowly returning to baseline (3 ± 1 PU) following 3 h of recovery

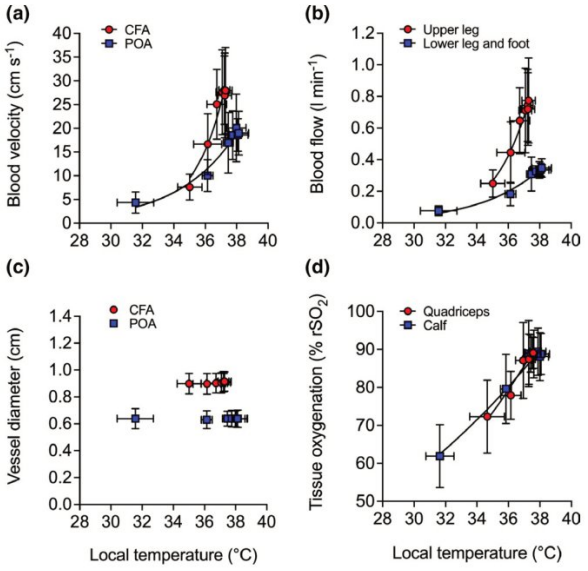

FIGURE 4 Relationship between the local temperature ($\overline{T}_{Leg}$) and local blood velocity (a), blood flow (b), vessel diameter (c), and tissue oxygenation (d) values during whole leg hyperthermia and recovery. Data represented as mean ± SD ($n = 9$). Vertical error bars signify the dependent variable SD, while horizontal error bars signify $\overline{T}_{Leg}$ SD, respectively

($p = 1.00$). At the systemic level, no significant changes were observed for systemic blood volume, red cell volume, plasma volume, and cardiac stroke volume (all $p > 0.5$; Table 1). However, increases of 8 ± 7 bpm in heart rate ($p = 0.002$) and 1.2 ± 0.7 L min^{-1} in cardiac output ($p = 0.038$) were observed at 3 h of whole leg heating (Table 1).

3.2 | Protocol 2: effects of upper leg heating on thermal, hemodynamic, and tissue oxygenation responses

Regional temperature responses

Full leg and core temperature responses are illustrated in Figure 5. As per the design, substantial increases in quadriceps and hamstrings T_{sk}, quadriceps T_m and $\overline{T}_{Upper\ leg}$ were observed following the two bouts of upper leg heating (all $p < 0.0001$). During the 30 min bout of upper leg cooling, however, upper leg T_{sk} dropped with hamstring T_{sk} declining below baseline ($\Delta = -2.4 \pm 2.5$ºC, $p = 0.0004$) whereas quadriceps T_{sk} remained elevated from baseline ($\Delta = +2.6 \pm 1.7$ºC, $p = 0.002$) (Figure 5a). Similar to quadriceps T_{sk}, T_m remained elevated above baseline during the cooling bout ($\Delta = +2.1 \pm 1.0$ºC, $p = 0.0001$) (Figure 5b).

TABLE 1 Influence of prolonged whole leg heating and subsequent recovery on central hemodynamics, common femoral artery blood flow, wave speed, arterial distensibility, and wave intensity parameters and popliteal artery blood flow

		Time (h)						
Variables	**Intervention**	**Baseline**	**1**	**2**	**3**	**4**	**5**	**6**
MAP (mmHg)		89 ± 7	88 ± 10	88 ± 9	91 ± 9	91 ± 8	90 ± 4	92 ± 5
$\dot{Q}$ (L min^{-1})		6.3 ± 0.6	6.8 ± 1.0	7.0 ± 0.8	7.5 ± 0.7*	6.7 ± 1.3	6.7 ± 1.7	6.7 ± 1.0
SV (ml)		111 ± 24	107 ± 19	111 ± 24	118 ± 30	116 ± 35	115 ± 41	114 ± 39
HR (beats min^{-1})		58 ± 10	64 ± 8*	65 ± 10*	66 ± 13*	60 ± 10	61 ± 11	63 ± 14
CFA blood flow (ml min^{-1})	Heated leg	320 ± 98	940 ± 226*†	1080 ± 202*†	1122 ± 250*†	513 ± 184	459 ± 134	438 ± 163
	Control leg	289 ± 142	365 ± 177	371 ± 108	360 ± 110	293 ± 67	323 ± 121	347 ± 136
POA blood flow (ml min^{-1})	Heated leg	78 ± 30	319 ± 106*†	382 ± 147*†	356 ± 60*†	145 ± 23	112 ± 33	82 ± 22
	Control leg	70 ± 34	101 ± 58	110 ± 40	120 ± 23	84 ± 19	74 ± 18	70 ± 30
Wave speed (m s^{-1})	Heated leg	20.4 ± 7.8	21.6 ± 10.6	20.6 ± 8.1	21.4 ± 10	22.1 ± 9.1	18.2 ± 6.2	19.3 ± 5.8
	Control leg	16.5 ± 9.3	16.1 ± 7.3	16.4 ± 6.3	16.9 ± 9.1	17.2 ± 3.4	15.4 ± 2.4	17.2 ± 4.4
Distensibility (×10^{-3} mmHg^{-1})	Heated leg	0.3 ± 0.2	0.3 ± 0.3	0.3 ± 0.3	0.3 ± 0.2	0.2 ± 0.2	0.3 ± 0.1	0.2 ± 0.1
	Control leg	0.3 ± 0.2	0.3 ± 0.3	0.3 ± 0.2	0.4 ± 0.2	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
FCW (cm^2 s^{-1})	Heated leg	1.6 ± 1.1	2.1 ± 0.8	2.5 ± 0.8	2.0 ± 0.7	1.9 ± 0.7	2.2 ± 0.9	2.6 ± 1.5
	Control leg	2.0 ± 0.8	2.2 ± 0.6	2.5 ± 1.1	1.8 ± 0.5	1.6 ± 0.6	1.7 ± 0.4	1.9 ± 1.0
FEW (cm^2 s^{-1})	Heated leg	0.5 ± 0.3	0.7 ± 0.3	0.7 ± 0.3	0.7 ± 0.3	0.8 ± 0.2	0.8 ± 0.4	0.8 ± 0.4
	Control leg	0.5 ± 0.3	0.6 ± 0.3	0.6 ± 0.3	0.7 ± 0.4	0.4 ± 0.1	0.6 ± 0.2	0.4 ± 0.2

Values are mean ± SD for nine participants, except for the wave intensity-derived parameters (n = 6). MAP: mean arterial pressure; $\dot{Q}$: cardiac output; SV: stroke volume; HR: heart rate; CFA: common femoral artery; POA: popliteal artery; FCW: forward compression waves; FEW: forward expansion waves. First 3 h represent whole leg heating responses, while hours 4 to 6 represent responses during the subsequent 3 h passive recovery.

∗ Different from baseline, $p < 0.05$.

† Different from respective control (contralateral) leg, $p < 0.05$.

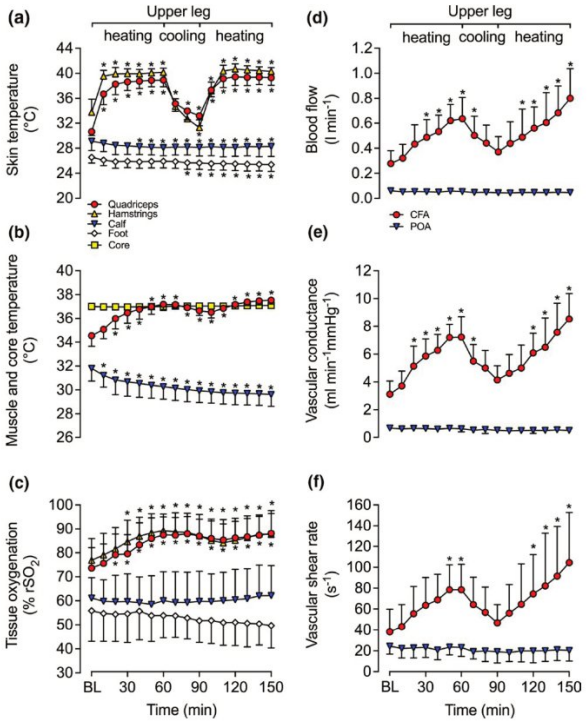

FIGURE 5 Core and leg temperatures (a, b), regional tissue oxygenation (c), blood flow (d), vascular conductance (e), and shear rate (f) in CFA and POA during upper leg heating, cooling and heating. Data represented as mean ± SD ($n = 8$). BL signifies baseline measurements. ∗ Different from baseline, $p < 0.05$

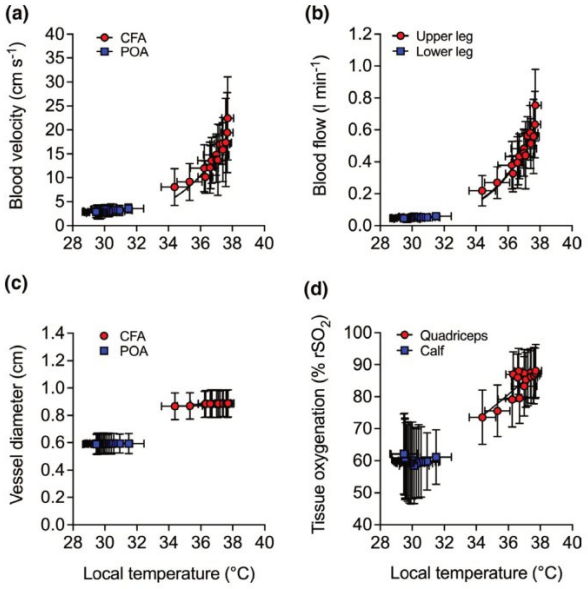

FIGURE 6 Relationship between the local temperature ($\overline{T}_{Leg}$) and local blood velocity (a), blood flow (b), vessel diameter (c), and tissue oxygenation (d) values during upper leg heating and cooling. Data represented as mean ± SD ($n = 8$). Vertical error bars signify the dependent variable SD, while horizontal error bars signify $\overline{T}_{Leg}$ SD, respectively

Consequently, $\overline{T}_{\text{Upper leg}}$ was elevated throughout the entirety of the protocol, increasing from 34.4 ± 0.8ºC to 37.7 ± 0.4ºC at 150 min ($p < 0.0001$). In contrast, in the lower leg, calf T_{sk} tended to decline ($p = 0.054$), while calf T_m and foot T_{sk} decreased −2.2 ± 0.4ºC and −1.2 ± 1.0ºC, respectively (both $p < 0.0001$) at 150 min. T_c and control leg quadriceps T_{sk} remained unaltered throughout the protocol ($p = 0.105$ and $p = 0.214$, respectively) (Figure 5a and 5b).

Leg blood flow, tissue oxygenation, and systemic hemodynamics

Complete blood flow responses for the CFA and POA are illustrated in Figure 5d. Following the first bout of 1 h upper leg heating, CFA blood flow increased 2.3-fold ($p < 0.0001$) and then declined toward baseline during the 30 min bout of upper leg cooling ($p = 0.639$) (Figure 5d). In the second bout of 1 h upper leg heating, CFA blood flow surpassed the magnitude obtained in the first bout of heating—increasing 2.9-fold and peaking at 801 ± 237 ml min^{-1} ($p < 0.0001$) (Figure 5d). These increases in CFA blood flow were exponentially related to an increasing $\overline{T}_{\text{Upper leg}}$ ($R^2 = 0.89$, $k = 0.40$ [0.31, 0.51]) (Figure 6b) and were the result of an increased blood velocity ($p = 0.001$) (Figure 6a), as CFA diameter remained unchanged ($p = 0.065$) (Figure 6c). Throughout the protocol, POA blood flow remained stable ($p = 0.149$) (Figure 5d). Accordingly, the calculated upper leg blood flow increased by 536 ± 243 ml min^{-1} following the second 1 h heating bout ($p < 0.0001$). Upper leg blood flow was higher than lower leg blood flow at all time points, ranging from a 3.6-fold difference at baseline to a 16-fold difference following the second bout of upper leg heating ($p < 0.0001$). In the control leg, CFA and POA blood flow pre- and post-protocol remained stable ($p = 0.369$ and $p = 0.150$, respectively).

Shear rate and vascular conductance mirrored the blood flow responses in both the CFA and POA: CFA shear rate and vascular conductance increased ~2-fold in the first bout and almost 3-fold in the second 1 h bout of upper leg heating (both $p < 0.0001$) (Figure 5). Conversely, POA shear rate and vascular conductance remained stable throughout the entire protocol (shear rate: $p = 0.153$; vascular conductance: $p = 0.107$) (Figure 5e and 5f). Furthermore, quadriceps skin blood flow increased from 7 ± 9 to 58 ± 27 PU during the first 1 h bout of upper leg heating. Quadriceps skin blood flow declined toward baseline at the end of the 30 min upper leg cooling bout ($p = 0.996$ vs. baseline), before increasing to 43 ± 28 PU ($p = 0.003$) once again during the second bout of upper leg heating. Tissue oxygenation responses are illustrated in Figure 5c. At all-time points, upper leg tissue oxygenation was higher than lower leg tissue oxygenation ($p < 0.0001$). During the first 1 h upper leg heating bout,

upper leg tissue oxygenation increased by 13 ± 4% rSO_2 units ($p < 0.0001$), increasing exponentially in response to $\overline{T}_{Upper\ leg}$ ($R^2 = 0.71$, $k = 0.05$ [0.03,0.07]) (Figure 6d). Subsequently, upper leg tissue oxygenation plateaued and remained elevated during the succeeding upper leg cooling and upper leg heating bouts ($p < 0.0001$) (Figure 5c). Conversely, calf and foot tissue oxygenation remained unchanged ($p = 0.350$ and $p = 0.074$, respectively) (Figure 5c). At the systemic level, heart rate, cardiac output, stroke volume, and mean arterial pressure remained stable with averages of 57 ± 5 bpm, 5.1 ± 0.8 L min^{-1}, 91 ± 15 ml, and 89 ± 14 mmHg, respectively (all $p > 0.05$) (Table 2).

3.3 | Protocol 3: effects of lower leg and foot heating on thermal, hemodynamic, and tissue oxygenation responses

Regional temperature responses. Full leg and core temperature responses are illustrated in Figure 7. As per the design, substantial increases in calf and foot T_{sk}, calf T_m and $\overline{T}_{Lower\ leg}$ were observed following the 1 h bout of lower leg and foot heating with $\overline{T}_{Lower\ leg}$ increasing from 32.0 ± 0.7°C to 37.7 ± 0.3°C ($p < 0.0001$). In contrast, $\overline{T}_{Upper\ leg}$ slowly declined throughout the protocol ($\Delta = -0.4 \pm 0.2$°C, $p = 0.003$). T_c remained unaltered ($p = 0.081$) (Figure 7b).

Leg blood flow, tissue oxygenation, and systemic hemodynamics. Complete blood flow responses for the CFA, SFA, PFA, and POA are illustrated in Figure 7. In response to lower leg and foot heating, CFA, SFA, and POA blood flow increased, while PFA blood flow remained stable ($p = 0.474$). Following 1 h of lower leg heating, CFA and SFA blood flow increased >2-fold ($p < 0.0001$) and POA blood flow increased 4.7-fold ($p < 0.0001$) (Figure 7d). The increase in POA blood flow was exponentially related

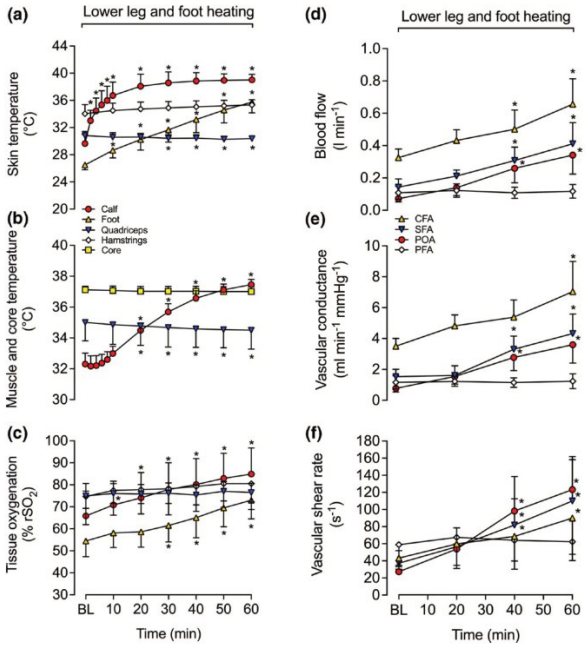

FIGURE 7 Core and leg temperatures (a, b), regional tissue oxygenation (c), blood flow (d), vascular conductance (e), and shear rate (f) in CFA, SFA, PFA, and POA during lower leg and foot heating. Data represented as mean ± SD ($n = 8$). BL signifies baseline measurements. * Different from baseline, $p < 0.05$

to increases in $\overline{T}_{Lower\ leg}$ ($R^2 = 0.99$, $k = 0.30$ [0.22,0.41]) (Figure 8b), and was attributed to an increased blood velocity ($p < 0.001$) (Figure 8a) as diameter remained unchanged ($p > 0.1$) (Figure 8c). Upper leg blood flow remained constant throughout the entire protocol at 276 ± 84 ml min^{-1} ($p = 0.257$) as the increases in CFA and SFA blood flow were proportional to the increase in lower leg blood flow. Similarly, CFA, SFA, and POA shear rate and vascular

TABLE 2 Influence of upper leg heating, cooling and heating on central hemodynamics and common femoral and popliteal artery blood flow

		Time (min)					
Variables	**Intervention**	**Baseline**	**30**	**60**	**90**	**120**	**150**
MAP (mmHg)		88 ± 16	83 ± 14	88 ± 14	88 ± 11	92 ± 12	94 ± 18
$\dot{Q}$ (L min^{-1})		4.9 ± 0.8	5.0 ± 0.7	5.0 ± 0.8	5.2 ± 0.9	5.2 ± 0.7	5.5 ± 1
SV (ml)		89 ± 13	92 ± 15	91 ± 17	93 ± 19	92 ± 16	101 ± 24
HR (beats min^{-1})		57 ± 5	57 ± 6	58 ± 9	57 ± 3	58 ± 5	56 ± 6
CFA blood flow (ml min^{-1})	Heated leg	278 ± 102	487 ± 142*	636 ± 170*	371 ± 122	561 ± 168*	801 ± 237*
	Control leg	299 ± 133	—	—	—	—	277 ± 127
POA blood flow (ml min^{-1})	Heated leg	60 ± 18	53 ± 18	54 ± 18	45 ± 19	47 ± 23	47 ± 18
	Control leg	61 ± 19	—	—	—	—	49 ± 23

Values are mean ± SD for eight participants, except for $\dot{Q}$ and SV values where $n = 6$. MAP: mean arterial pressure; $\dot{Q}$: cardiac output; SV: stroke volume; HR: heart rate; CFA: common femoral artery; POA: popliteal artery. Baseline to 60 min represents upper leg heating responses, 90 min represents upper leg cooling, and 120 min to 150 min represents the second bout of upper leg heating.

∗ Different from baseline, $p < 0.05$.

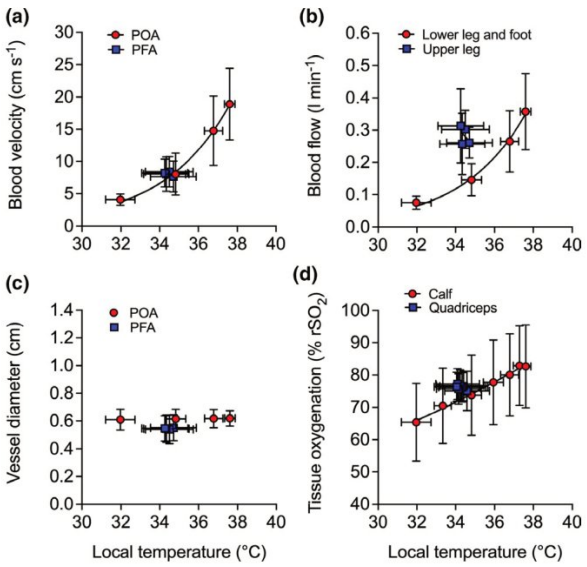

FIGURE 8 Relationship between the local temperature ($\overline{T}_{Leg}$) and local blood velocity (a), blood flow (b), vessel diameter (c), and tissue oxygenation (d) values during lower leg and foot heating. Data represented as mean ± SD ($n = 8$). Vertical error bars signify the dependent variable SD, while horizontal error bars signify $\overline{T}_{Leg}$ SD, respectively

conductance increased during the lower leg and foot heating protocol, with over 2-fold increases being observed at 1 h in both the CFA and SFA ($p < 0.0001$ for shear rate and vascular conductance in both arteries, respectively), while POA shear rate and vascular conductance increased ~4.5-fold at 1 h (both $p < 0.0001$). In contrast, no changes in PFA shear rate and vascular conductance were observed (shear rate: $p = 0.549$; vascular conductance: $p = 0.476$). In response to lower leg and foot heating, lower leg and foot tissue oxygenation increased by 18 ± 6% rSO_2 units, respectively ($p < 0.0001$). These increases in lower leg tissue oxygenation were exponentially related to increases in $\overline{T}_{\text{Lower leg}}$ ($R^2 = 0.99$, $k = 0.04$ [0.04, 0.05]), respectively (Figure 8d). No changes in quadriceps tissue oxygenation ($p = 0.210$; Figure 7c) or quadriceps skin blood flow ($p = 0.462$) were observed during lower leg and foot heating. However, similar to the control leg during whole leg heating, hamstrings tissue oxygenation increased slightly by 6 ± 3% rSO_2 units ($p = 0.015$; Figure 7c). At the systemic level, heart rate, cardiac output, stroke volume, and mean arterial pressure remained stable with averages of 59 ± 8 bpm, 5.2 ± 0.9 L min^{-1}, 89 ± 18 ml, and 93 ± 43 mmHg, respectively (all $p > 0.05$) (Table 3). In addition, no changes in control leg CFA, SFA, PFA, and POA blood flow pre- and post-protocol were observed (all $p > 0.05$).

3.4 | All protocols: comparison of changes in regional blood flow with whole leg, upper leg, and lower leg and foot hyperthermia

Individual and mean changes in whole leg, upper leg, and lower leg blood flow during the first hour of the three heating protocols are illustrated in Figure 9. The increase in whole leg blood flow was greater following 1 h of whole leg heating than during 1 h of upper- ($p = 0.007$) and lower leg heating ($p = 0.003$); however, upper leg and lower leg blood flow were not different from one another during whole leg heating ($p = 0.155$). During the upper leg heating protocol, the increase in whole leg blood flow was not different from the increase in upper leg blood flow ($p = 1.00$) but the elevation in upper leg blood flow was higher than that of lower leg blood flow ($p < 0.0001$). Last, during lower leg and foot heating, the increase in lower leg blood flow was similar to the increase in whole leg blood flow ($p = 0.982$), such that the change in lower leg blood flow was greater than that of

TABLE 3 Influence of lower leg and foot heating on central hemodynamics and common femoral and popliteal artery blood flow

		Time (min)			
Variables	**Intervention**	**Baseline**	**20**	**40**	**60**
MAP (mmHg)		93 ± 10	90 ± 9	93 ± 11	96 ± 18
$\dot{Q}$ (L min^{-1})		5.1 ± 0.9	5.2 ± 0.8	5.0 ± 1.0	5.3 ± 1.1
SV (ml)		89 ± 20	88 ± 15	89 ± 24	89 ± 14
HR (beats min^{-1})		59 ± 8	59 ± 9	58 ± 8	59 ± 8
CFA blood flow (ml min^{-1})	Heated leg	325 ± 53	432 ± 66	502 ± 119*	656 ± 157*
	Control leg	327 ± 85	—	—	314 ± 39
POA blood flow (ml min^{-1})	Heated leg	72 ± 21	140 ± 49	259 ± 89*	341 ± 117*
	Control leg	74 ± 22	—	—	64 ± 11

Values are mean ± SD for seven participants, except for blood flow values where $n = 8$. MAP: mean arterial pressure; $\dot{Q}$: cardiac output; SV: stroke volume; HR: heart rate; CFA: common femoral artery; POA: popliteal artery. Baseline to 60 min represents lower leg and foot heating responses.

* Different from baseline, $p < 0.05$.

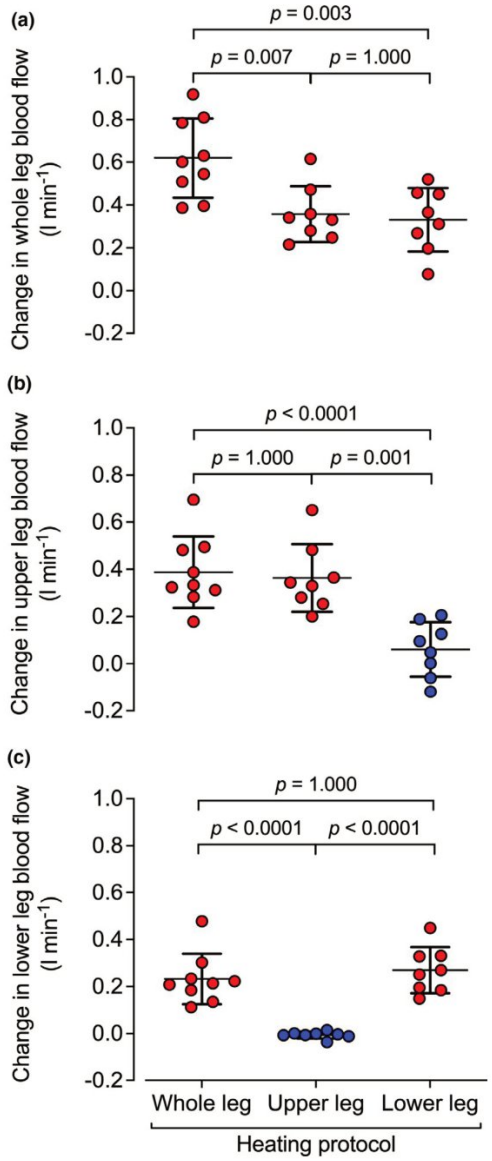

FIGURE 9 Changes in regional blood flow during 1 h whole leg and segmental leg heating. Circles depict the individual data points while the lines illustrate mean ± SD ($n = 25$). Red circles represent heated segments while blue circles represent control segments, respectively. The figure reports three levels of comparisons: whole leg versus upper leg blood flow, whole leg versus lower leg blood flow, and upper leg versus lower leg blood flow, respectively, with p values and half-tick down lines illustrating the differences. Note that increases in segmental blood flow reflect the changes in local temperature, regardless of the heating protocol

upper leg blood flow ($p = 0.008$). Moreover, these changes in upper leg and lower leg blood flow occurred exponentially to the change in temperature (Figure 10). Strong relationships between the change in upper leg blood flow and the change in upper leg temperature exist during whole leg

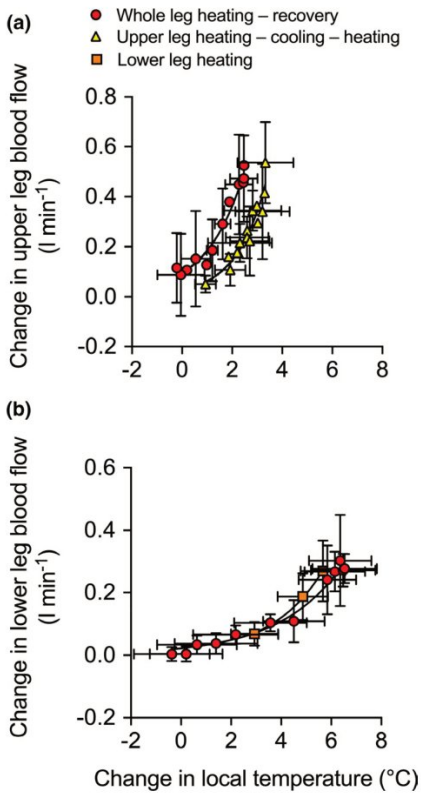

FIGURE 10 Relationship between the change in local temperature ($\overline{T}_{Leg}$) and the change in local blood flow during whole leg, upper leg, and lower leg and foot heating. Data represented as mean ± SD ($n = 25$). Vertical error bars signify Δ blood flow SD, while horizontal error bars signify Δ $\overline{T}_{Leg}$ SD, respectively

heating ($R^2 = 0.97$, $k = 0.67$ [0.57,0.79]) and upper leg heating ($R^2 = 0.89$, $k = 0.85$ [0.65,1.08]). Similarly, strong exponential relationships between the change in lower leg blood flow and the change in lower leg temperature exist during whole leg heating ($R^2 = 0.98$, $k = 0.40$ [0.33,0.49]) and lower leg and foot heating ($R^2 = 0.99$, $k = 0.49$ [0.21,0.94]).

3.5 | Wave speed, local arterial distensibility, and wave intensity parameters

Wave speed, distensibility, and wave intensity parameters measured at the CFA during prolonged whole leg heating and its subsequent recovery are reported in Table 1. Wave speed remained stable throughout the protocol in both legs ($p = 0.908$) with no differences being observed between the experimental and control leg ($p = 0.324$). Consequently, as arterial distensibility is calculated from wave speed, no changes were observed throughout the protocol ($p = 0.841$) or between legs ($p = 0.329$). Similarly, wave intensity parameters, forward compression, and forward expansion waves did not change in response to whole leg heating ($p = 0.218$

and 0.860, respectively), nor were there any differences between legs ($p = 0.371$ and 0.097, respectively).

4 | DISCUSSION

This study explored the relationships between local hyperthermia and the hemodynamic profiles of the leg major arteries and the oxygenation of the tissues they perfuse, comparing the responses of experimental and control legs as well as the upper and lower leg segments during prolonged whole leg and segmental leg heating. In line with the study's hypotheses, local macro- and microvascular blood flows were closely related to local temperature across all the experimental conditions producing large variations in local temperature, but essentially no changes in core temperature. Whole leg hyperthermia markedly increased blood flow and vascular conductance in the four major arteries during the 3 h heating protocol, then slowly declined during the subsequent recovery in association with the fall in local temperature. Segmental leg hyperthermia elicited comparable increases in regional blood flow to that of the regional hyperemia observed during whole leg hyperthermia. Additionally, increases in blood flow of the heated leg and leg segment occurred without noticeable changes to perfusion pressure or mean conduit artery diameter, while local tissue oxygenation, blood velocity, and blood flow were positively related to local temperature. Together, these findings support the notion that heat activates thermosensitive mechanisms in the leg microcirculation, thereby regulating the flow of blood through the human leg during local hyperthermia.

4.1 | Impact of regional hyperthermia on leg tissue perfusion

Local hyperthermia, whether it be through prolonged whole or segmental leg heating, resulted in sustained ≥3-fold increases in regional tissue perfusion. In all hyperthermic conditions, arterial blood flow was closely coupled with changes in local temperature—increasing exponentially with the rise in local hyperthermia. Of note is the strikingly similar increases in blood flow in the upper and lower leg during 1 h of segmental and whole leg hyperthermia (Figure 9). Previous studies have characterized the significant effects of segmental leg hyperthermia on global and local limb blood flow (Heinonen et al., 2011; Kuhlenhoelter et al., 2016; Romero et al., 2017; Thomas et al., 2017; Walsh et al., 2019), and the blood flow differences between the upper and lower leg under normothermia (Klein et al., 2003), and during whole body hyperthermia with lower leg occlusion (Chiesa et al., 2016). The present study is the first to comprehensively and directly compare the responses of the major leg arteries and the regional leg blood flow distribution during segmental and whole leg hyperthermia, in an attempt to isolate the effects of local thermosensitive regulatory mechanisms on thermal hyperemia. At baseline, we observed a ~ 3:1 distribution in blood flow between the upper and lower leg, in agreement with the previous literature (Chiesa et al., 2016; Klein et al., 2003). However, when upper and lower leg blood flow are expressed per 100 g of tissue, the blood flow values are similar, both at baseline and following 3 h of whole leg heating (2–3 and 9–10 ml min^{-1} 100 g^{-1}, respectively). This suggests that the approximately three times greater mass (Wang et al., 1999) and, by extension, more abundant muscle, skin, fat, and bone vasculature of the upper leg in comparison to the lower leg, might largely account for the higher absolute blood flow of the upper leg. Notwithstanding, we consistently observed lower baseline tissue oxygenation in the lower leg and foot compared to the upper leg, suggestive of greater basal oxygen extraction from the circulation in response to lower local perfusion and tissue temperature (Davis et al., 2006). These indications of a coupling between lower temperature and blood perfusion in the lower leg and foot are consistent with observations that the distal regions of the leg are more susceptible to ischemia in disease conditions such as peripheral arterial disease and diabetes (Hills et al., 2009; Hirsch et al., 2006; Ouriel, 2001). Yet, in the present study, both leg segments were found to be highly responsive to prolonged leg hyperthermia, with the relative increase in blood flow being greater in the lower leg than the upper leg (3.8- vs. 2.6-fold, respectively). Thus, the degree of tissue perfusion and temperature heterogeneity will, therefore, diminish with leg hyperthermia compared to control conditions (Figures 2 and 3). Nonetheless, the presently observed close temporal relationships between blood flow and local temperature and between regional tissue oxygenation and local temperature in both the experimental and control legs during the 6 h leg heating and recovery protocol, strongly support a causal link between local hyperthermia and hyperemia.

The blood flow responses of the major leg arteries to segmental leg heating reveal new insights into the regulation of tissue blood flow in human limbs during hyperthermia. Upper leg heating induced profound increases in thigh temperature and CFA blood flow (~3-fold), yet lower leg temperature and POA blood flow decreased somewhat or remained unchanged (Figure 5). The observed upper leg hyperemia during the first hour of upper leg heating elicited similar increases in upper leg blood flow to those observed during whole leg hyperthermia (~370 ml min^{-1}) and those reported in the literature during isolated limb heating and whole body heating (Chiesa et al., 2016; Heinonen et al., 2011; Pearson et al., 2011). In congruence with the findings during upper leg heating, lower leg and foot heating resulted in substantial increases in lower leg temperature and CFA, SFA, and POA blood flow, while PFA blood flow and upper leg temperature remained stable.

The observation that the increase in CFA and SFA blood flow—which feeds into the POA—is similar to the increase in POA blood flow, lends additional support to the argument that upper leg blood flow remained unchanged. Furthermore, the magnitude of the lower leg hyperemia was similar to that evoked by 1 h of whole leg hyperemia (241–270 ml min^{-1}; Figure 9). Moreover, increased tissue oxygenation—an index of microcirculatory blood flow—of the heated leg segment occurred in parallel to the regional hyperemia, while much like blood flow, no changes were observed in the non-heated adjacent leg segment. Collectively, the similar magnitude of regional blood flow and tissue oxygenation, regardless of whether the heating intervention is being applied to a leg segment or the whole leg, provides additional compelling evidence supporting local hyperthermia as the putative stimulus for the greatly enhanced hyperemia.

4.2 | Tissue perfusion regulation during local hyperthermia

Local hyperemia was associated with increases in local vascular conductance in all hyperthermic protocols, while perfusion pressure remained unchanged. A major aim of the present study was to identify the precise vascular locus in which hyperthermia modulates blood flow and tissue perfusion and in doing so, address the contribution between peripheral and central regulatory mechanisms. The primary forces that cause the movement of blood through a vessel are thought to be a positive pressure gradient and an increase in vascular conductance. Throughout all three heating protocols, no noticeable changes in perfusion pressure were observed—much like the majority of studies investigating the hemodynamic responses to local limb heating (Chiesa et al., 2016; Kalsi et al., 2017; Kuhlenhoelter et al., 2016; Pearson et al., 2011). In this study, segmental leg heating resulted in ≥3-fold increases in tissue perfusion, and considerable increases in regional tissue oxygenation and skin blood flow, while the adjacent leg segment displayed no changes in these variables. Moreover, we observed no changes in cardiac stroke volume or wave intensity-derived forward compression and forward expansion waves in the heated and control legs. This suggests that left ventricular contractility and late systolic flow deceleration were unchanged throughout the whole leg heating protocol even though cardiac output did increase to a similar extent as the rise in heated leg blood flow during the 3 h whole leg heating protocol (0.7–0.8 L min^{-1}). Hence, the activation of regulatory mechanisms and pathways in the peripheral circulation rather than augmented central hemodynamic forces (Blair et al., 1960; Edholm et al., 1957; Roddie et al., 1957; Rowell, 1974) must explain the robust increases in blood velocity and flow in the vasculature of the limb hyperthermic tissues.

The increase in blood flow to the heated leg region occurred in the face of a maintained diameter in all examined conduit arteries, including those of the control leg and/or control leg segment where blood velocity and flow did not change. The present findings agree with studies reporting no changes in conduit artery diameter during limb heating (Chiesa et al., 2016; Coombs et al., 2019, 2021; Pearson et al., 2011; Teixeira et al., 2017), but are at odds with studies showing decreases in POA diameter (Thomas et al., 2017). The unchanged conduit artery diameter, together with the general increases in local vascular conductance with whole leg and segmental leg hyperthermia, suggest that vasodilatation might have instead occurred in the downstream small arteries and resistance arterioles, and/or alternatively thermosensitive physical and chemical mechanisms governing blood's rheological properties and kinetic energy permitted the increase in microvascular blood velocity and flow.

Interestingly, the studies assessing the impact of temperature variations in in vitro skeletal muscle vessel preparations show, for the most part, that the temperature *per se* does not exert a direct effect on smooth muscle contractile function (Ives et al., 2011; Vanhoutte & Shepherd, 1970). This lends support to the view that heat is predominantly acting indirectly via changes in temperature-dependent blood viscosity, red blood cell deformability and dispersion, and/or intravascular vasodilatory mechanisms (Akyurekli et al., 1997; Artmann et al., 2008; Binzoni et al., 2012; Chiesa et al., ,2015, 2016; Heinonen et al., 2011; Stadler et al., 2012). Although not investigated herein, strong relationships exist between (1) increases in blood and tissue temperatures (Chiesa et al., 2015; Kalsi et al., 2017); (2) elevations in temperature and reductions in blood viscosity and frictional resistance (Çinar et al., 2001; Lim et al., 2010; Shin et al., 2004; Snyder, 1971); and (3) increases in temperature and rises in red cell deformability and dispersion (Çinar et al., 2001; Manteuffel-Szoege, 1960, 1969; Pinho et al., 2016). Hence, heat *per se* may reduce blood viscosity and vascular resistance, and increase red cell deformability, red cell dispersion, blood velocity, and blood kinetic energy. It, therefore, seems plausible that heat-modulated blood rheology explains at least part of the ≥3-fold elevation in thermal hyperemia.

Another likely possibility is that local hyperthermia induces downstream vasodilatation via heat-sensitive biochemical signals that (1) activate intravascular signaling-transduction mechanisms in the microvasculature such as shear-mediated nitric oxide release and cellular oxidative stress (Gifford et al., 2014; Kellogg et al., 1999; Laughlin et al., 2008; Minson et al., 2001; Paniagua et al., 2001; Romero et al., 2017) and/or (2) stimulate the release of vasoactive molecules from the circulating erythrocytes such as ATP (Kalsi et al., 2017; Kalsi & González-Alonso, 2012; Pearson et al., 2011). In support of the involvement of red cell

signaling mechanisms, close relationships have been reported between increases in temperature and erythrocyte-derived ATP release, but not other blood constituents (Etulain et al., 2011; Kalsi et al., 2017; Kalsi & González-Alonso, 2012), and between increases in plasma ATP and local limb hyperthermia (González-Alonso et al., 2015; Kalsi et al., 2017; Kalsi & González-Alonso, 2012; Pearson et al., 2011). Furthermore, increases in plasma ATP with intra-arterial infusion cause profound elevations in limb blood velocity and tissue perfusion, independent of temperature, metabolic or perfusion pressure changes (González-Alonso et al., 2008; Kalsi et al., 2017; Kalsi & González-Alonso, 2012). Taken together, the present and previous experimental evidence suggest that local hyperthermia likely increases local perfusion through the activation of vascular thermosensitive mechanisms that cause microvessel vasodilatation.

4.3 | Hyperthermia influence on local arterial distensibility

Another finding of the present study is that prolonged whole leg hyperthermia did not alter CFA distensibility in either the experimental or control legs. To our knowledge, this is the first study to directly assess the influence of limb hyperthermia on local arterial stiffness and distensibility using the ln(D)U-loop method. Despite the ≥3-fold increases in blood velocity, no changes were seen in wave speed or arterial distensibility. The present findings of local stiffness are in agreement with past whole body and two leg hyperthermia studies which reported no changes in regional (carotid–radial) arterial stiffness (Ganio et al., 2011; Moyen et al., 2016; Schlader et al., 2019). However, studies exploring the recovery following hyperthermia, reported decreases in peripheral and/or leg (femoral–ankle region) arterial stiffness alongside an elevated core temperature (Caldwell et al., 2017; Cheng et al., 2021; Lee et al., 2018; Sugawara & Tomoto, 2021; Thomas et al., 2017). The latter observations contrast with the unaffected or small changes in arterial stiffness/distensibility, core temperature, and arterial pressure observed in the present and previous single leg heating studies (Chiesa et al., 2015, 2016; Engelland et al., 2020; Takahashi et al., 2011). Previous studies have alluded to the possibility that a certain threshold of hyperthermic intensity—such that can initiate profound increases in core temperature and/or alterations in sympathetic activity—may be required to elicit reductions in arterial stiffness and associated increases in distensibility (Caldwell et al., 2017; Kaldur et al., 2016). Therefore, although further studies are warranted, the present findings indicate that single leg hyperthermia does not alter CFA stiffness and distensibility and thus, conduit artery vascular tone in conditions evoking no or negligible elevations in core temperature.

4.4 | Perspectives and significance

The present study provides substantial evidence to indicate that limb tissue blood flow during regional hyperthermia is controlled by highly localized events in the microcirculation, as opposed to central hemodynamic forces or thermal reflexes responding to increases in core temperature. In this construct, the heart accommodates the increase in leg tissue perfusion by augmenting cardiac output, rather than playing a major role in the control of tissue blood flow and its distribution, as demonstrated here by (1) the unchanged PFA blood flow, quadriceps tissue oxygenation, and skin blood flow during lower leg and foot heating, despite the substantial increases in CFA, SFA, and POA blood flow (Figure 7), and (2) the unchanged POA blood flow and calf and foot tissue oxygenation during upper leg heating in the face of markedly elevated CFA blood flow (Figure 5). Future studies can use the approach employed by Watanabe et al., (2020) during exercise to directly test this hypothesis, by simultaneously measuring vascular and cardiac function during passive regional and systemic hyperthermia.

The profound and sustained increases in blood flow and shear rate with prolonged leg heating may serve as effective hemodynamic stimuli for improving vascular health. Shear stress—which increased ≥3-fold in the present study—is widely accepted as an important stimulus for vascular remodeling (Girerd et al., 1996; Green et al., 2017; Vita et al., 2008; Zarins et al., 1987). Numerous intervention studies have investigated the effects of repeated two leg or whole body heating and the associated hyperemia on vascular health, reporting improvements in endothelial function (Brunt et al., 2016; Carter et al., 2014; Imamura et al., 2001; Kihara et al., 2002; Ohori et al., 2012). The advantage of the present local leg hyperthermia approach—in contrast to severe two leg or whole body heating interventions—is that the ensuing hemodynamic stimuli can be applied over prolonged periods without significant systemic physiological strain or thermal discomfort. Therefore, local limb heating may provide an effective alternative to promote beneficial arterial adaptations that improve vascular health in people with reduced or limited exercise capacity.

4.5 | Experimental considerations

Some experimental considerations should be acknowledged when interpreting the present findings. As reported in the methods, different individuals participated in the three protocols which make this study a between-subjects design as opposed to the gold standard within-subjects design. Nevertheless, the data from the three participants completing all three protocols and published data (Chiesa et al., 2015, 2016; Keller et al., 2010; Pearson et al., 2011) during 1 h

whole leg heating interventions in young healthy adults reveal comparable and reproducible leg blood flows to those observed in the present study (i.e., 0.5–0.6 ± 0.2 L min^{-1} increase in CFA blood flow). While the present protocols were different to address specific but complementary aims, the inter-protocol comparisons were conducted during the first hour of each protocol when the methodology was identical, other than the obvious distinction of the heated region. Consequently, the highly reproducible hemodynamic responses to local leg and segmental hyperthermia and the fact that the responses were compared during the same timeframe, strongly support that our data and interpretations are valid and robust.

5 | SUMMARY

In conclusion, the present study provides comprehensive and compelling evidence on the effects of local hyperthermia in human leg circulation. Prolonged whole leg hyperthermia produces a profound and sustained elevation in upper and lower leg blood flow, while segmental leg hyperthermia induces hyperemia to a magnitude that matches the regional hyperemia during whole leg heating without affecting blood flow, temperature or tissue oxygenation of the non-heated limb segment. Increases in local tissue oxygenation, blood flow, vascular conductance, and blood velocity were positively related to the rise in local temperature, yet these increases occurred without any changes to mean perfusion pressure, conduit artery diameter or wave intensity-derived parameters. Collectively, these findings support the notion that local hyperthermia increases peripheral tissue perfusion through the activation of local thermosensitive mechanisms. These mechanisms are proposed to increase microvascular blood flow by inducing blood rheology-mediated increases in vascular conductance and/or vasodilatation in the microcirculation. The markedly enhanced hyperemia and tissue oxygenation strongly support the therapeutic potential of local hyperthermia for the treatment of circulatory diseases and/or rehabilitation.

ACKNOWLEDGMENTS

We give special thanks to the participants in these studies. We also thank Alessandro Giudici, Madalina Negoita, Francesca R Cavallo, Kazuhito Watanabe, Scott Chiesa, and Tom Howes for their excellent technical support.

CONFLICTS OF INTERESTS

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

This study was performed at Brunel University London, Uxbridge, UK. NKE and JGA conceived and designed the research. NKE, ORG, AWK, and JGA acquired the data. NKE analyzed the data. NKE, AWK, and JGA interpreted the data. All authors revised the manuscript and provided intellectual feedback and agreed to be accountable for all aspects of the work.

DATA AVAILABILITY STATEMENT

The raw, unidentified data collected throughout this study is available via Brunel Figshare, an online data repository database. https://doi.org/10.17633/rd.brunel.14749386.

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How to cite this article: Koch Esteves, N., Gibson, O. R., Khir, A. W., & González-Alonso, J. (2021). Regional thermal hyperemia in the human leg: Evidence of the importance of thermosensitive mechanisms in the control of the peripheral circulation. *Physiological Reports*, 9, e14953. https://doi.org/10.14814/phy2.14953

Study 2. Thermo-haemodynamic coupling during regional thigh heating: Insight in to the importance of local thermosensitive mechanisms in blood circulation

Received: 28 September 2023 | Accepted: 2 January 2024

DOI: 10.1113/EP091556

RESEARCH ARTICLE

Experimental Physiology WILEY

Thermo-haemodynamic coupling during regional thigh heating: Insight into the importance of local thermosensitive mechanisms in blood circulation

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Handling Editor: David Edwards

Abstract

A positive relationship between local tissue temperature and perfusion exists, with isolated limb-segment hyperthermia stimulating hyperaemia in the heated region without affecting the adjacent, non-heated limb segment. However, whether partial-limb segment heating evokes a heightened tissue perfusion in the heated region without directly or reflexly affecting the non-heated tissues of the same limb segment remains unknown. This study investigated, in 11 healthy young adults, the lower limb temperature and haemodynamic responses to three levels of 1 h upper-leg heating, none of which alter core temperature: (1) whole-thigh (WTH; water-perfused garment), (2) quadriceps (QH; water-perfused garment) and (3) partial-quadriceps (PQH; pulsed shortwave diathermy) heating. It was hypothesised that perfusion would only increase in the heated regions. WTH, QH and PQH increased local heated tissue temperature by 2.9 ± 0.6, 2.0 ± 0.7 and 2.9 ± 1.3°C ($P < 0.0001$), respectively, whilst remaining unchanged in the non-heated hamstrings and quadriceps tissues during QH and PQH. WTH induced a two-fold increase in common femoral artery blood flow ($P < 0.0001$) whereas QH and PQH evoked a similar ~1.4-fold elevation ($P \leq 0.0018$). During QH and PQH, however, tissue oxygen saturation and laser-Doppler skin blood flow in the adjacent non-heated hamstrings or quadriceps tissues remained stable ($P > 0.5000$). These findings in healthy young humans demonstrate a tight thermo-haemodynamic coupling during regional thigh heating, providing further evidence of the importance of local heat-activated mechanisms on the control of blood circulation.

KEYWORDS
blood flow, haemodynamics, heat, thermal mechanisms

1 | INTRODUCTION

A well-established, positive relationship exists between local tissue temperature and blood flow during whole-body or local-limb hyperthermia (Chiesa et al., 2016; Heinonen et al., 2011; Johnson & Proppe, 1996; Kalsi et al., 2017; Koch Esteves et al., 2021; Pearson et al., 2011). Recent research from our laboratory demonstrated that segmental-limb heating – that is, isolated upper- and lower-leg heating – produces comparable magnitudes of conduit artery hyperaemia for their respective limb segment during whole-leg heating, without affecting tissue temperature or perfusion in the adjacent non-heated segment (Koch Esteves et al., 2021). These findings suggest that during segmental-limb heating, adjacent limb segments are not affected by central neural reflexes (Barcroft et al., 1947; Johnson et al., 1976) or indirect heating, whether it be through conductive or convective heat transfer via the tissues and blood, respectively (González-Alonso, 2012; Incropera et al., 1996; Xiang & Liu, 2008).

Explanations for the observed maintenance of adjacent, non-heated segment perfusion could be that it is due to (a) an insufficient hyperthermic stimulus to evoke reflex drives (Taylor et al., 1984) or (b) an excessively steep positive temperature gradient between the heated and non-heated segments, whereby heat – whether via conductance in the tissues or convection in the blood – is being quickly dissipated in the normothermic limb segment as per Pennes's bioheat equation (Arkin et al., 1994; Fiala & Havenith, 2015; Incropera et al., 1996; Pennes, 1948). However, whether this phenomenon would hold true within a limb segment where the heated and non-heated areas are in proximity remains unclear. For instance, would solely heating the whole or a portion of the quadriceps increase temperature and thus blood flow in the hamstrings and non-heated quadriceps section or would the hyperaemia be solely confined in the heated area? Research exploring the clinical application of targeted hyperthermia on tumours has found large increases in tissue perfusion and oxygenation of the heated tissue (Bosque et al., 2021; Elming et al., 2019; Song, 1984), while the temperature of the adjacent healthy tissue remained unaltered (Kim et al., 1977; LeVeen et al., 1976; Suit & Gerweck, 1979). Although the unchanged temperature of the adjacent tissue is indicative of a maintained tissue perfusion, direct measures of macro- and microcirculation tissue perfusion in healthy limb tissue are required to verify or refute this assumption.

Consequently, the aim of the present study was to comprehensively assess the relationship between local limb-segment heating and the ensuing hyperaemia, specifically investigating temperature, tissue oxygenation and haemodynamic responses in the major arteries of the human leg during whole-thigh heating (WTH), quadriceps heating (QH) and partial-quadriceps heating (PQH). It was hypothesised that: (a) all local hyperthermia interventions would increase the blood flow profiles of the femoral arteries which supply the upper leg, with the magnitude of hyperaemia being proportional to the volume of heated tissue, and (b) muscle and skin temperature, tissue perfusion, oxygen saturation and skin blood flow would remain unchanged in the adjacent non-heated areas.

Highlights

- **What is the central question of the study?**
  Limb-segment hyperthermia increases perfusion without evoking haemodynamic changes in the adjacent limb-segment: does this hold true within a small limb-area?
- **What is the main finding and its importance?**
  Local whole-thigh, quadriceps and partial-quadriceps heating increased tissue temperature, blood flow and oxygen saturation in the heated region without affecting these parameters in the non-heated area within the same segment. These findings further support the notion that local thermosensitive mechanisms are the primary regulators of thermal hyperaemia.

2 | METHODS

2.1 | Ethical approval

The study was approved by the Brunel University London Research Ethics Committee (38109-MHR-Oct/2022-41690-2) and was performed in accordance with the *Declaration of Helsinki*, except for registration in a database. All participants provided informed written consent prior to their participation in the present study following a detailed written and verbal explanation of the experimental protocol.

2.2 | Participants

Eleven healthy, physically active adults (4 women) participated in the present study. Participants had a mean ± SD age of 22 ± 6 years, a height of 174.4 ± 8.8 cm and body mass of 76.1 ± 13.1 kg (Table 1). Participants were considered healthy and physically active following the completion of a health questionnaire and a basic cardiovascular screening. Participants refrained from heavy exercise for 48 h, alcohol consumption for 24 h and caffeine consumption for 12 h before the commencement of the protocols. Moreover, female participants were requested to avoid scheduling their laboratory visit during their menses.

2.3 | Experimental protocols

The present study consisted of three protocols: (a) WTH, (b) QH and (c) PQH, which were conducted over two visits (Figure 1). Protocol 1 was completed during visit A whilst protocols 2 and 3 were completed during another visit, B, with the order being counterbalanced among

participants. Upon arriving at the laboratory, participants were asked to weigh themselves in a semi-nude state and then had their height measured (Seca 798 scale, Hamburg, Germany) and in the case for visit 1 only, had their leg anthropometric measurements recorded. The latter data allowed an estimate of leg composition using the method reported by Jones and Pearson (1969). Seven leg circumferences were taken at the gluteal furrow, one-third subischial (one-third of the distance between the gluteal furrow and the popliteal crease), the minimum circumference above the knee, the maximum circumference at the knee joint, the minimum circumference below the knee, the maximum circumference at the calf and the minimum circumference at the ankle joint. Additionally, skinfold measurements were obtained at the following four sites: one-third subischial (anterior and posterior sites) and at the maximum calf circumference (lateral and medial sites) using skinfold callipers (Jones & Pearson, 1969). Subsequently, participants sat on a custom-built bed within a climate chamber room set at an ambient temperature and humidity of 22°C and 30–40%, respectively.

2.3.1 | Protocol 1: effects of WTH

Participants were instrumented with ECG electrodes, temperature thermistors, tissue oxygenation optode pads and the finometer upper-arm and middle finger cuffs (described below). The experimental protocol was initiated with baseline haemodynamic measurements of the common femoral artery (CFA), superficial femoral artery (SFA), profunda (deep) femoral artery (PFA) and popliteal artery (POA) in the right leg. Next, participants were fitted with a custom-made water-perfusion trouser on their right upper leg, which was then wrapped in a survival blanket to optimise the heating procedure by limiting heat loss from the trouser to the surrounding environment. The trouser was connected to a thermostatically controlled water circulator (Julabo F-34, Seelbach, Germany), which continuously circulated water at a temperature of 50°C. During the 1 h heating protocol, blood flow was measured every 15 min at the CFA, SFA, PFA and POA of the right, experimental leg (Figure 1).

2.3.2 | Protocol 2: effects of QH

Following instrumentation of ECG electrodes, temperature thermistors, tissue oxygenation optode pads and finometer cuffs – much like protocol 1 – participants had baseline haemodynamic measurements of the CFA, SFA, PFA and POA in the right leg. Subsequently, participants were fitted with a custom-made water-perfusion trouser which solely covered the top of their right upper leg (i.e. the quadriceps). A survival blanket was then placed on top of the heated trouser to limit heat loss; however, care was taken not to cover the remainder of the upper leg (i.e. the hamstrings) which was left exposed. The trouser was heated as described in protocol 1. During the 1 h heating protocol, blood flow was measured every 15 min at the CFA, SFA, PFA and POA of the right, experimental leg (Figure 1).

2.3.3 | Protocol 3: effects of PQH

This protocol followed immediately after the completion of protocol 2. Baseline haemodynamic measurements of the CFA, SFA, PFA and POA were recorded in the left leg. PQH was conducted using a pulsed shortwave diathermy (MegaPulse II, EMS Physio, Wantage, UK) at 800 pulses per second, with a pulse duration of 400 μs. The heating drum has a surface area of ~200 cm^2. Thus, if one assumes that whole-thigh and quadriceps hyperthermia heat 100 and 50% of total upper-leg surface area, respectively, using the anthropometric measures calculated from the present cohort, partial-quadriceps hyperthermia heated ~8% of the upper-leg (i.e. 16% of the quadriceps). During the 1-h heating protocol, blood flow was measured every 15 min at the CFA, SFA, PFA and POA of the left, experimental leg (Figure 1).

2.4 | Temperature measurements

Core temperature (T_c) was measured using a rectal probe (Ret-1 Special, Physitemp, Clifton, NJ, USA) which was self-inserted 15 cm past the sphincter muscle. Tympanic temperature (T_{Tym}) was measured using a commercially available thermometer (Thermoscan 7, Braun, Germany). Skin temperature (T_{sk}) was measured in the quadriceps at two different locations (one proximal and one distal) and the hamstrings of the experimental leg using commercially available thermistors (IT-18, Physitemp, Clifton, NJ, USA) which were securely held in place using medical tape. Muscle temperature (T_m) in the proximal portion of the vastus lateralis muscle (during all three protocols), the distal portion of the vastus lateralis muscle (during protocol 3), and in the middle of the biceps femoris muscle of the right leg (during protocols 1 and 2) was measured using thermistors (T-204f, Physitemp) inserted via an 18G catheter ~3 cm deep into the muscle. T_c, T_{sk} and T_m were recorded online using a commercially available thermocouple metre (TC-2000, Sable Systems International, Las Vegas, NV, USA) collected at 1000 Hz through a data acquisition system (PowerLab 26T, ADInstruments, Dunedin, New Zealand), and exported in 30-s bins using a commercially available data acquisition software (LabChart 7, ADInstruments). Data were then imported and analysed in Microsoft Excel software, and reported as 2-min averages. Temperature data are reported every 5 min for the first 15 min of protocols 1 and 2 to better characterise the sharp increase in tissue temperatures and then every 15 min, in parallel with haemodynamic measurements, for the remainder of the protocols. During protocol 3, temperature data are reported at baseline, 30 and 60 min due to interference between the thermocouple metre and diathermy unit. In addition, mean tissue temperature for the heated regions was calculated using:

$$\bar{T}_{\text{Whole-thigh}} = \left(\frac{T_{\text{m,quad1}} + T_{\text{m,ham}}}{2} \times 0.92\right) + \left(\frac{T_{\text{sk,quad1}} + T_{\text{sk,quad2}} + T_{\text{sk,ham}}}{3} \times 0.08\right),$$

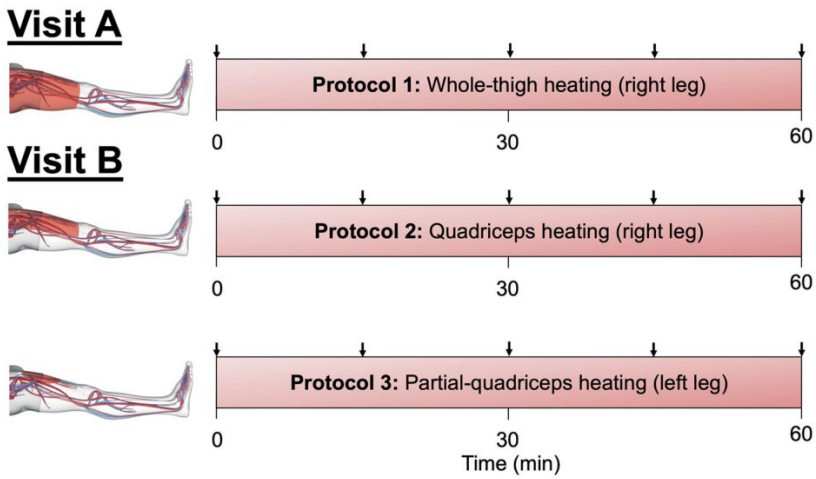

FIGURE 1 Schematic representation of experimental protocol. Arrows illustrate the times in which an ultrasound blood flow measurement was conducted. Blood flow was measured at the common, superficial and profunda femoral arteries and popliteal artery. Core temperature, leg muscle and skin temperatures, leg tissue oxygen saturation, leg skin blood flow and central haemodynamics were measured continuously throughout the protocol. Protocol 1 was conducted during visit A, and protocols 2 and 3 on visit B. The respective visits were randomised and counterbalanced.

$$\bar{T}_{\text{Quadriceps}} = (T_{\text{m,quad1}} \times 0.92) + \left(\frac{T_{\text{sk,quad1}} + T_{\text{sk,quad2}}}{2} \times 0.08\right) \text{ and } \bar{T}_{\text{Partial-quadriceps}} = (T_{\text{m,quad1}} \times 0.92) + (T_{\text{sk,quad1}} \times 0.08),$$

where quad1, quad2 and ham represent the proximal and distal portions of the quadriceps and hamstrings, respectively. Mean tissue temperature formulas were created using previously reported volume ratios of the different tissue compartments in the leg (Wang et al., 1999).

2.5 | Haemodynamic measurements

Heart rate was continuously measured using a three-lead echocardiogram. Also, arterial blood pressure was measured non-invasively – at the same time points as arterial blood flow measurements – using infrared photoplethysmography (Finometer, Finapres Medical Systems, Enschede, Netherlands), through a cuff on the middle finger of the right hand. Blood flow was measured at set time points (Figure 1) – recording two 12-s Doppler images – throughout the protocols in the various arteries using a duplex Doppler ultrasound system (Vivid E95, GE Medical Systems, Little Chalfont, UK) with a 9-MHz linear array transducer probe (GE Medical Systems) at an insonation angle of ≤60°, with sample volume positioned in the centre of the artery. Before commencing baseline blood flow measures, arterial sites for the CFA, SFA, PFA and POA, in the right leg during protocol 1 and both legs for protocols 2 and 3, were located and marked to ensure blood flow measures which were consistently measured at the same site. SFA and PFA blood flow measurements were acquired at a distance of ≥2 cm from the femoral bifurcation to prevent turbulent flow disruption to the measurements, and thus improve validity of measures. Blood flow (ml min^{-1}) was calculated using the following equation: $\text{BF} = V_{\text{mean}} \times \pi \times (\frac{D_{\text{mean}}}{2})^2 \times 60$, where V_{mean} is the average centreline blood velocity (cm s^{-1}) and D_{mean} (cm) is the average internal diameter calculated using: $D_{\text{mean}} = \frac{1}{3}(D_{\text{systole}}) + \frac{2}{3}(D_{\text{diastole}})$ (Rådegran, 1997). Furthermore, upper-leg blood flow was calculated as the difference between whole-leg blood flow (CFA) and lower-leg blood flow (POA).

Shear rate (SR) was calculated using: $\text{SR} = \frac{4 \times V_{\text{mean}}}{D_{\text{mean}}}$, where V_{mean} is mean blood velocity. Additionally, vascular conductance (VC) was

TABLE 1 Participant demographic and anthropometric characteristics.

Variable	Value
Age (years)	22 ± 6
Sex (*n* (%))	
Female	4 (36)
Male	7 (64)
Height (cm)	174.4 ± 8.8
Mass (kg)	76.1 ± 13.1
Right leg volume (l)	12.2 ± 2.4
Right leg lean volume (%)	74.7 ± 9.1
Right leg non-lean volume (%)	25.3 ± 9.1
Left leg volume (l)	12.2 ± 2.3
Left leg lean volume (%)	74.9 ± 9.1
Left leg non-lean volume (%)	25.1 ± 9.1

Values are means ± SD, except where stated otherwise, for 11 participants.

calculated using: VC = BF/MAP, represented as ml min^{-1} $mmHg^{-1}$, BF is blood flow (ml min^{-1}) and MAP is mean arterial pressure (mmHg). Blood flow was analysed offline using a commercially available software (EchoPAC, GE Medical, Horton, Norway). Blood velocity was averaged over two 12-s Doppler images, and average diameter was determined from four 2D B-mode images. Furthermore, central haemodynamic data were collected at 1000 Hz using a commercially available data acquisition system (PowerLab 26T, ADInstruments) and exported in 30-s bins using a commercially available data acquisition software (LabChart 7, ADInstruments). Following exportation, data were imported and analysed in Microsoft Excel software. Data are reported as 2-minute averages throughout the three protocols. Furthermore, quadriceps skin blood flow was measured in all three protocols via laser-Doppler flowmetry (PeriFlux Flowmetry System, Perimed, Järfälla, Denmark), reported in perfusion units (PU). The probe was attached to the skin of the thigh, specifically on the distal portion of the vastus lateralis. During protocols 1 and 2, skin blood flow was measured under the heated region; however, during protocol 3, it was measured in the non-heated area of the quadriceps.

2.6 | Tissue oxygen saturation measures

Direct and continuous measurements of regional tissue haemoglobin oxygen saturation were obtained in the experimental upper legs using a near-infrared spectroscopy unit (NIRS; INVOS 5100C Cerebral Oximeter; Somanetics Corp, Troy, MI, USA). The optodes were placed on the skin surrounding the quadriceps and hamstrings of the experimental upper leg, with the same positioning as the T_{sk} thermistors, and taped to reduce interference from external light sources. It was not possible to measure tissue oxygen saturation at the heated region during protocol 3 – that is, proximal quadriceps under the diathermy unit – due to interference between the near-infrared spectroscopy and diathermy units.

2.7 | Statistical analysis

Statistical analysis was conducted using R Studio (version 2022.07.1+554, Team (2022)). An independent Student's *t*-test was conducted to identify any differences in anthropometric data between the right and left leg. In addition, linear mixed-effects models were employed to investigate differences between protocols and over time in all measured variables – that is, central and local haemodynamics, temperature and tissue oxygenation – during the three heating protocols. The linear mixed-effects models were conducted following the confirmation of the data's normality via the Shapiro–Wilk test and Mauchly's test of sphericity. Following the linear mixed-effects models, once a significant interaction between protocol and time was found, a Bonferroni *post hoc* test was conducted to locate the specific time points at which those changes occurred. Significance was set at $P < 0.05$. Note, if no time points or protocols are specified, *P*-values were with reference to the interaction (time × protocol) following the linear mixed-effects model; if time points or protocols are specified, then *P*-values were obtained from the *post hoc* test. Results are expressed as means ± SD, with all data corresponding to the experimental legs. Moreover, linear, exponential and polynomial regression curve fit tests were performed using GraphPad Prism (version 8, GraphPad Software, La Jolla, CA, USA) to assess the relationships among various key data, with R^2 representing the goodness of fit. Subsequently, Akaike's information criterion was used to evaluate which model provides the most appropriate fit.

3 | RESULTS

3.1 | Demographic and anthropometric characteristics

Demographic and anthropometric data for the participants are reported in Table 1. No differences were observed in leg volume ($P = 0.748$) or proportion of lean to non-lean mass ($P = 0.797$) between the right and left legs.

3.2 | Regional and core temperature responses to whole-thigh, quadriceps and partial-quadriceps heating

Leg muscle and skin temperatures are illustrated in Figure 2, whilst core and tympanic temperatures are reported in Table 2. As per experimental design, all three heating protocols induced significant increases in tissue temperature of their respective heated regions ($P = 0.0145$), whilst the measured unheated regions during QH and PQH remained unchanged (Figure 2). Specifically, WTH increased $\bar{T}_{Whole-thigh}$ to 37.3 ± 0.4°C ($\Delta = +2.9 \pm 0.6$°C; $P < 0.0001$), QH increased $\bar{T}_{Quadriceps}$ to 37.2 ± 0.3°C ($\Delta = +2.0 \pm 0.7$°C; $P < 0.0001$) and PQH increased $\bar{T}_{Partial-quadriceps}$ to 36.8 ± 1.3°C ($\Delta = +2.9 \pm 1.3$°C; $P < 0.0001$), with these temperatures representing the average tissue temperature of the respective heated regions. No between-protocol differences were observed in the tissue temperature of the heated regions. Moreover, core temperature was stable during QH and PQH but decreased marginally following 1 h of WTH protocol ($\Delta = -0.2 \pm 0.3$; $P < 0.001$) (Table 2). No between-protocol differences in core temperature were observed (Table 2). Lastly, no differences across time or between protocols were observed in tympanic temperature ($P = 0.2664$) (Table 2).

3.3 | Leg haemodynamics, tissue oxygen saturation and systemic haemodynamics during whole-thigh, quadriceps and partial-quadriceps heating

Complete haemodynamic responses during 1 h of WTH, QH and PQH for the CFA, SFA, PFA and POA are reported in Table 3 and

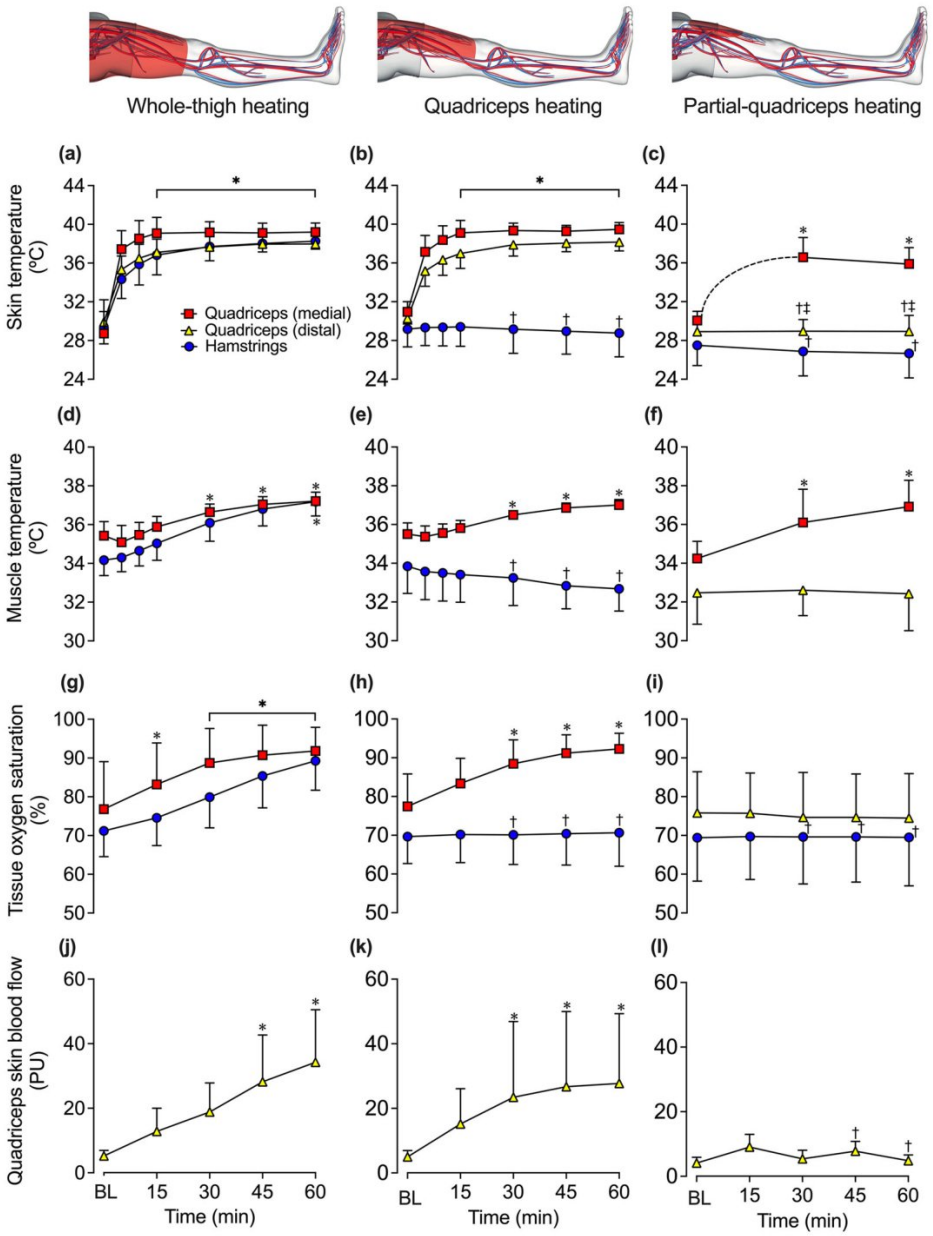

FIGURE 2 Skin and muscle leg temperatures (a–f), regional tissue oxygen saturation (g–i) and skin blood flow (j–l) during whole-thigh (a, d, g, j), quadriceps (b, e, h, k) and partial-quadriceps (c, f, i, l) hyperthermia. Data represented as means ± SD ($n = 11$). BL signifies baseline measurements. Due to the fewer data points recorded during partial-quadriceps heating, a curved dotted line between BL and 30 min quadriceps (medial) skin temperature data points was plotted to illustrate the predicted change in temperature over time based on the literature (Hafen et al., 2018). Quadriceps regional tissue oxygenation and skin blood flow were measured within the heated area during protocols 1 and 2; however, it was only measured outside the heated proximal quadriceps area during protocol 3. *Different from baseline across time within the same protocol, $P < 0.05$. †Different from whole-thigh heating, $P < 0.05$. ‡Different from quadriceps heating, $P < 0.05$.

Figure 3. Femoral artery blood flow increased steadily during WTH with CFA, SFA and PFA blood flow increasing ~2-fold above baseline (Δ = +0.31 ± 0.16, +0.13 ± 0.12 and +0.13 ± 0.07 l min^{-1}, respectively) following 1 h (all $P < 0.0001$). One hour of QH resulted in a smaller magnitude of upper-leg tissue perfusion in comparison to WTH ($P = 0.0080$; Figure 4) with CFA blood flow increasing ~1.4-fold (Δ = +0.15 ± 0.09 l min^{-1}, $P = 0.0018$); however, no significant changes were observed in SFA ($P = 0.0836$) and PFA ($P = 0.5546$) blood flow. PQH increased CFA and PFA blood flow by ~1.4–1.6-fold (Δ = +0.15 ± 0.12 and +0.07 ± 0.06 l min^{-1}, $P = 0.0006$ and

TABLE 2 Influence of whole-thigh, quadriceps and partial-quadriceps heating on body temperature, and central haemodynamics.

	Time (min)				
Variable	**0**	**15**	**30**	**45**	**60**
T_c (°C)					
Whole-thigh heating	37.2 ± 0.2	37.0 ± 0.2	37.0 ± 0.2*	37.0 ± 0.3*	37.0 ± 0.3*
Quadriceps heating	37.1 ± 0.2	36.9 ± 0.2	36.9 ± 0.2	36.9 ± 0.2	36.9 ± 0.2
Partial-quadriceps heating	36.9 ± 0.2	–	36.9 ± 0.2	–	37.1 ± 0.2
T_{Tym} (°C)					
Whole-thigh heating	36.9 ± 0.2	36.8 ± 0.2	36.8 ± 0.2	36.7 ± 0.2	36.7 ± 0.2
Quadriceps heating	36.7 ± 0.4	36.7 ± 0.3	36.7 ± 0.4	36.7 ± 0.4	36.6 ± 0.4
Partial-quadriceps heating	36.7 ± 0.4	–	36.7 ± 0.4	–	36.7 ± 0.4
HR (beats min^{-1})					
Whole-thigh heating	64 ± 7	65 ± 8	64 ± 8	65 ± 7	66 ± 6
Quadriceps heating	60 ± 8	59 ± 7	60 ± 7	59 ± 8	59 ± 8
Partial-quadriceps heating	59 ± 9	57 ± 6	59 ± 8	58 ± 8	62 ± 8
MAP (mmHg)					
Whole-thigh heating	87 ± 8	89 ± 8	89 ± 10	88 ± 14	90 ± 13
Quadriceps heating	92 ± 13	88 ± 13	90 ± 11	92 ± 11	90 ± 10
Partial-quadriceps heating	93 ± 10	93 ± 11	97 ± 11	98 ± 14	95 ± 13

Values are means ± SD for 11 participants. *Different from baseline, $P < 0.05$. Abbreviations: HR, heart rate; MAP, mean arterial pressure; T_c, core temperature; T_{Tym}, tympanic temperature.

$P = 0.0002$, respectively) whilst SFA blood flow remained unchanged ($P = 1.000$). These increases in upper-leg blood flow were not lower than those observed during WTH ($P = 0.3392$) and QH ($P = 1.0000$) (Figure 4). During all thigh heating protocols, POA blood flow remained unchanged ($P = 0.0642$) (Figure 4). Whole-leg blood flow was related (second-order polynomial) to increases in mean tissue temperature of the heated region during WTH ($R^2 = 0.35$), QH ($R^2 = 0.19$) and PQH ($R^2 = 0.22$) (Figure 5). No relationship was observed between mean tissue temperature and diameter (Figure 5); as such, no changes in diameter were observed in the CFA ($P = 0.4231$), SFA ($P = 0.6718$) and PFA ($P = 0.0642$). Correspondingly, relationships (second order polynomial) were observed between blood velocity and mean tissue temperature during WTH ($R^2 = 0.54$), QH ($R^2 = 0.27$) and PQH ($R^2 = 0.36$) (Figure 5), which mirrored the changes in blood flow. Moreover, quadriceps skin blood flow increased ~6.6-fold ($P < 0.0001$) and ~5.6-fold ($P < 0.0001$) during WTH and QH, respectively, but remained unchanged during PQH ($P = 1.0000$) as the optode was placed outside the heated area (Figure 2).

Similar responses to blood flow were observed in VC and SR during the three heating protocols (Figure 5 and Table 3). Following 1 h of WTH, VC and SR increased ~1.7–2.1-fold and ~1.6–2-fold, respectively, in all three femoral arteries (all $P < 0.0001$). During QH, CFA VC and SR increased ~1.4-fold ($P = 0.0019$ and $P = 0.0077$), respectively, and was lower than WTH ($P = 0.0050$ and 0.0130). However, during QH, no differences in SR across time were observed in the SFA and PFA ($P \geq 0.1401$) and as such, SFA and PFA SR and PFA VC were lower than WTH (all $P < 0.05$) whilst SFA VC was not ($P = 0.1328$). Conversely, PQH instigated ~1.4 increases in CFA VC ($P = 0.0033$) and SR ($P = 0.0006$), and ~1.6 in PFA VC ($P = 0.0005$) and SR ($P < 0.0001$). VC and SR were lower during PQH in comparison to whole-thigh for all arteries ($P < 0.05$) apart from CFA and PFA SR which were not different ($P = 0.2807$ and $P = 0.4048$, respectively). No differences, however, were observed between QH and PQH (all $P = 1.0000$).

Tissue oxygen saturation responses are illustrated in Figure 3. During WTH, quadriceps and hamstrings tissue oxygen saturation increased steadily, peaking at 92 ± 6% units (Δ = +15 ± 8% units, $P < 0.0001$) and 89 ± 8% units (Δ = +18 ± 5% units, $P < 0.0001$), respectively. Similarly, following 1 h of QH, quadriceps tissue oxygen saturation increased to 92 ± 4% units (Δ = +15 ± 6% units, $P < 0.0001$), which was similar to that observed during WTH ($P = 1.000$). Conversely, hamstring tissue oxygen saturation remained unchanged during QH ($P = 1.000$), which differed from the responses observed during WTH ($P < 0.0001$). There were no changes observed in quadriceps ($P = 0.5270$) and hamstring ($P = 0.9960$) tissue oxygen saturation during PQH, as both sites were measured outside the heated area. As such, quadriceps tissue oxygen saturation was lower following 1 h of PQH in comparison to WTH ($P < 0.0001$) and QH ($P < 0.0001$). Similarly, hamstring tissue oxygen saturation was lower following 1 h of PQH in comparison to WTH ($P < 0.0001$) but not different from QH ($P = 1.000$). Lastly, at the systemic haemodynamic level, no differences in heart rate and mean arterial pressure were observed during or between any of the three heating protocols ($P = 0.1689$ and $P = 0.7958$, respectively) (Table 2).

TABLE 3 Influence of whole-thigh, quadriceps and partial-quadriceps heating on leg haemodynamics.

	Time (min)				
Variable	**0**	**15**	**30**	**45**	**60**
CFA blood flow (l min^{-1})					
Whole-thigh heating	0.33 ± 0.10	0.39 ± 0.13	0.45 ± 0.11*	0.59 ± 0.23*	0.64 ± 0.20*
Quadriceps heating	0.34 ± 0.08	0.37 ± 0.09	0.44 ± 0.16	0.48 ± 0.16*	0.48 ± 0.12*†
Partial-quadriceps heating	0.33 ± 0.09	0.38 ± 0.13	0.41 ± 0.13	0.48 ± 0.18*	0.48 ± 0.14*
SFA blood flow (l min^{-1})					
Whole-thigh heating	0.17 ± 0.03	0.19 ± 0.06	0.22 ± 0.05	0.26 ± 0.11*	0.30 ± 0.12*
Quadriceps heating	0.17 ± 0.04	0.20 ± 0.06	0.19 ± 0.06	0.23 ± 0.10	0.23 ± 0.07†
Partial-quadriceps heating	0.18 ± 0.06	0.19 ± 0.05	0.19 ± 0.06	0.19 ± 0.6	0.21 ± 0.08†
PFA blood flow (l min^{-1})					
Whole-thigh heating	0.12 ± 0.04	0.15 ± 0.07	0.17 ± 0.07*	0.23 ± 0.09*	0.25 ± 0.10*
Quadriceps heating	0.12 ± 0.04	0.12 ± 0.04	0.14 ± 0.04	0.14 ± 0.03†	0.16 ± 0.04†
Partial-quadriceps heating	0.11 ± 0.03	0.13 ± 0.07	0.14 ± 0.05	0.16 ± 0.05	0.18 ± 0.05*
POA blood flow (l min^{-1})					
Whole-thigh heating	0.09 ± 0.02	0.09 ± 0.03	0.08 ± 0.03	0.09 ± 0.04	0.09 ± 0.03
Quadriceps heating	0.09 ± 0.04	0.10 ± 0.03	0.09 ± 0.03	0.10 ± 0.03	0.09 ± 0.03
Partial-quadriceps heating	0.10 ± 0.04	0.09 ± 0.03	0.08 ± 0.02	0.08 ± 0.03	0.07 ± 0.02
CFA vascular conductance (ml min^{-1} $mmHg^{-1}$)					
Whole-thigh heating	3.6 ± 1.1	4.1 ± 1.3	5.0 ± 1.3	6.6 ± 2.6*	7.1 ± 1.9*
Quadriceps heating	3.8 ± 1.1	4.3 ± 1.2	5.0 ± 1.6	5.3 ± 1.7*	5.4 ± 1.2*†
Partial-quadriceps heating	3.5 ± 0.9	4.0 ± 1.4	4.2 ± 1.4	4.9 ± 1.6†	5.1 ± 1.2*†
SFA vascular conductance (ml min^{-1} $mmHg^{-1}$)					
Whole-thigh heating	1.9 ± 0.4	2.0 ± 0.6	2.5 ± 0.6	3.0 ± 1.3*	3.3 ± 1.2*
Quadriceps heating	1.9 ± 0.5	2.3 ± 0.7	2.2 ± 0.7	2.5 ± 1.0	2.6 ± 0.6†
Partial-quadriceps heating	2.0 ± 0.6	2.0 ± 0.5	2.0 ± 0.5	2.0 ± 0.5†	2.2 ± 0.8†
PFA vascular conductance (ml min^{-1} $mmHg^{-1}$)					
Whole-thigh heating	1.3 ± 0.5	1.5 ± 0.7	1.9 ± 0.8*	2.5 ± 1.2*	2.7 ± 1.0*
Quadriceps heating	1.3 ± 0.5	1.5 ± 0.5	1.6 ± 0.5	1.6 ± 0.5†	1.8 ± 0.5†
Partial-quadriceps heating	1.2 ± 0.3	1.4 ± 0.6	1.4 ± 0.5	1.6 ± 0.5†	1.9 ± 0.4*
POA vascular conductance (ml min^{-1} $mmHg^{-1}$)					
Whole-thigh heating	1.0 ± 0.3	0.9 ± 0.3	0.9 ± 0.3	1.0 ± 0.4	1.0 ± 0.3
Quadriceps heating	1.0 ± 0.4	1.1 ± 0.3	1.0 ± 0.3	1.1 ± 0.3	1.0 ± 0.2
Partial-quadriceps heating	1.1 ± 0.3	0.9 ± 0.3	0.9 ± 0.3	0.7 ± 0.4	0.8 ± 0.3
CFA shear rate (s^{-1})					
Whole-thigh heating	51 ± 16	56 ± 15	67 ± 21	81 ± 18*	90 ± 22*
Quadriceps heating	51 ± 15	54 ± 13	62 ± 17	69 ± 20*	70 ± 17*†
Partial-quadriceps heating	48 ± 13	55 ± 15	57 ± 12	68 ± 19*	69 ± 18*
SFA shear rate (s^{-1})					
Whole-thigh heating	53 ± 19	56 ± 16	68 ± 20	77 ± 24*	87 ± 30*
Quadriceps heating	56 ± 17	59 ± 13	57 ± 15	69 ± 20	68 ± 16
Partial-quadriceps heating	55 ± 14	56 ± 13	57 ± 11	58 ± 14	62 ± 20†
PFA shear rate (s^{-1})					
Whole-thigh heating	63 ± 16	73 ± 22	86 ± 22	113 ± 29*	126 ± 39*
Quadriceps heating	62 ± 17	66 ± 14	74 ± 16	77 ± 19	87 ± 29†
Partial-quadriceps heating	60 ± 14	68 ± 16	74 ± 27	88 ± 29	102 ± 41*

(Continues)

TABLE 3 (Continued)

Variable	Time (min)				
	0	15	30	45	60
POA shear rate (s^{-1})					
Whole-thigh heating	38 ± 13	36 ± 12	35 ± 9	39 ± 13	39 ± 13
Quadriceps heating	37 ± 8	38 ± 8	37 ± 5	40 ± 9	38 ± 8
Partial-quadriceps heating	42 ± 9	36 ± 10	35 ± 7	34 ± 8	31 ± 9

Values are means ± SDs for 11 participants. *Different from baseline, $P < 0.05$. †Different from whole-thigh heating, $P < 0.05$. Abbreviations: CFA, common femoral artery; PFA, profunda femoral artery; POA, popliteal artery; SFA, superficial femoral artery.

4 | DISCUSSION

The present study explored the relationship between local tissue temperature and perfusion during three levels of regional thigh heating to gain further insight into the importance of local thermosensitive mechanisms in the control of blood circulation. As per the study's primary hypothesis, all heating protocols increased CFA blood flow, with WTH evoking a larger magnitude of hyperaemia than QH, but surprisingly, not greater than PQH. Moreover, in line with the secondary hypothesis, tissue temperature, blood flow and oxygen saturation remained unchanged in the respective non-heated hamstrings, quadriceps and lower-leg segment. Together, the present findings demonstrate a close coupling among tissue perfusion, oxygen saturation and temperature during regional thigh heating, which further substantiates the local origin of the mechanisms involved in the control of blood circulation during isolated hyperthermia.

4.1 | Influence of regional thigh heating on tissue thermo-haemodynamics

In this study, all three modalities of upper-leg heating – WTH, QH and PQH – evoked sustained 1.4–2-fold increases in CFA blood flow (Figure 3). In line with previous studies, local hyperthermia-induced increases in leg tissue perfusion, assessed via CFA blood flow, occurred in relation to increases in local tissue temperature (Chiesa et al., 2016; Koch Esteves et al., 2021, 2023). A previous study from our laboratory revealed that the magnitude of hyperaemia was associated with the volume of the heated limb segment, with whole-leg heating evoking the largest degree of hyperaemia, proportionally followed by upper- and lower-leg heating, respectively (Koch Esteves et al., 2021). The present study extends those findings by showing that the hyperaemia during 1 h QH and WTH (+0.15 and +0.31 l min^{-1}, respectively) was proportional to that previously reported during 1 h whole-leg heating (+0.62 l min^{-1}) (Koch Esteves et al., 2021). These observations together substantiate the impact of volume of heated tissue on the magnitude of hyperaemia evoked by the same type of thermal intervention (whole-leg > whole-thigh > quadriceps). Furthermore, in line with the literature, WTH stimulated a two-fold increase in upper-leg blood flow without affecting the unheated lower leg or contralateral leg (Koch Esteves et al., 2021). A novel finding was that the 1.4-fold increase in upper-leg blood flow during PQH and QH was confined to the directly heated tissues. This interpretation is supported by the observations that hamstrings tissue oxygen saturation – a surrogate for tissue perfusion (Davis et al., 2006) – and hamstring skin and muscle temperature in the experimental leg remained unchanged during QH and that the temperature, skin blood flow and tissue oxygen saturation of the unheated area of the quadriceps (≤18 cm distal to the heated source) was unaffected during PQH (Figure 2). Collectively, these data indicate that tissue blood flow during isolated heating, including a small area of the quadriceps muscles and overlying subcutaneous fat and skin, is regulated in direct response to increases in local tissue temperature.

4.2 | Interaction among heating modalities and tissue temperature in the regulation of tissue perfusion

A pertinent finding from the present study is that the type of thermal intervention can influence the magnitude of thermal stimulus and hyperaemia. The present study used a hot water-perfused garment during WTH and QH, and diathermy during PQH. Interestingly, the magnitude of hyperaemia induced by 1 h of PQH was not different from WTH and QH despite only targeting approximately 8% of the upper-leg's total surface area. Diathermy is known to increase muscle temperature at a depth of ~3 cm by 3–4°C within 30 min (Benincá et al., 2021; Draper et al., 2013; Garrett et al., 2000; Hafen et al., 2018). In this study, PQH increased muscle temperature from 34°C to 37°C. Although a similar peak quadriceps muscle temperature of ~37°C was observed in the three protocols, the magnitude of increase was different with WTH, QH and PQH increasing quadriceps muscle temperature by 1.8, 1.5 and 2.7°C, respectively. The thermal stimulus was, therefore, potentially greater during PQH, which could explain the significant hyperaemia despite the smaller heated area. Previous studies which have employed local leg heating and leg cooling following heating or exercise provide strong evidence that tissue perfusion of the temperature-manipulated region more closely mirrors deep muscle (2–3 cm) temperature in comparison to skin temperature (Caldwell et al., 2016; Chiesa et al., 2016; Gregson et al., 2011; Heinonen et al., 2011; Mawhinney et al., 2013, 2017; Pearson et al., 2011; Sekins et al., 1984). Most striking is that diathermy has been shown to increase

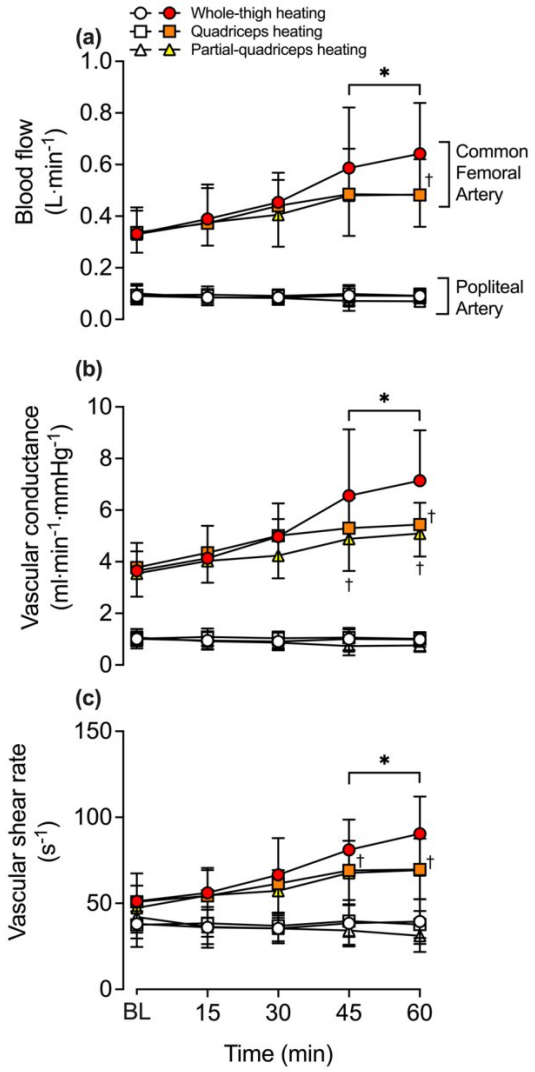

FIGURE 3 Blood flow (a), vascular conductance (b) and shear rate (c) during whole-thigh, quadriceps and partial-quadriceps hyperthermia. Data represented as means ± SD ($n = 11$) for the common femoral (CFA) and popliteal (POA) arteries, represented by filled and open symbols, respectively. BL signifies baseline measurements. *Different from baseline across time within the same protocol, $P < 0.05$. †Different from whole-thigh heating.

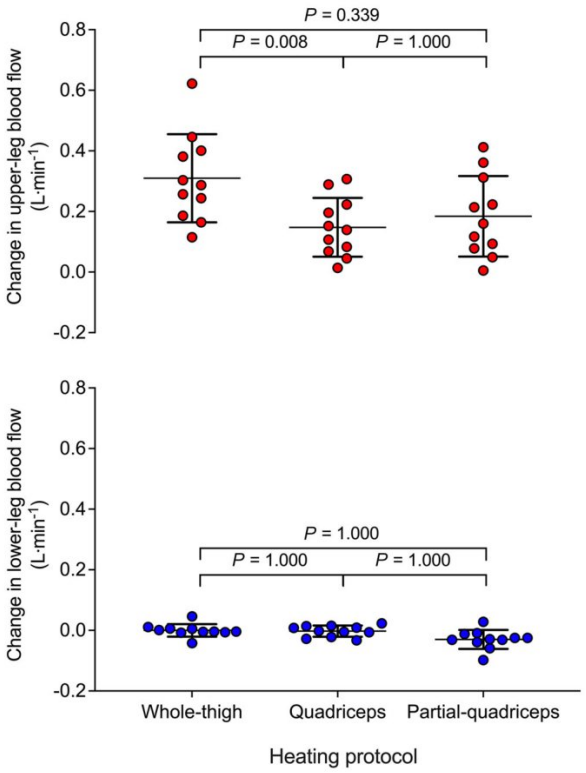

FIGURE 4 Individual changes in regional blood flow following 1 h whole-thigh, quadriceps and partial-quadriceps hyperthermia. Circles depict the individual data points while the lines illustrate means ± SD ($n = 11$). Red circles represent heated segments while blue circles represent control segment.

thigh muscle temperature from ~34°C to ~42°C during simultaneous skin cooling with a concomitant elevation in muscle blood flow from ~3 ml min^{-1} 100 g^{-1} up to ~48 ml min^{-1} 100 g^{-1} (Sekins et al., 1980, 1982, 1984). Taken together, these findings indicate that the level of muscle thermal stimulus also impacts the magnitude of rise in tissue perfusion.

Temperature, tissue perfusion and tissue oxygen saturation remained unchanged in the non-heated limb hamstrings and proximal quadriceps tissues during QH and PQH (Figure 2). Limb tissue perfusion during systemic hyperthermia is postulated to be mediated through local and central thermosensitive mechanisms, working either separately or in tandem (Chiesa et al., 2019; Johnson & Proppe, 1996). The present finding that local tissue perfusion and temperature were unchanged in the non-heated areas alongside a maintained core temperature aligns with past studies advocating for the predominant role of local thermosensitive mechanisms in hyperthermia-induced hyperaemia (Chiesa et al., 2015, 2016; Kalsi et al., 2017; Koch Esteves et al., 2021). However, this finding conflicts with past studies that have demonstrated the influence of an augmentation in central haemodynamics, such as a cutaneous vasodilatory reflex drive, during whole-body and/or local-limb heating where blood flow increases not only in heated limb but also in the non-heated or cooled contralateral limb (Caldwell et al., 2016; Heinonen et al., 2011; Johnson et al., 1976; Mallette et al., 2016; Taylor et al., 1984). In those studies, core temperature was regarded as the primary stimulus underlying the global haemodynamic adjustments: a 1°C increase in core temperature is associated with an approximately nine-fold, 3 l min^{-1}, and 35 beats min^{-1} increase in skin blood flow, cardiac output and heart rate, respectively (Chiesa et al., 2019; Johnson & Park, 1979). In support, Heinonen et al. (2011) found that during unilateral calf heating where core temperature remained unchanged, blood flow

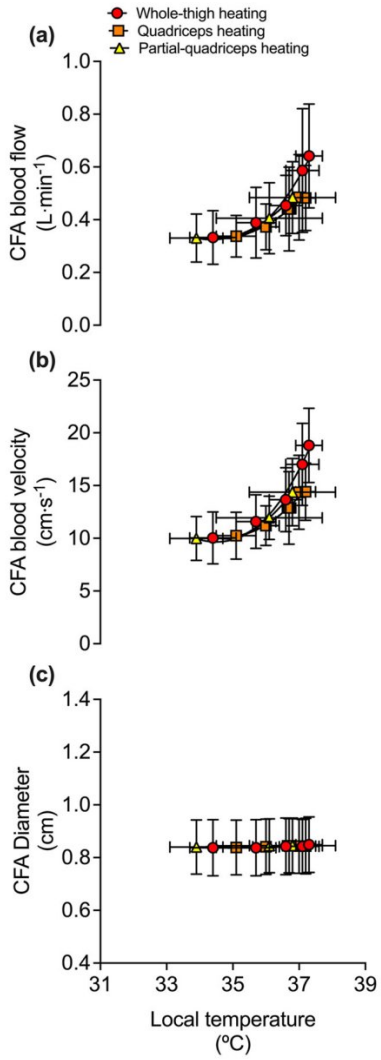

FIGURE 5 Relationship between the mean local tissue temperature and common femoral artery (CFA) blood flow (a), blood velocity (b) and arterial diameter (c) during whole-thigh, quadriceps and partial-quadriceps hyperthermia. Local temperature signifies the corresponding average heated tissue temperature for the protocol, as discussed in the methods – that is, $\bar{T}_{Whole-thigh}$, $\bar{T}_{Quadriceps}$ and $\bar{T}_{Partial-quadriceps}$. Data represented as means ± SD ($n = 11$). Vertical error bars signify blood flow SD, while horizontal error bars signify local temperature SD. Lines represent the exponential fit of the data.

increased in the heated calf but not in the control, contralateral calf. However, during whole-body heating where core temperature increased by 1°C, blood flow not only increased in the heated calf but also in the non-heated, contralateral calf alongside large increases in local vascular conducance (Heinonen et al., 2011). The observed hyperaemia in the unheated contralateral calf supports that core temperature can indeed stimulate a vasodilatory reflex drive (Heinonen et al., 2011). This scenario is different from the present experimental conditions where core body temperature remained unaltered. During the isolated hyperthermia conditions of this study, local thermosensitive mechanisms play a primary role in the regulation of peripheral tissue perfusion. In support of the existence of local temperature-sensitive regulatory mechanisms, a recent study utilising a 3-day-old chick embryo model revealed that after the heart has been arrested with KCl, blood velocity in the vitelline vessel still increased ~ 3.7-fold in response to infrared radiation (heat energy) but ceased completely when the heat source was taken away. Hence, the present data together with previous observations in the literature (Manteuffel-Szoege, 1960, 1969) indicate that a heat-dependent mechanism can operate in the circulatory system (Li & Pollack, 2023).

The present observations raise the question of why increases in temperature and perfusion were confined to the area directly under the heat source. Heat can be transferred between biological tissues through two pathways: intercellular conductive and vascular convective heat transfer (González-Alonso, 2012; Khaled & Vafai, 2003). Conduction is the transfer of heat energy through a positive temperature gradient across media in direct contact (Incropera et al., 1996), and is known to be a relatively slow process as it is reliant upon a positive temperature gradient and the thermal conductivity of the surrounding tissues (González-Alonso, 2012). This pathway is responsible for the transfer of heat from the water-perfused garment to the upper leg skin during WTH and QH via a positive thermal gradient between the garment and skin. On the other hand, given that the upper leg contains a dense vascular network consisting of arterioles, post-capillary venules, capillaries and their cellular constituents (Guven et al., 2020), convection – the transfer of heat through the movement of blood – plays a significant role in heat transfer within a limb (Fiala & Havenith, 2015; Incropera et al., 1996). The resultant concomitant increase in internal limb tissue perfusion during local heating further increases convective heat transfer through the various microvessels perfusing the internal tissues, exchanging heat in the capillary beds until it reaches equilibrium with the surrounding tissues (Chato, 1980; Fiala & Havenith, 2015). Whilst the blood perfusing the heated tissues can act as a heat source to the neighbouring unheated tissues (Baish et al., 1986), the large disparity between heated and non-heated tissue volume as well as the vast network of vessels means that the heat is quickly dissipated in the surrounding unheated tissues and/or heat transfer is very small between the heated and non-heated areas. This is supported by past studies demonstrating a 5–10°C difference in tissue temperature between the heated tumour and adjacent tissues during targeted tumour hyperthermia, though the distance between the heated tumour and unheated adjacent tissue is unknown (Kim et al., 1977; LeVeen et al., 1976; Suit & Gerweck, 1979). Consequently, the present findings provide evidence that local heating of a limb segment does not cause a heightened tissue perfusion to the proximal non-heated tissues which quickly dissipate the heat, likely due to a combination of an insufficient thermal stimulus and the human body's ability to maintain thermal homoeostasis when heating is applied to a small segment of the human limb.

4.3 | Experimental considerations

There are several methodological considerations in this study. First, during QH and PQH, it is not possible to directly measure which tissues are being perfused via the femoral conduit arteries. Consequently, tissue oxygen saturation measures of the heated and non-heated regions were used to assess regional tissue perfusion. Whilst there is some debate as to whether tissue oxygen saturation data solely reflect muscle perfusion or a combination of cutaneous and muscle perfusion, there is a consensus that it does indeed provide a measure of tissue perfusion (Choo et al., 2017; Davis et al., 2006; Koch Esteves et al., 2021; Pearson et al., 2011). Thus, we are confident in our interpretation of the data. Second, during PQH, baseline skin and muscle temperatures were lower than in the other two protocols as the leg was left exposed in ambient conditions for >1 h whilst protocol 2 was being performed on the right leg. This introduced challenges when making between-protocol comparisons. Third, during PQH, the electromagnetic waves from the diathermy unit interfered with some of the data recording equipment. Hence, we were only able to collect temperature data by pausing the diathermy unit for 1 min. While this does not directly affect the quality or validity of the present findings, it would have been of great value to have continuously measured the increase in temperature at the onset of heating as diathermy has been shown to rapidly increase muscle temperature (Benincá et al., 2021; Draper et al., 2013; Garrett et al., 2000; Hafen et al., 2018). Fourth, the sum of SFA and PFA blood flow does not perfectly match the measured CFA blood flow. This is likely due to the difficulty faced when attempting to obtain high-quality images of the PFA, in comparison to other main conduit leg arteries, as the PFA is highly influenced by the individual vessel anatomy (Hussain, 1997; Tomaszewski et al., 2017; Koch Esteves & Chiesa, 2021). Nonetheless, this potential limitation does not affect the interpretation and conclusion of the present findings. Lastly, whilst female participants were requested not to attend during menses, the cycle phase and use of hormonal contraceptives were not controlled for. Thus, whilst we acknowledge that not obtaining cycle and contraceptive information is a limitation, we believe that our data and findings remain valid and reliable.

4.4 | Summary

Passive WTH, QH and PQH evoked 1.4–2-fold increases in leg blood flow which mirrored the increases in local tissue temperature. Moreover, the QH and PQH protocols increased tissue perfusion to their respective heated regions without affecting perfusion and tissue oxygen saturation of the non-heated area within the same segment, likely due to an unchanged tissue temperature. These results therefore indicate a strong relationship between local tissue temperature and perfusion even in a small area of a human lower limb. Ultimately, this close relationship, regardless of the volume of the heated limb, further highlights the significance of local thermosensitive mechanisms in the regulation of tissue perfusion during hyperthermia.

AUTHOR CONTRIBUTIONS

This study was performed at Brunel University London, Uxbridge, UK. Nuno Koch Esteves, Jeneil McDonald and José González-Alonso conceived and designed the research. Nuno Koch Esteves, Jeneil McDonald and José González-Alonso acquired the data. Nuno Koch Esteves analysed the data. Nuno Koch Esteves and José González-Alonso interpreted the data. All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

ACKNOWLEDGEMENTS

We give special thanks to the participants without whom, this study would not have been possible. Additionally, we would like to thank David McDougall for his assistance with the graphics.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

FUNDING INFORMATION

No funding was received for this work.

DATA AVAILABILITY STATEMENT

The raw, unidentified data collected throughout this study will be made available via Brunel Figshare, an online data repository database.

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How to cite this article: Koch Esteves, N., McDonald, J., & González-Alonso, J. (2024). Thermo-haemodynamic coupling during regional thigh heating: Insight into the importance of local thermosensitive mechanisms in blood circulation. *Experimental Physiology*, 1–14.
https://doi.org/10.1113/EP091556

Study 3. Lower limb hyperthermia augments functional hyperaemia during small muscle mass exercise similarly in trained elderly and young humans

Received: 4 May 2023 | Accepted: 23 June 2023

DOI: 10.1113/EP091275

Experimental Physiology WILEY

RESEARCH ARTICLE

Lower limb hyperthermia augments functional hyperaemia during small muscle mass exercise similarly in trained elderly and young humans

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Handling Editor: Paul Fadel

Abstract

Heat and exercise therapies are recommended to improve vascular health across the lifespan. However, the haemodynamic effects of hyperthermia, exercise and their combination are inconsistent in young and elderly people. Here we investigated the acute effects of local-limb hyperthermia and exercise on limb haemodynamics in nine healthy, trained elderly (69 ± 5 years) and 10 young (26 ± 7 years) adults, hypothesising that the combination of local hyperthermia and exercise interact to increase leg perfusion, albeit to a lesser extent in the elderly. Participants underwent 90 min of single whole-leg heating, with the contralateral leg remaining as control, followed by 10 min of low-intensity incremental single-leg knee-extensor exercise with both the heated and control legs. Temperature profiles and leg haemodynamics at the femoral and popliteal arteries were measured. In both groups, heating increased whole-leg skin temperature and blood flow by 9.5 ± 1.2°C and 0.7 ± 0.2 L min^{-1} (>3-fold), respectively ($P < 0.0001$). Blood flow in the heated leg remained 0.7 ± 0.6 and 1.0 ± 0.8 L min^{-1} higher during exercise at 6 and 12 W, respectively ($P < 0.0001$). However, there were no differences in limb haemodynamics between cohorts, other than the elderly group exhibiting a 16 ± 6% larger arterial diameter and a 51 ± 6% lower blood velocity following heating ($P < 0.0001$). In conclusion, local hyperthermia-induced limb hyperperfusion and/or small muscle mass exercise hyperaemia are preserved in trained older people despite evident age-related structural and functional alterations in their leg conduit arteries.

KEYWORDS
ageing, blood flow, exercise, haemodynamics, heat

1 | INTRODUCTION

Ageing is associated with numerous structural–functional alterations in the peripheral vasculature and the myocardium. These typically include increases in arterial diameter, wall thickness and stiffness, left ventricular hypertrophy, elevations in arterial blood pressure and reductions in maximal cardiac output (Ferrari et al., 2003; Hossack & Bruce, 1982; Nichols et al., 2022; Rosenthal, 1987; Sonesson et al., 1993; Thijssen et al., 2016). Maximal limb hyperaemia (functional hyperaemia) is also reduced in elderly populations during maximal aerobic exercise, a physical effort that engages a large muscle mass (Beere et al., 1999; Poole et al., 2003; Proctor et al., 1998, 2004; Wahren et al., 1974). Findings during small muscle mass knee-extensor exercise are inconsistent, however, with studies showing a blunted hyperaemic response to low- to maximal-intensity exercise in sedentary and normally active elderly participants (Donato et al., 2006; Lawrenson et al., 2003; Mortensen et al., 2012) but not in life-long endurance-trained elderly counterparts (Mortensen et al., 2012). The age-related attenuation in functional hyperaemia during low-intensity knee-extensor exercise is surprising given that a number of studies have shown no differences in lower-limb blood flow between young and aged adults during matched low-intensity cycling exercise in sedentary (Beere et al., 1999; Poole et al., 2003), normally active (Proctor et al., 2003) and endurance-trained (Wahren et al., 1974) participants. To address this discrepancy in the literature and minimise the influences of different levels of aerobic fitness and cardiovascular capacity, the impact of ageing on functional hyperaemia can be investigated in exercised-trained elderly people during low-intensity exercise.

Hyperthermia – which can also instigate profound increases in limb blood flow – may provide further insight into the age-related alterations in vascular function. A wealth of literature exists on the blood flow responses to passive hyperthermia in young and aged subjects indicating that elderly adults have an attenuated hyperthermia-induced elevation in forearm perfusion (Armstrong & Kenney, 1993; Minson et al., 1998; Rooke et al., 1994; Sagawa et al., 1988) and leg perfusion (Kenny et al., 2017; Romero et al., 2017). There are discrepancies in the literature, though, as two studies have reported comparable magnitude of hyperperfusion between aged cohorts in the human forearm (Kenny et al., 2017) and leg (Engelland et al., 2020). Moreover, the impact of superimposing local hyperthermia to exercise remains unresolved irrespective of the population. Three studies in young individuals show no effect of localised thigh heating during knee-extensor exercise (Ferguson et al., 2006), whole-body heating during prolonged submaximal single-leg and two-leg cycling (Savard et al., 1988), or incremental two-leg cycling to exhaustion (Trangmar et al., 2017). Conversely, two recent investigations revealed an additive effect of whole-body hyperthermia in young participants, with functional hyperaemia increasing by 0.6–0.7 L min^{-1} during single-leg knee-extensor exercise in comparison to normothermic conditions (Chiesa et al., 2015; Pearson et al., 2011). To our knowledge, the effect of lower-limb hyperthermia on the magnitude of functional hyperaemia during small muscle mass exercise in elderly adults has never been investigated. Addressing this gap in knowledge is important in determining the potential of local heating and light exercise as a therapeutic intervention to improve health in people with reduced functional capacity.

New Findings

- **What is the central question of the study?**
  Ageing is postulated to lead to underperfusion of human limb tissues during passive and exertional hyperthermia, but findings to date have been equivocal. Thus, does age have an independent adverse effect on local haemodynamics during passive single-leg hyperthermia, single-leg knee-extensor exercise and their combination?
- **What is the main finding and its importance?**
  Local hyperthermia increased leg blood flow over three-fold and had an additive effect during knee-extensor exercise with no absolute differences in leg perfusion between the healthy, exercise-trained elderly and the young groups. Our findings indicate that age per se does not compromise lower limb hyperaemia during local hyperthermia and/or small muscle mass exercise.

The aim of the present study was to comprehensively examine and compare the haemodynamic responses to single-leg hyperthermia, one-legged knee-extensor exercise and combined single-leg hyperthermia and knee-extensor exercise in aged and young adults. It was hypothesised that: (a) single-leg hyperthermia and one-legged knee-extensor exercise would increase limb blood flow, which would be tightly coupled to increases in local limb temperature and/or metabolic demands; and (b) elderly participants would demonstrate an attenuated hyperaemic response to single-leg hyperthermia and knee-extensor exercise, in comparison to the young adult group.

2 | METHODS

2.1 | Ethical approval

The study was approved by the Brunel University London Research Ethics Committee (31692-A-Nov/2021-34810-2) and was performed in accordance with the *Declaration of Helsinki*, except for registration in a database. All participants provided informed written consent prior to their participation in the present study.

TABLE 1 Participant demographic and anthropometric characteristics.

Variables	Elderly ($n = 9$)	Young ($n = 10$)
Age (years)	69 ± 5	26 ± 7
Sex, *n* (%)		
Female	3 (30%)	3 (30%)
Male	6 (60%)	7 (70%)
Height (cm)	172.9 ± 4.8	171.7 ± 6.8
Mass (kg)	68.7 ± 11.7	71.0 ± 9.7
Right leg volume (L)	10.2 ± 1.6	11.0 ± 1.9
Right leg lean volume (%)	76.3 ± 7.9	78.6 ± 6.7
Right leg non-lean volume (%)	23.7 ± 7.9	21.4 ± 6.7
Left leg volume (L)	9.8 ± 1.9	11.3 ± 1.8
Left leg lean volume (%)	76.3 ± 8.1	78.7 ± 6.9
Left leg non-lean volume (%)	23.7 ± 8.1	21.3 ± 6.9

Values are means ± SD for nine elderly and 10 young participants.

2.2 | Participants

A group of nine healthy elderly adults (three women) and a group of 10 healthy young (three women) individuals participated in this study. The elderly adults had a mean ± SD age of 69 ± 5 years, a height of 172.9 ± 4.8 cm and body mass of 68.7 ± 11.7 kg, whereas the corresponding values for the young cohort were 26 ± 7 years, 171.7 ± 6.8 cm and 71.0 ± 9.7 kg (Table 1). Prior to the start of the study, informed written consent was obtained from all participants following a detailed written and verbal explanation of the experimental protocol. Participants were considered healthy and trained following the completion of a health questionnaire and a basic cardiovascular screening. All participants regularly engaged in structured sports, endurance and/or strength and conditioning training 3–7 times per week, with each session lasting 30–120 min. There were no differences in exercise frequency, duration and modalities between aged cohorts. Participants refrained from heavy exercise for 48 h, alcohol consumption for 24 h and caffeine consumption for 12 h before the commencement of the protocols. Moreover, young female participants, who had not undergone menopause, were requested to schedule their laboratory visit during the first 7 days follow menses – that is, the early follicular phase – as it is commonly associated with the lowest levels of oestrogen and progesterone.

2.3 | Experimental protocols

Participants were asked to consume their usual breakfast and report to the laboratory between 08.00 and 10.00 h. They were weighed in a semi-nude state and had their height (SECA 798 Scale, SECA, Hamburg, Germany) and leg anthropometric measurements recorded. The latter data allowed an estimate of leg composition using the method reported by Jones and Pearson (1969). Seven leg circumferences were taken at the gluteal furrow, one-third subischial (one-third of the distance between the gluteal furrow and the popliteal crease), the minimum circumference above the knee, the maximum circumference at the knee joint, the minimum circumference below the knee, the maximum circumference at the calf and the minimum circumference at the ankle joint. Additionally, skinfold measurements were obtained at the following four sites: one-third subischial (anterior and posterior sites) and at the maximum calf circumference (lateral and medial sites) using skinfold callipers (Jones & Pearson, 1969). Subsequently, participants sat in a semi-recumbent position on the chair of a custom-built knee-extensor ergometer within the laboratory at an ambient temperature and humidity of 21°C and 30–40%, respectively. Participants were then instrumented with ECG electrodes and the finometer upper-arm and middle finger cuffs to allow the assessment of central haemodynamics.

2.3.1 | Familiarisation protocol

The familiarisation protocol commenced with a basic cardiovascular screening where participants had their ECG, cardiac output, stroke volume and leg blood flow at the common and superficial femoral arteries (SFAs) measured and evaluated. If participants reported abnormal values which were indicative of any underlying health issues, their participation in the study was terminated and they were recommended to schedule an appointment with their health practitioner. Following the successful completion of the health screening, participants had their left ankle and foot inserted and strapped into the boot of a modified dynamic knee-extensor exercise Monark ergometer. Participants were familiarised with the one-legged knee-extensor exercise (Andersen & Saltin, 1985), exercising on an unloaded ergometer for 5 min (Figure 1). Once the familiarisation with their left leg was completed, the participants' right ankle and foot were inserted and strapped into the boot of the ergometer, and the familiarisation protocol was repeated once more.

2.3.2 | Experimental protocol

The schematic for the experimental protocol is illustrated in Figure 1. Following the successful completion of the familiarisation protocol, participants rested for the following 30 min. During this time, they were instrumented with tissue oxygenation optode pads and temperature thermistors (described below). Once the participant was successfully instrumented and completed the 30-min rest period, the experimental protocol was initiated with baseline measurements of the common femoral artery (CFA), SFA, profunda (deep) femoral artery (PFA) and popliteal artery (POA) in both the right and left legs. Next, participants were fitted with a custom-made water-perfusion trouser on their right leg, which was then wrapped in a survival blanket to optimise the heating procedure by limiting heat loss from the trouser to the surrounding environment. The trouser was connected to a thermostatically controlled water circulator (Julabo F-34, Seelbach,

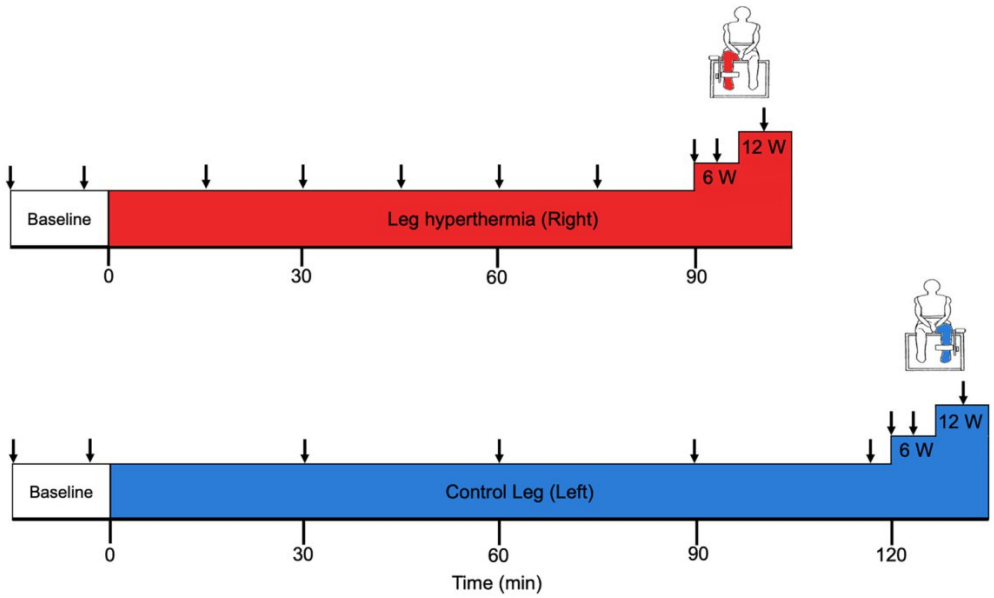

FIGURE 1 Schematic of experimental protocol. Downward arrows illustrate the times in which an ultrasound blood flow measurement was conducted. Blood flow was measured at the common, superficial and profunda femoral arteries and popliteal artery during rest and passive heating; however, blood flow was solely measured at the common and superficial femoral arteries during exercise. Core temperature, leg temperatures, leg tissue oxygen saturation and central haemodynamics were measured continuously throughout the protocol.

Germany), which continuously circulated water at a temperature of 58°C (Figure 1). While the water temperature leaving the water circulator was substantially hotter than that commonly reported in the literature (~48°C) – including other previous studies from our laboratory (Koch Esteves et al., 2021) – the actual skin temperature was similar to those in previous studies due to the larger heat loss through the smaller diameter tubing and thus reduced flow rate in the custom-made water-perfused trouser. During the 1.5-h right leg heating protocol, blood flow was measured every 15 min at the CFA, SFA, PFA and POA in the heated leg, and every 30 min in the control (left) leg.

Following passive whole leg heating, participants performed incremental one-legged knee-extensor exercise. The one-legged knee-extensor exercise consisted of two 5-min bouts, the first at 6 W and the second at 12 W (Figure 1). Intensity was controlled by increasing the resistance on the flywheel via metal weights. To account for the minor variations in cadence – that is, differences in the number of knee extensions per minute – individual work rates were calculated for each stage using the following formula: $\text{Work Rate} = \overline{\text{Cadence}} \times \text{Resistance}$. During the exercise protocol, blood flow was measured at the CFA and SFA of the right leg during the middle of each exercise bout – that is, at 2.5 and 7.5 min. Once the exercise protocol was completed, the heated trouser was removed, thus concluding the protocol for the right leg. Subsequently, the control left leg was inserted and strapped into the boot of the exercise ergometer. The exercise protocol for the left leg was the same to the right leg protocol, except for the fact that the heated water-perfused trouser was not utilised. Baseline blood flow measures were taken at the CFA, SFA, PFA and POA of the left leg. This was succeeded by an incremental exercise protocol (6 and 12 W for 5 min each) with blood flow being assessed at the same time points and vessels as in the heat leg (Figure 1).

2.4 | Temperature measurements

Core temperature (T_c) was measured using a rectal probe (Ret-1 Special, Physitemp, Clifton, NJ, USA) which was self-inserted 15 cm past the sphincter muscle. Skin temperature (T_{sk}) in the quadriceps, hamstrings and calf for both legs was measured using commercially available thermistors (IT-18, Physitemp) which were securely held in place using medical tape. T_c and T_{sk} were recorded online using a commercially available thermocouple metre (TC-2000, Sable Systems International, Las Vegas, NV, USA) connected to a data acquisition system (PowerLab 26T, ADInstruments, Dunedin, New Zealand). Foot T_{sk} was collected via wireless temperature loggers (DS1922L iButton Thermochron, Measurement Systems Ltd, Newbury, UK). Following the protocol, foot temperature was exported from the wireless temperature loggers in 30-s bins using a specialist logging software (iButtons, Measurement Systems Ltd). Data were then imported and analysed in Microsoft Excel software, reported as 2-min averages. In addition, mean skin leg temperature ($\bar{T}_{Leg}$) was calculated as an unweighted average of quadriceps, hamstrings, calf and foot T_{sk}. Similarly, mean skin upper-leg temperature ($\bar{T}_{Upper-Leg}$) was calculated as the unweighted average of quadriceps and hamstrings T_{sk}, and

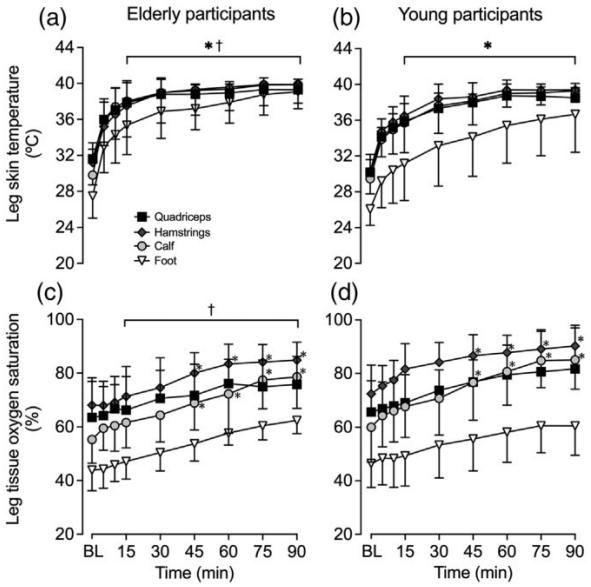

FIGURE 2 Heated (experimental) leg temperatures (a, b) and regional tissue oxygen saturation (c, d) during whole-leg hyperthermia, in elderly (a, c) and young (b, d) participants. Data presented as means ± SD (elderly: $n = 9$; young: $n = 10$). BL signifies baseline measurements. *Different from baseline, $P < 0.05$. †Different from control, young participants, $P < 0.05$.

mean skin lower-leg temperature ($\bar{T}_{\text{Lower-Leg}}$) was calculated as the unweighted average of calf and foot T_{sk}.

2.5 | Haemodynamic measurements

Heart rate was continuously measured using a three-lead echocardiogram. Also, arterial blood pressure and cardiac output were measured non-invasively – at the same time points as arterial blood flow measurements – using infrared photoplethysmography (Finometer, Finapres Medical Systems, Amsterdam, Netherlands), through a cuff on the middle finger of the right hand. Cardiac output was calculated as heart rate × stroke volume, where stroke volume was estimated using the ModelFlow method, which incorporated corrections for age, height and weight (Beatscope, Finapres Medical Systems) (Wesseling et al., 1993). Blood flow was measured at set time points – recording two 12-s Doppler images – throughout the protocols in the various arteries using a duplex Doppler ultrasound system (Vivid E95, GE Healthcare, Chalfont St Giles, Buckinghamshire, UK) with a 9-MHz linear array transducer probe (GE Healthcare) at an insonation angle of ≤60°, with sample volume positioned in the centre of the artery. The water-perfusion heated trouser had custom-made openings which allowed the probe to be placed on the skin with minimal heat loss. Before commencing baseline blood flow measures, arterial sites for the CFA, SFA and PFA in both legs were located and marked to ensure blood flow measurements were consistently made at the same site. SFA and PFA blood flow measurements were acquired at a distance of ≥2 cm from the femoral bifurcation to prevent turbulent flow disruption to the measurements, and thus improve validity of measures. Blood flow (mL min^{-1}) was calculated using the following equation: $\text{BF} = V_{\text{mean}} \times \pi \times (\frac{D_{\text{mean}}}{2})^2 \times 60$, where V_{mean} is the average centreline blood velocity (cm s^{-1}), and D_{mean} (cm) is the average internal diameter calculated using: $D_{\text{mean}} = \frac{1}{3}(D_{\text{systole}}) + \frac{2}{3}(D_{\text{diastole}})$ (Rådegran, 1997). It was not possible to directly measure PFA blood flow during the exercise protocol; therefore, during exercise, PFA blood flow was estimated using the following formula: PFA blood flow = (CFA − SFA) blood flow.

Shear rate (SR) was calculated using: $\text{SR} = \frac{4 \times V_{\text{mean}}}{D_{\text{mean}}}$, where V_{mean} is the mean blood velocity. Additionally, vascular conductance (VC) was calculated using: VC = BF/MAP, where VC is in mL min^{-1} mmHg^{-1}, BF is blood flow (mL min^{-1}) and MAP is mean arterial pressure (mmHg). Blood flow was analysed offline using commercially available software (EchoPAC, GE Medical, Horton, Norway). Blood velocity was averaged over two 12-s Doppler images, and average diameter was determined from four 2D B-mode images. Furthermore, central haemodynamic and temperature data were collected at 1000 Hz using a commercially available data acquisition system (PowerLab 26T, ADInstruments) and exported in 30-s bins using a commercially available data acquisition software (LabChart 7, ADInstruments). Following exportation, data were imported and analysed in Microsoft Excel. Data are reported as 2-min averages.

2.6 | Tissue oxygen saturation measures

Direct and continuous measurements of regional tissue haemoglobin oxygen saturation were obtained in the experimental and control legs using two near-infrared spectroscopy units with four optodes each (NIRS; INVOS 5100C Cerebral Oximeter; Somanetics Corp, Troy, MI, USA). The optodes were placed on the skin surrounding the quadriceps, hamstrings, calves and feet of both legs and taped to reduce interference from external light sources.

2.7 | Statistical analysis

Statistical analysis was conducted using R Studio (version 2022.07.1+554, R Core Team (2022)). A two-way ANOVA was conducted to discover any differences in demographic and anthropometric data between the elderly and young cohorts, as well as identifying any potential anthropometric differences between legs. Moreover, three-way repeated measures ANOVA was performed to investigate differences in haemodynamics, flow profiles, tissue oxygenation saturation and temperature between age cohorts and between and within the experimental and control legs over time during the passive heating protocol and between workloads during knee-extensor exercise protocols, respectively. The repeated measures ANOVA was conducted following the confirmation of the data's

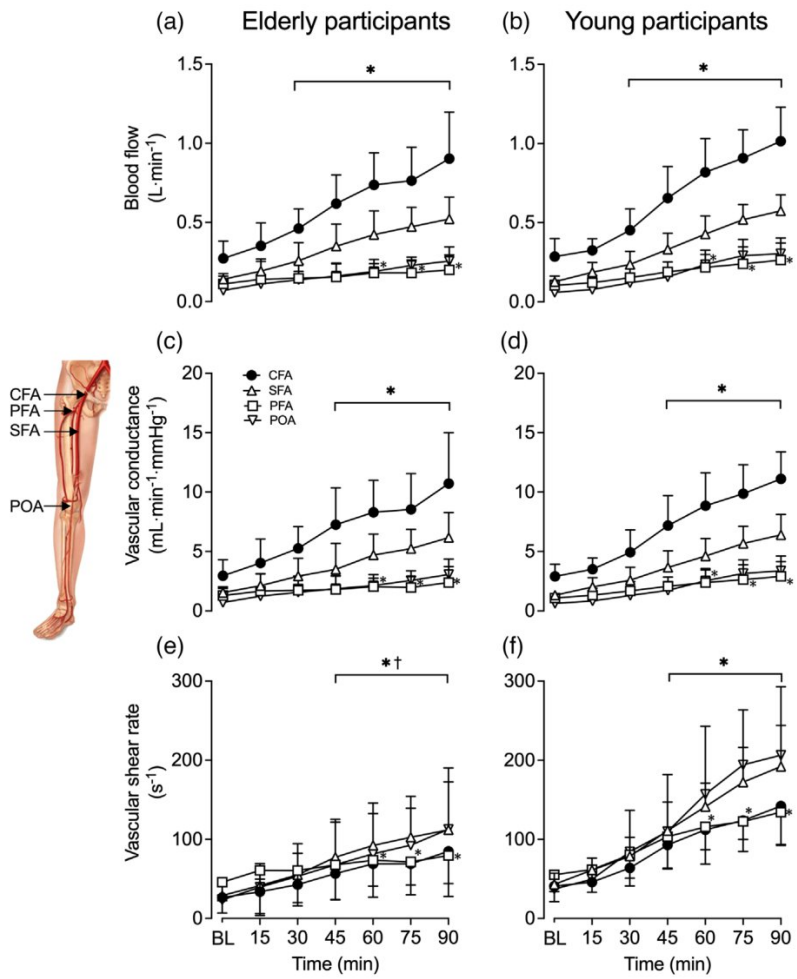

FIGURE 3 Blood flow (a, b), vascular conductance (c, d) and shear rate (e, f) during whole-leg hyperthermia in the common (CFA), superficial (SFA) and profunda (PFA) femoral arteries and the popliteal (POA) of the heated (experimental) leg, in elderly (a, c, e) and young (b, d, f) participants. Data presented as means ± SD (elderly: $n = 9$; young: $n = 10$). BL signifies baseline measurements. *Different from baseline, $P < 0.05$. †Different from control, young participants, $P < 0.05$.

normality via the Shapiro–Wilk test and Mauchly's test of sphericity. In addition, two-way repeated measures ANOVA was conducted to investigate differences between age cohorts and over time in systemic variables – that is, heart rate, cardiac output, mean arterial pressure and core temperature – during the passive heating protocol. Following the two/three-way repeated measures ANOVA, once a significant effect was found, Tukey's *post hoc* test was performed to locate the specific time points at which those changes occurred. Results are expressed as means ± SD. Significance is set at $P < 0.05$. Moreover, linear, exponential and polynomial regression curve fit tests were performed using GraphPad Prism (version 8, GraphPad Software, La Jolla, CA, USA) to assess the relationships among various key data. Subsequently, Akaike's information criterion was used to evaluate which model provides the most appropriate fit. Where an exponential curve fit was appropriate, the equation $y = y_0 \times e^{kx}$ was used, where y_0 is the y value (parameter investigated) when x (time) is zero and k is the rate constant. Significance of this fit is reported through 95% confidence intervals on the estimated value of k with the null value being $k = 0$.

3 | RESULTS

3.1 | Demographic and anthropometric characteristics

Demographic and anthropometric data for the participants are reported in Table 1. Other than age ($P < 0.0001$), no differences were observed in height, mass, leg volume and lean mass between the two cohorts. Moreover, no anthropometric differences were found between the right and left legs in either group.

3.2 | Effects of passive single-leg heating on thermal, haemodynamic and tissue oxygen saturation responses in healthy aged and young participants

3.2.1 | Regional and core temperatures

Experimental leg T_{sk} values during passive heating—measured at the quadriceps, hamstrings, calf and foot—are illustrated in Figure 2, whilst

T_c and $\bar{T}_{Leg}$ for both the experimental and control legs are reported in Table 3. As per experimental design, leg T_{sk} progressively increased at all measured sites in the heated leg following 15 min of heating (all $P < 0.0001$), whereas $\bar{T}_{Leg}$ for the control leg remained unchanged ($P = 0.9989$) (Table 3). Following 90 min of passive heating, both cohorts showed similar increases with $\bar{T}_{Leg}$ for the experimental leg averaging 9.6 ± 1.3°C ($P < 0.0001$) in the aged cohort and 9.4 ± 1.2°C ($P < 0.0001$) in the young cohort, respectively (Figure 2). However, T_c remained unchanged throughout the entirety of the heating protocol ($P = 0.9359$) and was not different between cohorts ($P = 0.7400$) (Table 3).

3.2.2 | Leg blood flow, tissue oxygen saturation and systemic haemodynamics

Complete haemodynamic responses during 90 min of passive whole-leg heating for the CFA, SFA, PFA and POA of the experimental leg are reported in Figure 3, whilst whole-leg blood flow for the control leg is reported in Table 2. In the heated leg, blood flow in the CFA, SFA and POA increased progressively up to values ≥3.5-fold above baseline (Δ = 0.68 ± 0.21, 0.42 ± 0.11 and 0.22 ± 0.10 L min^{-1}, respectively), whilst PFA blood flow increased ~2-fold (Δ = 0.11 ± 0.09 L min^{-1}) following 90 min (all $P < 0.0001$). Elevations in whole-leg arterial blood velocity, blood flow and tissue oxygen saturation were exponentially related to increases in leg skin temperature—for both the aged participants (CFA blood velocity: $R^2 = 0.75$, $k = 0.25$ (0.04, 0.71); CFA blood flow: $R^2 = 0.77$, $k = 0.27$ (0.05, 0.70); tissue oxygen saturation: $R^2 = 0.74$, $k = 0.03$ (0.01, 0.05)) and young participants (CFA blood velocity: $R^2 = 0.85$, $k = 0.29$ (0.10, 0.64); CFA blood flow: $R^2 = 0.86$, $k = 0.29$ (0.10, 0.62); tissue oxygen saturation $R^2 = 0.88$, $k = 0.03$ (0.02, 0.04)) (Figure 4). No relationships were observed between leg tissue skin temperature and diameter, which remained unchanged in all measured arteries (all $P \geq 0.948$) (Figure 4). Moreover, segmental blood flow (i.e. upper-leg and lower-leg) was exponentially related to increases in local skin temperature (upper leg: $R^2 = 0.68$, $k = 0.46$ (0.03, 1.33); lower leg: $R^2 = 0.84$, $k = 0.27$ (0.07, 0.56)) and young participants (upper leg: $R^2 = 0.74$, $k = 0.27$ (0.05, 0.85); lower leg: $R^2 = 0.94$, $k = 0.34$ (0.20, 0.53)) (Figure 5). Arterial blood flow increased in parallel to blood velocity – ≥3.5-fold in CFA, SFA and POA and ~2-fold in PFA (all $P < 0.0001$). Despite no changes in arterial diameter over time, differences in absolute diameter were observed between the two age cohorts for all arteries, with the elderly cohort showing a 16 ± 6% larger diameter ($P < 0.0001$) average obtained by comparing diameters across all arteries at baseline for both legs (Table 2). Correspondingly, the elderly cohort demonstrated a 25 ± 12% lower blood velocity at baseline conditions averaged across all arteries, with the difference between cohorts further increasing to 51 ± 6% following 90 min of heating ($P < 0.0001$) (Table 2). Similar responses were observed in vascular conductance and SR in all four arteries of the heated leg (Figure 3). Vascular conductance and SR increased ≥3.4-fold in the CFA, SFA and POA and ≥2.1-fold in the PFA (all $P < 0.0001$). No changes

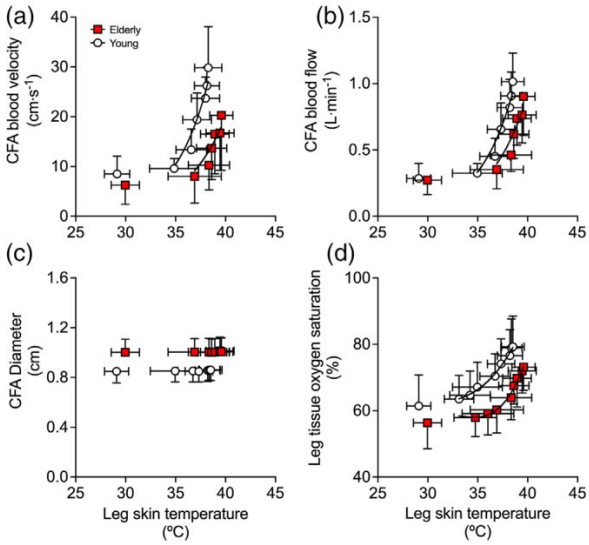

FIGURE 4 Relationship between the mean leg skin temperature common femoral artery (CFA) blood velocity (a), blood flow (b), diameter (c) and mean leg tissue oxygen saturation (d) during whole-leg hyperthermia in elderly and young participants. Data presented as means ± SD (elderly: $n = 9$; young: $n = 10$). Vertical error bars signify local blood flow SD, while horizontal error bars signify local temperature SD. Lines represent the exponential fit of the data.

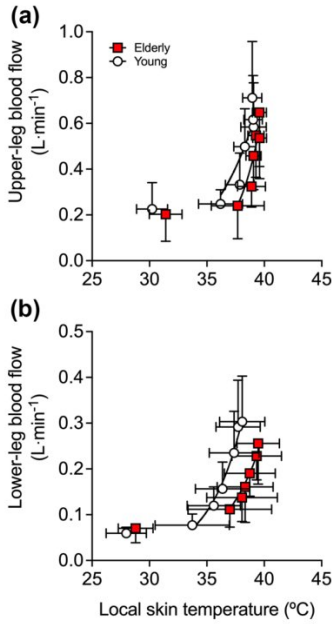

FIGURE 5 Relationship between the local skin temperature and local blood flow during whole-leg hyperthermia in elderly and young participants. Data presented as means ± SD (elderly: $n = 9$; young: $n = 10$). The graphs illustrate the relationship between upper-leg blood flow and upper-leg skin temperature (a), and lower-leg blood flow and lower-leg skin temperature (b). Vertical error bars signify local blood flow SD, while horizontal error bars signify local temperature SD. Lines represent the exponential fit of the data.

TABLE 2 Influence of whole-leg heating and subsequent one-legged knee extensor exercise on body and skin temperatures and central haemodynamics.

		Passive leg heating					Control	
		Rest			One-legged knee extensor exercise		One-legged knee extensor exercise	
		Time (min)			Workload (W)		Workload (W)	
Intervention	Baseline	30	60	90	6	12	6	12
T_c (°C)								
Elderly	36.9 ± 0.4	36.9 ± 0.4	37.0 ± 0.4	37.2 ± 0.4	37.3 ± 0.4†,‡	37.3 ± 0.4†,‡	37.4 ± 0.4†	37.4 ± 0.4†
Young	37.1 ± 0.3	36.9 ± 0.2	36.9 ± 0.2	37.0 ± 0.2	37.1 ± 0.2	37.1 ± 0.2	37.2 ± 0.1	37.2 ± 0.1
$\bar{T}_{Leg}$ (°C)								
Experimental leg								
Elderly	30.0 ± 1.4†	38.3 ± 2.1*,†,‡	38.9 ± 1.4*,†,‡	39.6 ± 1.2*,†,‡	38.2 ± 1.3‡	37.4 ± 1.0‡	33.9 ± 0.9‡	33.3 ± 0.9‡
Young	29.1 ± 1.3	36.7 ± 1.5*	38.2 ± 1.3*	38.5 ± 1.1*	37.9 ± 1.4	37.7 ± 1.6	33.7 ± 0.7	33.3 ± 0.7
Control leg								
Elderly	29.9 ± 1.5	29.6 ± 1.5	29.3 ± 1.5	29.1 ± 1.5	28.6 ± 1.2	28.6 ± 1.3	28.7 ± 1.3	29.1 ± 1.7
Young	28.7 ± 0.9	28.2 ± 0.8	28.1 ± 0.8	28.2 ± 0.9	28.1 ± 1.2	28.0 ± 1.3	28.2 ± 1.2	28.4 ± 1.2
MAP (mmHg)								
Elderly	93 ± 12	91 ± 14	91 ± 8	87 ± 13	115 ± 19*	110 ± 20*	122 ± 22*	121 ± 20*
Young	98 ± 22	94 ± 12	95 ± 13	92 ± 13	111 ± 24*	112 ± 26*	118 ± 25*	119 ± 24*
$\dot{Q}$ (L min^{-1})								
Elderly	4.5 ± 1.1†	4.0 ± 1.2†	4.7 ± 2.1†	4.7 ± 1.9†	6.2 ± 1.6*,†	8.0 ± 0.8*,†	5.3 ± 1.2*,†	6.4 ± 1.7*,†
Young	5.0 ± 1.0	5.7 ± 0.8	5.5 ± 0.7	5.8 ± 0.7	7.9 ± 1.9*	8.9 ± 2.0*	7.4 ± 1.6*	8.1 ± 1.8*
HR (beats min^{-1})								
Elderly	57 ± 10†	58 ± 12†	56 ± 10†	61 ± 12†	81 ± 24*,†	79 ± 19*,†	80 ± 26*,†	80 ± 22*,†
Young	66 ± 9	62 ± 12	64 ± 9	69 ± 9	92 ± 22*	100 ± 24*	89 ± 26*	96 ± 32*

Values are means ± SD for nine elderly participants and 10 young participants. Experimental leg refers to right, heated leg, whilst the control leg refers to the left, contralateral leg. Values for rest, represent the responses during 90 min of passive whole-leg heating. Leg skin temperature is presented as an unweighted mean average collected at four different sites: quadriceps, hamstrings, calf and foot. *Different from baseline, $P < 0.05$. †Different from respective young, control group at the same time point, $P < 0.05$. ‡Different from respective contralateral, control leg, $P < 0.05$. Abbreviations: HR, heart rate; MAP, mean arterial pressure; $\bar{T}_{Leg}$, mean leg skin temperature; $\dot{Q}$, cardiac output.

TABLE 3 Influence of whole-leg heating and subsequent one-legged knee extensor exercise on arterial blood flow and tissue oxygen saturation.

		Passive leg heating					Control	
		Rest			One-legged knee extensor exercise		One-legged knee extensor exercise	
		Time (min)			Workload (W)		Workload (W)	
Intervention	Baseline	30	60	90	6	12	6	12
CFA blood flow (L min^{-1})								
Experimental leg								
Elderly	0.27 ± 0.10	0.46 ± 0.12*,‡	0.74 ± 0.20*,‡	0.90 ± 0.30*,‡	2.93 ± 0.48*,‡	3.31 ± 0.49*,‡	–	–
Young	0.29 ± 0.11	0.45 ± 0.13*	0.82 ± 0.21*	1.02 ± 0.22*	2.42 ± 0.83*,‡	2.96 ± 0.70*,‡	–	–
Control leg								
Elderly	0.29 ± 0.08	0.26 ± 0.07	0.24 ± 0.08	0.24 ± 0.09	–	–	2.15 ± 0.52*,‡	2.34 ± 0.39*,‡
Young	0.29 ± 0.10	0.27 ± 0.09	0.26 ± 0.07	0.27 ± 0.08	–	–	1.87 ± 0.49*	2.24 ± 0.43*
CFA blood velocity (cm s^{-1})								
Experimental leg								
Elderly	6.3 ± 3.9†	10.3 ± 5.0†,‡	16.5 ± 8.0*,†,‡	20.3 ± 11.0*,†,‡	63.8 ± 19.9*,†,‡	70.1 ± 8.9*,†,‡	–	–
Young	8.5 ± 3.6	13.4 ± 4.2	23.7 ± 4.3	29.8 ± 8.3	67.4 ± 26.3*,‡	83.3 ± 23.3*,‡	–	–
Control leg								
Elderly	6.3 ± 2.2†	5.7 ± 1.8†	5.4 ± 2.3†	5.6 ± 3.3†	–	–	48.5 ± 16.4*,†	51.5 ± 9.2*,†
Young	8.3 ± 2.9	7.7 ± 2.3	7.7 ± 2.1	7.8 ± 1.7	–	–	52.3 ± 17.1*	62.1 ± 13.4*
CFA diameter (cm)								
Experimental leg								
Elderly	1.00 ± 0.10†	1.00 ± 0.11†	1.00 ± 0.11†	1.01 ± 0.11†	1.01 ± 0.10†	1.00 ± 0.04†	–	–
Young	0.85 ± 0.09	0.85 ± 0.09	0.85 ± 0.09	0.86 ± 0.09	0.88 ± 0.08	0.88 ± 0.08	–	–
Control leg								
Elderly	1.00 ± 0.10†	0.99 ± 0.10†	0.99 ± 0.10†	0.99 ± 0.10†	–	–	0.99 ± 0.10†	0.98 ± 0.05†
Young	0.86 ± 0.10	0.86 ± 0.09	0.86 ± 0.09	0.86 ± 0.10	–	–	0.88 ± 0.08	0.88 ± 0.08

(Continues)

TABLE 3 (Continued)

		Passive leg heating					Control			
		Rest			One-legged knee extensor exercise		One-legged knee extensor exercise			
		Time (min)			Workload (W)		Workload (W)			
Intervention	Baseline	30	60	90	6	12	6	12		
Tissue oxygen saturation (%)										
Experimental leg										
Elderly	56 ± 8†	64 ± 6*,†,‡	70 ± 9*,†,‡	73 ± 7*,†,‡	69 ± 6†,‡	68 ± 6†,‡	71 ± 9†,‡	71 ± 9†,‡		
Young	61 ± 9	70 ± 7*	77 ± 8*	79 ± 9*	76 ± 6	76 ± 6	79 ± 8	78 ± 8		
Control leg										
Elderly	57 ± 8†	57 ± 7†	55 ± 10†	59 ± 8†	56 ± 9†	56 ± 8†	54 ± 6†	50 ± 13†		
Young	62 ± 8	64 ± 6	65 ± 5	67 ± 6	64 ± 9	64 ± 8	65 ± 7	65 ± 7		

Values are means ± SD for nine elderly participants and 10 young participants. Experimental leg refers to right, heated leg, whilst the control leg refers to the left, contralateral leg. Tissue oxygen saturation reflects changes in regional tissue haemoglobin oxygen saturation. Values for rest, represent the responses during 90 min of passive whole-leg heating. *Different from baseline, $P < 0.05$. †Different from respective young, control group at the same time point, $P < 0.05$. ‡Different from respective contralateral, control leg, $P < 0.05$. Abbreviation: CFA, common femoral artery.

in control leg blood flow and vascular conductance were observed in all arteries ($P \geq 0.9999$), nor were there any differences in arterial blood flow ($P \geq 0.0720$) or vascular conductance ($P \geq 0.5030$) between the cohorts. However, the elderly cohort showed a 51 ± 7% lower SR in all arteries during baseline conditions, which was further exacerbated to 73 ± 7% following 90 min of heating (all $P < 0.0001$), associated with a 16 ± 6% larger diameter.

Tissue haemoglobin oxygen saturation increased gradually at all four sites (quadriceps, hamstrings, calf and foot) of the heated leg, with mean leg tissue oxygen saturation increasing 17 ± 6% units ($P < 0.0001$) in the aged cohort and 18 ± 6% units ($P < 0.0001$) in the young cohort following 90 min of heating (Figure 2 and Table 2). No differences in tissue oxygen saturation were observed in the control leg ($P = 0.9531$). However, differences between the age cohorts were observed, with the elderly cohort exhibiting a lower mean leg tissue oxygen saturation in the heated leg (73 ± 7 vs. 79 ± 9%; $P < 0.0001$) and control leg (59 ± 8 vs. 67 ± 6%; $P < 0.0001$). At the systemic haemodynamic level, no changes were observed for heart rate ($P = 0.6465$), cardiac output ($P = 0.9390$) and mean arterial pressure ($P = 0.8920$) during 90 min of heating (Table 3). However, heart rate ($P = 0.0006$) and cardiac output ($P < 0.0001$) were lower in the aged cohort in comparison to their younger counterparts (Table 3).

3.3 | Effects of incremental low-intensity knee extensor exercise and single-leg heating on thermal, haemodynamic and tissue oxygen saturation responses in healthy aged and young participants

3.3.1 | Regional and core temperatures

As per the experimental design, heated leg T_{sk} was substantially higher at all measured sites in comparison to the control leg ($P < 0.0001$) (Figure 6 and Table 3). When comparing the two legs during their respective exercise protocols, $\bar{T}_{Leg}$ was 9.3 ± 1.5°C higher in the heated leg ($P < 0.0001$). Additionally, no differences were observed in $\bar{T}_{Leg}$ between workloads ($P = 0.345$) or age cohorts ($P = 0.164$) (Figure 6 and Table 3). Conversely, small but significant differences in T_c were observed between leg exercise protocols and age cohorts: T_c was 0.1 ± 0.1°C lower during the heated leg exercise protocol in comparison to the control leg exercise protocol ($P = 0.0441$), and 0.2 ± 0.1°C higher in the aged cohort ($P = 0.0143$) (Table 3).

3.3.2 | Leg blood flow, tissue oxygen saturation and systemic haemodynamics

Whole-leg (CFA) haemodynamics during exercise are reported in Figure 7 and Table 2, whilst SFA and PFA blood flows are reported in Figure 8. In absolute terms, CFA blood flow in the heated leg was 0.65 ± 0.56 and 1.04 ± 0.83 L min^{-1} higher at 6 and 12 W, respectively,

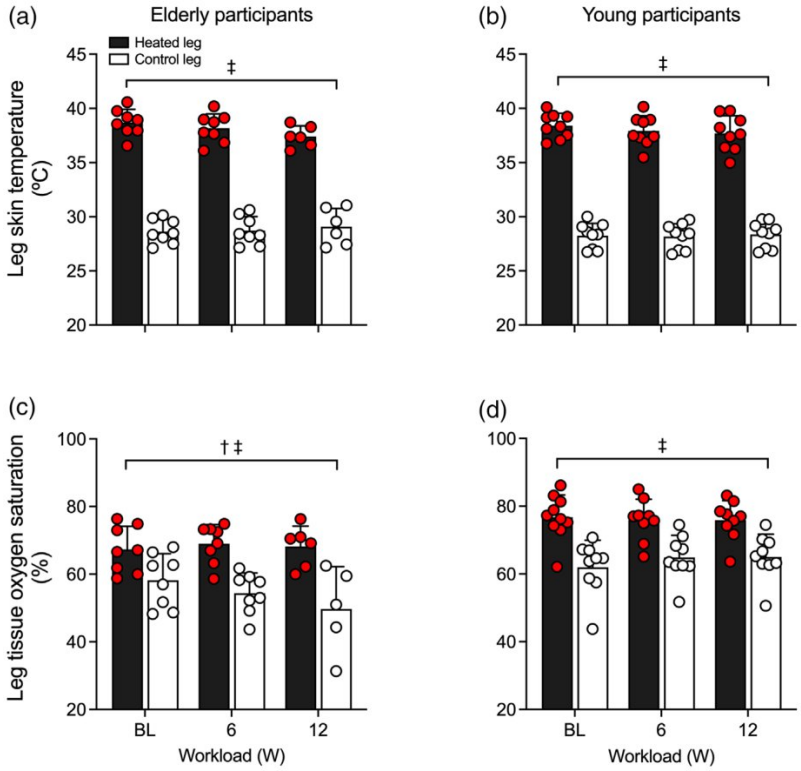

FIGURE 6 Heated (experimental) leg skin temperature (a, b) and tissue oxygen saturation (c, d) during one-legged knee extensor exercise with heating, in elderly (a, c) and young (b, d) participants. Data presented as means ± SD (elderly: $n = 9$; young: $n = 9$). Circles symbolise individual data points. Baseline (BL) measurements signify the measurements taken immediately prior to the start of exercise, following 90 min of whole-leg heating. Leg skin temperature and tissue oxygen saturation are presented as an unweighted mean average collected at four different sites: quadriceps, hamstrings, calf and foot. *Different from baseline, $P < 0.05$. †Different from control, young participants, $P < 0.05$.

in comparison to the control exercise condition (Figure 7). Similar responses were observed in the SFA and PFA, with heated exercise inducing 0.36 ± 0.18 and 0.36 ± 0.30 L min^{-1} higher blood flows than the control exercise for the SFA at 6 and 12 W, respectively, and 0.28 ± 0.49 and 0.36 ± 0.53 L min^{-1} for the PFA at 6 and 12 W, respectively (all $P < 0.0001$). However, no differences were observed between age cohorts ($P \geq 0.1131$). As reported earlier, arterial diameter did not change during the protocol ($P \geq 0.700$), although the average diameter was larger in the elderly cohort ($P < 0.0001$). Whole-leg vascular conductance was 7 ± 5 and 8 ± 7 mL min^{-1} mmHg^{-1} higher in the heated leg compared to the control leg during 6 and 12 W of exercise, respectively (Figure 7). No differences were observed for vascular conductance between the age cohorts ($P = 0.1540$). Likewise, CFA SR was 61 ± 65 and 73 ± 60 s^{-1} higher in the heated leg during 6 and 12 W of exercise, respectively (Figure 7). Lastly, differences were observed between legs ($P < 0.0001$) and between cohorts ($P = 0.0011$), with the elderly cohort reporting a reduced SR across both exercise workloads and legs (Figure 7).

No differences were observed in mean leg tissue oxygen saturation during exercise, across both workloads ($P = 0.9880$). However, differences were observed between legs and between age cohorts, with the heated leg showing a higher mean leg tissue oxygen saturation across both workloads (73 ± 7 vs. 51 ± 8%, respectively, $P < 0.0001$) and the elderly cohort reporting a lower mean leg tissue oxygen saturation during heated-leg exercise (76 ± 6 vs. 69 ± 6%, respectively, $P < 0.0001$) (Figure 6 and Table 2). At the systemic haemodynamic level, no differences in arterial pressure or cardiac output were observed between the heated- and control-leg exercise protocols (all $P \geq 0.2160$) (Table 3). However, differences were observed between age cohorts for heart rate ($P = 0.0122$) and cardiac output ($P < 0.0001$), with the elderly cohort reporting lower values during the exercise protocols (Table 3).

4 | DISCUSSION

The present study sought to characterise the haemodynamic profiles of the major leg arteries in response to single-leg hyperthermia, single-leg knee-extensor exercise, and their combination in healthy, active elderly and young participants and in doing so, gain insight into the impact of local temperature and age on the control of limb perfusion. In line with our primary hypothesis, blood flow was tightly coupled to the rise in local temperature and metabolic demand, with the combination of local limb hyperthermia and exercise producing a higher level of

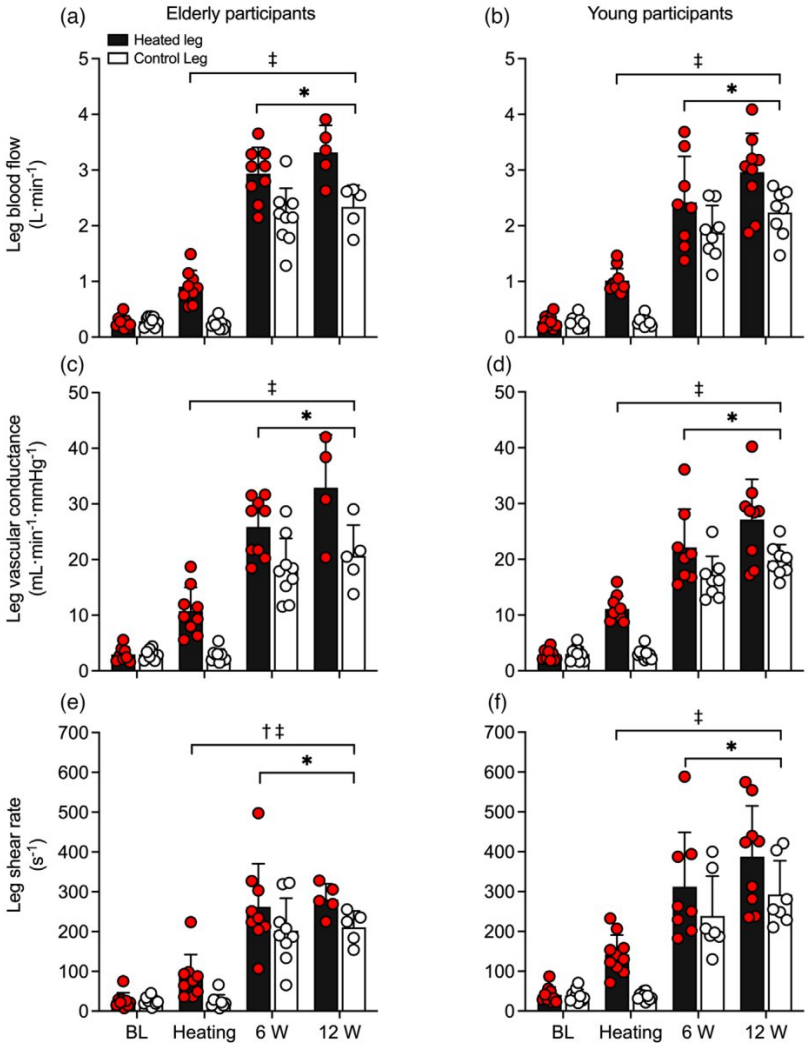

FIGURE 7 Blood flow (a, b), vascular conductance (c, d), and shear rate (e, f) during one-legged knee extensor exercise with and without heating, in the common femoral artery of the heated (experimental) leg and control (contralateral) leg, in elderly (a, c, e) and young (b, d, f) participants. Data presented as means ± SD (elderly: $n = 9$; young: $n = 9$). Circles symbolise individual data points. *Different from baseline (BL) prior to the commencement of exercise (measurements taken following 90 min of whole-leg heating), $P < 0.05$. †Different from respective young, control group at the same time point, $P < 0.05$. ‡Different from respective contralateral, control leg, $P < 0.05$.

hyperaemia than exercise alone. However, contrary to our secondary hypothesis, no differences in the hyperaemic response to local limb hyperthermia, normal exercise or hyperthermic exercise were observed between the elderly and young cohort. Importantly, the equal hyperaemia happened despite the elderly group exhibiting a lower blood velocity during all three interventions. Collectively, the present findings suggest that age indeed induces structural and functional vascular adaptation—as shown by the age-related differences in arterial diameter, blood velocity and shear stress – but it does not alter the magnitude of hyperaemia during local limb hyperthermia, small muscle mass exercise or combined limb hyperthermia and exercise.

4.1 | Effect of hyperthermia on the hyperaemic response to low-intensity knee-extensor exercise in old and young humans

An important finding of this study is that local hyperthermia augmented macro- and microcirculatory blood flow similarly at rest and during single-leg exercise, irrespective of age. Ninety minutes of passive single-leg heating increased $\bar{T}_{Leg}$ by 9.5°C, inducing an average 0.6–0.7 L min^{-1} elevation in whole-leg blood flow in the elderly and young cohorts. The relationships between the rise in local temperature (>35°C skin temperature) and tissue perfusion for both the upper and the lower leg in both groups are consistent

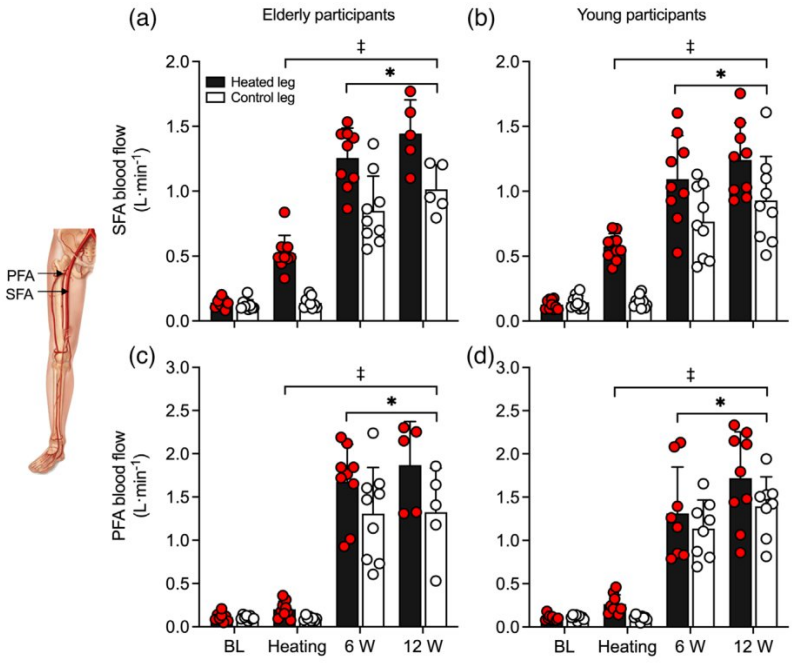

FIGURE 8 Blood flow during one-legged knee extensor exercise with and without heating, superficial (SFA) (a, b) and profunda (PFA) (c, d) femoral arteries of the heated (experimental) leg and control (contralateral) leg, in elderly (a, c) and young (b, d) participants. Data presented as means ± SD (elderly: $n = 9$; young: $n = 9$). Circles symbolise individual data points. Note: during exercise, PFA blood flow was calculated indirectly as the difference between CFA and SFA blood flow. *Different from baseline (BL) prior to the commencement of exercise (measurements taken following 90 min of whole-leg heating), $P < 0.05$. †Different from respective young, control group at the same time point, $P < 0.05$. ‡Different from respective contralateral, control leg, $P < 0.05$.

with previous findings from our laboratory during isolated leg heating in young participants (Figure 5) (Chiesa et al., 2016; Koch Esteves et al., 2021; Pearson et al., 2011). Similarly, the addition of single-leg hyperthermia during knee-extensor exercise in both cohorts produced a 0.7 L min^{-1} larger leg hyperaemia at 6 W and 1.0 L min^{-1} at 12 W, in comparison to exercise alone. Functional hyperaemia during control-leg exercise was also independent of age, as whole-leg blood flow was comparable between the elderly and young cohorts—2.2 versus 1.9 L min^{-1} and 2.3 versus 2.2 L min^{-1} at 6 and 12 W, respectively (Figure 7). The magnitude of the local hyperthermia-induced hyperaemia was, therefore, remarkably similar at rest and during single-leg knee-extensor exercise in both groups.

An age-old question is whether heat stress provides an additive stimulus for functional hyperaemia, with the premise that tissue temperature and perfusion are elevated compared to control conditions. Evidence to date is equivocal, however, with several past studies reporting no differences in leg blood flow during two-legged cycling exercise (Savard et al., 1988; Trangmar et al., 2017) and single-leg knee extensor exercise (Ferguson et al., 2006; Savard et al., 1988), whilst others reported an increased flow of 0.6–0.7 L min^{-1} between hyperthermic and control conditions (Chiesa et al., 2015; Pearson et al., 2011). Exercise duration, intensity and mass of active muscle all influence the balance between heat production and heat transfer, which ultimately affect local muscle and blood temperatures (González-Alonso et al., 2000, 2015; Saltin & Hermansen, 1966; Saltin et al., 1968). Since local tissue perfusion is strongly related to local skin and muscle temperature (Koch Esteves et al., 2021), it is plausible that some of the aforementioned studies did not find an effect of environmental heat stress or external heating on functional hyperaemia because these interventions did not sufficiently increase deep tissue temperature above the control exercise condition (Ferguson et al., 2006; Savard et al., 1988; Trangmar et al., 2017). In this light, it is important to consider that whole-leg exercise – for example, cycling – increases tissue and blood temperatures in the whole leg whereas knee-extensor exercise increases temperature solely in the quadriceps muscle (González-Alonso et al., 2000). Localised thigh heating, on the other hand, increases temperature and perfusion only in the upper-leg segment (Koch Esteves et al., 2021). Thus, the larger differences in tissue temperature across the whole leg between the present heated and control exercise protocols likely rationalise the additive effect of leg hyperthermia on functional hyperaemia.

While the influence of combined local-leg heating and exercise on whole-leg hyperaemia was consistent in both groups, the precise distribution of the additional exertional hyperaemia remains uncertain. Assuming that lower-leg perfusion—supplied by the POA—remained stable during knee-extensor exercise, which following passive leg heating increased by ~0.2 L min^{-1} (Figure 3), it is estimated that upper-leg hyperaemia was 0.5–0.8 L min^{-1} higher during combined leg hyperthermia and exercise at the two workloads examined, in comparison to control exercise. Additionally, if the hyperthermia-induced hyperaemia is equally distributed among the thigh tissues, we estimated that 0.2–0.4 L min^{-1} would be perfusing the anterior thigh tissues, with a significant portion added to the normal exercise hyperaemia perfusing the active quadriceps muscle, which would amount to a 9–18% elevation in exertional hyperaemia. Evidence in the literature supports this argument. On one hand, muscle perfusion and tissue oxygen saturation increase and leg arteriovenous oxygen difference declines in response to passive local hyperthermia (Chiesa et al., 2015; Heinonen et al., 2011; Keller et al., 2010; Pearson et al., 2011). Moreover, leg (quadriceps) $\dot{V}_{O_2}$ is maintained during

knee-extensor exercise under hyperthermic conditions in association with compensatory reductions in arteriovenous oxygen differences (Chiesa et al., 2015; Pearson et al., 2011). The higher tissue oxygen saturation seen in this study during leg heating and knee-extensor exercise suggests that a higher muscle hyperaemia and corresponding increased oxygen delivery were met by a lower oxygen extraction from the circulation in both the elderly and young cohorts (Figure 5). Together, these findings reveal that hyperthermia induces increases in muscle perfusion during knee-extensor exercise despite an unchanged metabolic demand. Furthermore, these observations lend support to the notion that the rise in local temperature is a putative stimulus for augmenting muscle tissue perfusion during low-intensity small muscle mass exercise in both young and elderly individuals.

4.2 | Effect of age on the thermal mechanisms enhancing skeletal muscle perfusion during low-intensity knee-extensor exercise

The literature surrounding the effect of age on functional hyperaemia in response to hyperthermia and exercise is riddled with inconsistencies, yet the prevailing view is that age attenuates functional hyperaemia. According to Darcy's law of flow – the hydraulic equivalent of Ohm's law – skeletal muscle perfusion is determined by vascular conductance and perfusion pressure gradient. Using mean arterial pressure as a surrogate, no differences in the perfusion pressure gradient were observed during single-leg heating and between the elderly and young cohorts (Table 3). Hence, hyperperfusion during single-leg heating was solely associated with increases in vascular conductance (Figure 3). A blunted vascular conductance is commonly reported in elderly individuals during hyperthermia and exercise and postulated as a primary mediator of the age-associated attenuation in tissue perfusion (Kenney, 2001; Martin et al., 1995; Hearon Jr & Dinenno, 2016). Reduced bioavailability of endothelium-derived vasoactive substances (i.e. nitric oxide) and compromised functional sympatholysis – that is, a reduced ability to counteract vasoconstriction and maintain adequate blood flow in the presence of elevated sympathetic nerve activity – have been implicated in this phenomenon (Hearon Jr & Dinenno, 2016). From a global haemodynamic perspective, an age-related attenuation in hyperaemia during local lower-leg hyperthermia (Romero et al., 2017) and knee-extensor exercise (Donato et al., 2006; Lawrenson et al., 2003; Mortensen et al., 2012) has been associated with a lower vascular conductance in elderly sedentary and normally active subjects, which in some cases is largely the result of an elevated mean arterial pressure (Donato et al., 2006; Lawrenson et al., 2003; Mortensen et al., 2012). In contrast, the present elderly cohort demonstrated a comparable vascular conductance, mean arterial pressure and blood flow to their younger counterparts (Figure 7) in line with recent studies during local two-leg hyperthermia (Engelland et al., 2020) and submaximal knee-extensor exercise in endurance-trained aged cohorts (Mortensen et al., 2012). The discrepancies among studies may be explained, at least in part, by regular participation in physical activity, which has been shown to preserve arterial responsiveness to vasodilator infusion and functional sympatholysis with age (Kruse et al., 2018; Mortensen et al., 2012; Piil et al., 2018). Alternatively, functional sympatholysis may not impose a limitation on the hyperaemic response in the present study because – unlike two-leg heating (Engelland et al., 2020), whole-body hyperthermia (Crandall et al., 1999; Cui et al., 2004) and two-legged cycling (Katayama & Saito, 2019) – single lower-leg heating and moderate-intensity dynamic knee-extensor exercise (30 W) consistently or transiently reduce muscle sympathetic nerve activity compared to baseline values (Katayama & Saito, 2019; Takahashi et al., 2011). Moreover, circulating noradrenaline levels remain low (~1–2 nmol L^{-1}) when core temperature is unchanged during heating and moderate intensity single-leg knee-extensor exercise (Chiesa et al., 2019; Pearson et al., 2011; Powers et al., 1982). It therefore appears that lifelong regular engagement in physical activity and the low vasoconstrictor activity during single-leg hyperthermia and exercise contribute to the preservation of functional hyperaemia during local limb heating and small muscle mass exercise.

Increases in leg vascular conductance were approximately 50% higher during heated-leg exercise than control-leg exercise; however, no changes in conduit arterial diameter were observed (Figure 7). According to the Hagen–Poiseuille law, vascular conductance (the inverse of resistance) is predominantly determined by vessel diameter and blood viscosity. It is possible that vasodilatation occurred downstream in the small arteries and resistance arterioles (i.e. microcirculation), thereby contributing to the increases in blood velocity and flow in both age groups (Koch Esteves et al., 2021). During passive hyperthermia in young adults, myriad potential thermosensitive mechanisms have been shown to positively impact blood velocity and thus blood flow. Thermal stimuli could activate intravascular signalling transduction mechanisms (Gifford et al., 2014; Kellogg et al., 1999; Laughlin et al., 2008; Minson et al., 2001; Paniagua et al., 2001) and/or stimulate the release of vasoactive molecules from erythrocytes such as ATP (Kalsi & González-Alonso, 2012; Kalsi et al., 2017; Pearson et al., 2011), which in turn induce vasodilatation of the resistance vessels. Additionally, reductions in blood viscosity and frictional resistance (Çinar et al., 2001; Lim et al., 2010; Shin et al., 2004; Snyder, 1971) in conjunction with increases in red blood cell deformability and dispersion (Çinar et al., 2001; Manteuffel-Szoege, 1960, 1969; Pinho et al., 2016) may also play a role in the observed hyperthermia-induced hyperaemia. However, ageing has been found to negatively impact these thermosensitive and rheological mechanisms, evoking increases in blood viscosity (Carallo et al., 2011; Simmonds et al., 2013), reductions in red blood cell deformability (Simmonds et al., 2013) and an impaired response to various vasodilators (Holowatz & Kenney, 2010; Mortensen et al., 2012; Wray & Richardson, 2015; Hearon Jr & Dinenno, 2016). Regular engagement in physical activity has been shown to preserve these mechanisms (Ernst, 1987; Groot et al., 2016; Mortensen et al., 2012; Simmonds et al., 2013) and thus may explain why the present elderly and young cohorts showed comparable magnitudes of hyperaemia. Nonetheless, if these thermosensitive vascular mechanisms and blood rheological properties do

indeed worsen with age, they could at least in part elucidate why the present elderly cohort exhibited a lower blood velocity following heating, knee-extensor exercise and their combination.

The present elderly cohort also displayed a larger diameter across all measured conduit arteries which was sufficient to compensate for the lower blood velocity (Figure 4). An age-related increase in arterial diameter is well-established in the literature (Gonzales et al., 2009; Hirata et al., 2006; Kawasaki et al., 1987; Sandgren et al., 1999), with the aortic diameter increasing by as much as 24% between the ages of 25 and 70 years (Sonesson et al., 1993). These increases are generally associated with an increase in arterial stiffness (Kawasaki et al., 1987; Sonesson et al., 1993) and are therefore indicative of an impaired endothelial-dependent vasodilator function (Anderson, 2006). However, Hickson et al. (2010) postulated that an increase in (aorta) diameter without a proportional increase in arterial wall thickness may be an adaptation to offset the age-related increase in arterial stiffness. In this construct, one could speculate that the increased femoral artery diameter in the elderly cohort is an adaptive response to maintain flow rate as blood velocity is attenuated, a hypothesis that warrants further investigation.

4.3 | Experimental considerations

There are several methodological considerations in this study. First, the present experimental design included two fixed absolute exercise workloads – 6 and 12 W – for all participants. Absolute, low-intensity workloads were selected as the approach allowed for the direct comparison of hyperaemia between the control and hyperthermic conditions, and between old and young cohorts. Whilst utilising relative workloads would have merit, it is important to acknowledge that the overtime power output variability per kick is large during single-leg knee-extensor exercise (González-Alonso et al., 2000). Thus, using relative workloads—especially if differences in workloads were less than 6 W would have introduced additional variability between protocols and cohorts. Although comparing absolute workloads can pose some limitations (Donato et al., 2006), the present observation that exercise hyperaemia was not different in the elderly and young cohorts suggests that the likely higher relative intensity in the elderly did not affect the outcomes of the study. Second, arterial leg blood velocity, flow and tissue oxygen saturation did not initially increase as rapidly, and to the same magnitude, as local skin temperature. Figures 4 and 5 clearly show that whole-leg hyperaemia lagged in comparison to the rapid increase in mean leg skin temperature. This initial uncoupling of temperature and haemodynamics was likely due to internal tissue temperature not being measured; thus, it could not be included in the calculation of mean leg temperature and help establish the impact of whole-leg tissue hyperthermia on limb haemodynamics. When using hot-water-perfused garments, as in this study, deep tissue temperature takes longer to increase because conductive heat transfer relies on a positive temperature gradient between the heated skin and the cooler deep tissues. This likely explains why the present correlations were not as strong as those reported in our previous study, which had estimates of regional leg hyperthermia (Koch Esteves et al., 2021). Notwithstanding this limitation, our data lend further support to the notion that internal temperature and concomitant increases in deep tissue blood flow contribute to the observed hyperthermia-induced limb hyperaemia (Chiesa et al., 2016; Heinonen et al., 2011; Koch Esteves et al., 2021; Pearson et al., 2011). Third, participants were considered *trained* due to their regular participation in structured exercise, which was assessed via a self-completed questionnaire as opposed to an objective quantification of exercise capacity (e.g. a maximal oxygen consumption test). Although this modality of training status assessment is liable to self-desirability bias (Nederhof, 1985), we are confident that our participants are indeed trained – particularly our elderly cohort – as their leg muscle mass was comparable to the young cohort. This differs from non-trained elderly individuals where leg muscle mass is decreased by ~16% when compared to young 25-year-old adults (Naruse et al., 2023). Consequently, the present elderly cohort was classified as *trained* as they did not present characteristics of normal age-related sarcopenia. Whilst this study attempted to isolate the effect of age on the haemodynamic responses to single-limb hyperthermia and exercise, rather than the commonly associated age-related reductions in physical activity, the present findings should not be extrapolated to aged sedentary individuals who will exhibit significant sarcopenia. Last, although lower limb hyperthermia increased blood flow in all major vessels in both the elderly and young groups, the magnitude of increase in blood flow was greater in SFA than PFA. This could be due to the thermal intervention inducing a smaller increase in deep tissue temperature, which is typically warmer than the superficial tissues (Savard et al., 1988), thus producing a weaker thermal stimulus for vasodilatation and/or haemorheological changes. Moreover, measurements of PFA blood flow are influenced by the individual vessel anatomy (Tomaszewski et al., 2017), making it more difficult to obtain high-quality images compared to the other main leg conduit arteries (Hussain, 1997; Koch Esteves & Chiesa, 2021). Based on PFA blood flow estimates from the measurements of CFA and SFA blood flow, it appears that the magnitude of increase in PFA blood flow was underestimated. Therefore, this limitation does not have a bearing on the conclusions of the study.

4.4 | Summary

Passive lower limb heating increased leg blood flow and vascular conductance over three-fold – alongside increases in regional tissue haemoglobin oxygen saturation – which occurred in parallel to increases in local temperature. Moreover, passive leg heating had an additive effect on blood flow during knee-extensor exercise with the combination of hyperthermia and exercise exhibiting the largest magnitude of functional hyperaemia. Notably, no differences in the functional hyperaemic responses were observed between the healthy, active elderly and the young cohorts despite the larger femoral artery diameter, and lower central haemodynamics and blood velocity in the elderly. These findings reject the idea that age per se

compromises local hyperthermia-induced limb hyperperfusion or small muscle group functional hyperaemia, notwithstanding structural age-related differences in the vasculature. Further research is warranted to investigate the hyperaemic responses to local limb heating and larger muscle mass exercise – such as walking – in elderly active and sedentary participants to establish the safety and effectiveness of combined local heating and exercise in enhancing circulatory function and vascular health.

AUTHOR CONTRIBUTIONS

This study was performed at Brunel University London, Uxbridge, UK. Nuno Koch Esteves and José González-Alonso conceived and designed the research. Nuno Koch Esteves and José González-Alonso acquired the data. Nuno Koch Esteves analysed the data. Nuno Koch Esteves, Ashraf W. Khir and José González-Alonso interpreted the data. All authors revised the manuscript and provided intellectual feedback. All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

ACKNOWLEDGEMENTS

We give special thanks to the participants without whom, this study would not have been possible. We also thank Jeneil Mcdonald and Patrick Cazorla for their excellent assistance with the data collection, Dr Francesca R. Cavallo for her support with R coding and Dr Steven Trangmar for his editorial comments.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

FUNDING INFORMATION

This study was completed without external funding.

DATA AVAILABILITY STATEMENT

The raw, unidentified data collected throughout this study will be made available via Brunel Figshare, an online data repository database.

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How to cite this article: Koch Esteves, N., Khir, A. W., & González-Alonso, J. (2023). Lower limb hyperthermia augments functional hyperaemia during small muscle mass exercise similarly in trained elderly and young humans. *Experimental Physiology*, *108*, 1154–1171. https://doi.org/10.1113/EP091275

Viewpoint: Passive leg movement: a novel method to assess vascular function during passive leg heating?

Received: 17 September 2021 | Accepted: 7 October 2021

DOI: 10.1113/EP090033

Experimental Physiology WILEY

VIEWPOINT

Passive leg movement: A novel method to assess vascular function during passive leg heating?

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Edited by: David Edwards

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Over the last decade, passive leg movement (PLM) has emerged as an increasingly popular alternative to flow-mediated dilatation (FMD) for the non-invasive assessment of vascular endothelial function. Passive leg movement involves continuous measurement of common femoral artery (CFA) blood flow (BF) by Doppler ultrasound as the participant's leg is passively extended and flexed at a fixed rate (60 movements/min) for 1–2 min. The assessment is based on the notion that the mechanical deformation of vessels caused by the movement stimulates a predominantly nitric oxide (NO)-mediated vasodilatory response (Mortensen et al., 2012), the magnitude of which is believed to be indicative of vascular endothelial function. Despite a growing appreciation of the many potential benefits of this new, easy-to-administer and non-invasive technique, many questions remain unanswered regarding the optimization of the method and the interpretation of the results.

In the October issue of *Experimental Physiology*, Shields et al. (2021) used local passive leg heating to investigate the extent to which differences in baseline BF might impact estimates of vascular function measured during PLM. Although the authors are to be commended for a well-planned and carefully executed study, their paper (as is often the case in newly burgeoning fields of research) introduced as many exciting new research questions as it answered. As far as their primary aim was concerned, their results are clear: at high levels of baseline BF (in this case, almost double normal resting values) PLM responses are affected, with the overall peak BF response found to be greater (~100 ml/min), but relative changes from baseline ($BF_{\Delta peak}$) smaller. Thus, they draw the reasonable conclusion that taking care to obtain true baseline BF values before conducting PLM is paramount to the reliability of this technique. A welcome bonus to using a local passive heating model to manipulate baseline BF in their study, however, is that Shields et al. (2021) also provide the first data, to our knowledge, in which vascular responsiveness to local hyperthermia has been reported using PLM. Although not the primary aim of their study, these new data are interesting and, at times, somewhat surprising and warrant further scrutiny.

Previous studies using the current gold-standard non-invasive test of vascular function (FMD) have generally demonstrated unchanged or even improved vascular function during acute passive heating (Romero et al., 2017). Given the agreeability between FMD and PLM outcomes, one might therefore have expected the reactive hyperaemic response to be enhanced after upper or lower leg heating and, in absolute terms (i.e., BF_{peak}), this was true. However, as pointed out by Shields et al. (2021), the relative change from baseline when combining heating and PLM was, in fact, lower than that observed during normothermic conditions, suggesting that acute heating might, in fact, attenuate (rather than augment) vascular responsiveness. Given the ever-growing interest in passive heating as a potential therapeutic intervention to improve vascular function, this finding raises several questions regarding both the intervention itself and the applicability of PLM to assess its response.

Firstly, could the timing of the measurement explain these apparently discrepant results? In the study by Shields et al. (2021), passive heating was used solely as a tool to manipulate baseline BF and, as a result, the authors rightly initiated PLM during (or immediately after) heating, when 'resting' BF levels were at their peak (approximately double baseline values). An interesting and clinically relevant area of future research might be to assess PLM responses after a post-heating return to true resting values, thereby assessing

whether the sustained exposure to increased shear stress during heating might improve longer-term endothelial function in the resting state.

Secondly, how should the observation of higher absolute but lower relative BF changes demonstrated by Shields et al. (2021) be interpreted? Although the greater absolute BF_{peak} is probably the consequence of a greater dual stimulus to both skin and muscle (i.e., the combination of skin and muscle-directed hyperthermic hyperaemia and muscle-directed mechanical hyperaemia), the finding of a reduced relative increase in profunda/deep femoral artery (DFA) BF during heating suggests a reduction in skeletal muscle vascular responsiveness. Given extraordinary capacity of skeletal muscle for vasodilatation, this finding seems surprising and certainly warrants further investigation. Although only speculative, one potential explanation might lie in the method by which DFA BF has been quantified here, with the authors opting to estimate the value as the difference between CFA BF and superficial femoral artery (SFA) BF. This technique is understandable and probably unavoidable owing to the extreme difficulty, if not impossibility, of measuring DFA during PLM. Nonetheless, it should be noted that this method has been shown by both ourselves and others to overestimate DFA BF in comparison to direct measures (Chiesa et al., 2016; Koch Esteves et al., 2021). Indeed, the finding of a significantly higher DFA than SFA during baseline measures in their study (DFA, 213 ± 89 ml/min; SFA, 135 ± 63 ml/min) is the exact opposite to what we and others have observed with direct measures (DFA, ~130 ml/min; SFA, ~170 ml/min; Chiesa et al., 2016; Hussain, 1997; Koch Esteves et al., 2021). Given the obvious importance of baseline values in calculating subsequent $BF_{\Delta peak}$, demonstrated by Shields et al. (2021) in their primary aim, an overestimated DFA BF at baseline might explain, at least in part, why the relative $BF_{\Delta peak}$ was attenuated. Further research using pharmaceutical interventions to manipulate BF independently (e.g., sodium nitroprusside infusion) might help to clarify these answers.

In summary, Shields et al. (2021) demonstrate that obtaining a true baseline is vital for the interpretation of vascular function using PLM. Furthermore, although the present study was not centred around the effects of acute passive heating, their findings provide exciting future directions for research assessing the applicability of PLM to assess vascular function in conditions where 'baseline' BF is elevated (e.g., heat stress/exercise). Passive leg movement remains a promising tool for vascular research, but further investigation is required to establish a robust protocol that can be used in various conditions.

COMPETING INTERESTS

None declared.

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How to cite this article: Koch Esteves, N., & Chiesa, S. T. (2021). Passive leg movement: A novel method to assess vascular function during passive leg heating? *Experimental Physiology*, *106*, 2335–2336. https://doi.org/10.1113/EP090033

Conference Abstracts

Regional Thermal Hyperaemia—Evidence of a Critical Role of Local Thermosensitive Mechanisms in the Control of the Human Leg Circulation During Hyperthermia

Oral presentation at the CHLS conference at Brunel University London, London, UK, 2019

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Hyperthermia is thought to increase limb tissue blood flow (BF) through activation of thermosensitive mechanisms. However, the precise vascular locus in which hyperthermia causes vasodilatation and increases in BF in the different segments of the leg is not fully understood. This study tested the hypothesis that temperature-sensitive mechanisms alter limb haemodynamics by acting in the microcirculation, downstream from the conduit arteries. **Methods**: Twenty healthy participants (31 ± 13 years) participated in three protocols. Leg temperature profiles and haemodynamics of the common, superficial and profunda femoral arteries and popliteal artery were measured during each protocol: (1) 3 h of whole-leg heating (WLH); (2) 1 h of upper-leg heating (ULH), 30 min cooling and another 1h of ULH; (3) 1 h of lower-leg heating (LLH). **Results**: WLH induced a sustained ≥3-fold elevation in upper- and lower-leg BF proportional to increases in leg temperature (T_{Leg}). Regionally, ULH increased upper-leg BF as WLH, but without changing lower $T_{Leg,}$ tissue oxygenation or BF whereas LLH increased lower-leg BF, comparable to WLH, without altering upper T_{Leg}, tissue oxygenation or profunda femoral artery BF. **Conclusion:** These findings support that microcirculatory thermosensitive mechanisms control leg circulation during local hyperthermia and that sustained regional thermal hyperaemia is a robust physiological process with potential for treatment of peripheral circulatory disorders.

Regional Thermal Hyperaemia—Evidence of a Critical Role of Local Thermosensitive Mechanisms in the Control of the Human Leg Circulation During Hyperthermia

Virtual poster presentation at the CHMLS conference at Brunel University London, London, UK, 2020

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Hyperthermia is thought to increase limb tissue blood flow (BF) through the activation of thermosensitive mechanisms within the limb vasculature, but the precise vascular locus in which hyperthermia modulates perfusion remains elusive. We tested the hypothesis that temperature-sensitive mechanisms alter limb haemodynamics by regulating microvascular BF. **Methods:** Temperature and oxygenation profiles and leg haemodynamics of the common femoral artery (CFA) and popliteal artery (POA) were measured in eight healthy participants during 1 h of upper-leg heating (ULH) followed by 30 min of cooling and 1 h bout of ULH. **Results:** Ensuing the two bouts of ULH, upper leg temperature (T_{Leg}) increased by 3.3 ± 1.1 ºC ($p < 0.0001$). This was followed by a ~3-fold elevation in upper-leg BF and a 14 ± 4 % units in quadriceps tissue oxygenation during the first 1 h bout of ULH ($p < 0.0001$). Upper-leg BF and tissue oxygenation then declined towards baseline during the 30 min cooling bout, before upper-leg BF increased >5-fold during the second 1 h bout of ULH ($p < 0.0001$). Throughout the entirety of the protocol, central haemodynamics, arterial diameter, and lower $T_{Leg,}$ tissue oxygenation and POA BF remained unchanged. **Conclusion:** These observations support the notion that heat-activated haemovascular mechanisms in the microcirculation regulate limb tissue perfusion during local hyperthermia.

Regional Thermal Hyperaemia in the Human Leg: Insight into the Role of Thermosensitive Mechanisms in the Control of the Peripheral Circulation

Poster presentation at the virtual Physiological Society's Future Physiology conference, 2021

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Hyperthermia is thought to increase limb tissue blood flow through the activation of thermosensitive mechanisms within the limb vasculature (Kalsi *et al.*, 2017). However, the precise vascular locus in which hyperthermia modulates perfusion, specifically in the different segments of the human leg, remains elusive. We tested the hypothesis that temperature-sensitive mechanisms alter limb haemodynamics by regulating microvascular blood flow. **Methods**: A cohort of healthy males and females (30 ± 12 years; mean ± SD) participated in three protocols: (a) 3 h of whole-leg heating followed by 3 h of recovery (n = 9); (b) 1 h of upper-leg heating followed by 30 min of cooling and 1 h bout of upper-leg heating (n=8) and (c) 1 h of lower-leg heating (n=8). Leg haemodynamics of the common (CFA), superficial (SFA) and profunda (PFA) femoral arteries and popliteal artery (POA) of the experimental and control leg were measured at regular intervals (every 10–30 min depending on the protocol) throughout protocols using a duplex Doppler ultrasound system. Furthermore, temperature and oxygenation profiles of the experimental and control leg were measured continuously throughout the protocols. Two-way repeated measures ANOVA tests were performed to investigate differences in haemodynamics, flow profiles and temperature between and within the experimental and control leg/leg segment over time. Additionally, regression analysis was performed to assess the relationship between various key data. **Results**: Whole-leg heating increased experimental average leg temperature (T_{Leg}) by 4.2 ± 1.2 ºC (p < 0.0001) and blood flow in the CFA, SFA, PFA and POA by ≥3-fold, whilst core and control-leg temperatures and haemodynamics remained

stable. Upper- and lower-leg blood flow increased by 525 ± 199 and 278 ± 49 mL·min^{-1} (p<0.0001), respectively, in direct response to the rise in local T_{Leg} (R^2 = 0.95) and then declined during recovery. Furthermore, upper-leg heating increased upper T_{Leg} by 3.3 ± 1.1 ºC (p < 0.0001) and upper-leg blood flow by 536 ± 162 mL·min^{-1} (p < 0.0001), without any changes to lower T_{Leg} tissue oxygenation or blood flow. These are comparable to whole-leg heating responses. Conversely, lower-leg heating increased lower T_{Leg} and blood flow—5.7 ± 0.9 ºC and 270 ± 98 mL·min^{-1} (p < 0.0001)—without altering upper T_{Leg}, tissue oxygenation or skin and PFA blood flow. Neither of three protocols resulted in changes to systemic haemodynamics, perfusion pressure or conduit artery diameter across all vessels (p > 0.1). Throughout all three protocols, changes in blood flow were directly related to changes in local T_{Leg} (R^2 = 0.97). **Conclusion**: Findings demonstrate that whole-leg hyperthermia induces profound and sustained elevations in upper- and lower-limb blood flow and that segmental hyperthermia matches the regional thermal hyperaemia, without causing thermal or haemodynamic alterations in the non-heated limb segment. These observations support the notion that heat-activated haemovascular mechanisms in the microcirculation regulate limb tissue perfusion during hyperthermia. Importantly, the markedly enhanced hyperaemia and tissue oxygenation lend support to the therapeutic potential of local hyperthermia for treatment of circulatory diseases.

Understanding the Thermal Mechanisms Controlling the Human Leg Circulation to Exploit its Therapeutic Application

Virtual poster presentation at the Doctoral Researchers conference at Brunel University London, London, UK, 2021

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The role of local thermosensitive mechanisms in the control of tissue blood flow remains poorly understood. We tested the hypothesis that heat-sensitive mechanisms play a crucial role in the regulation of leg blood flow in humans. **Methods**: Leg temperature, tissue oxygenation and blood flow of the major leg arteries were measured in eight participants during two 1 hour upper-leg heating bouts, interspersed by 30 minutes of cooling. **Results**: Upper-leg temperature, blood flow and tissue oxygenation increased 3.3 ± 1.1ºC, ~3-fold and 14 ± 4 % units, respectively (all $p < 0.0001$) during the first heating bout, declined towards baseline during cooling and increased further during the second heating bout (all $p < 0.0001$). Conversely, lower-leg temperature, blood flow, tissue oxygenation and central haemodynamics remained unchanged throughout. **Conclusion:** The present novel findings demonstrate that heat-activated microvascular mechanisms regulate human leg blood flow during local hyperthermia and provide strong support for the therapeutic use of local hyperthermia for treatment of circulatory diseases.

Lower limb hyperthermia similarly augments functional hyperaemia during knee-extensor exercise in trained elderly and young humans

Presented at the ECSS congress in Paris, France, 2023

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Exercise and heat therapies are commonly recommended to improve vascular function and health in young and elderly people (Hellsten & Nyberg, 2016; Green *et al.*, 2017). Ageing is associated with reduced limb tissue and systemic perfusion during passive and exertional whole-body hyperthermia (Minson *et al.*, 1998) and large-muscle groups exercise (Wahren *et al.*, 1974); however, evidence in isolated limb hyperthermia, small-muscle group exercise and its combination is conflicting (Kenney *et al.*, 2021). Here, we tested the hypothesis that the combination of single-leg hyperthermia and knee-extensor exercise would have an additive effect on limb haemodynamics, albeit lower in the elderly group. **Methods:** Nine trained, healthy elderly (69 ± 5 years) and ten young (26 ± 7 years) adults underwent 90 min of single-leg heating followed by 10 min of low-intensity single-leg knee-extensor exercise at 6 W and 12 W work rate (5 min each), with both the heated and control legs. Temperature and tissue oxygenation profiles, and leg haemodynamics at the femoral and popliteal arteries were measured. **Results:** In both groups, heating increased whole-leg skin temperature, blood flow and tissue oxygenation by 9.3 ± 1.6 ºC, 0.7 ± 0.2 L·min^{-1} (>3-fold), and 18 ± 6 % units ($p<0.0001$), respectively. Larger increases in leg blood flow were seen in the heated leg in comparison to the control leg during exercise (3.1 ± 0.6 vs 2.3 ± 0.4 L·min^{-1}, $p < 0.0001$) as leg blood flow was tightly coupled to leg temperature ($R^2 = 0.77$, $k = 0.27$ [0.05, 0.70]). Shear rate increased 3-fold during leg heating, 8-fold during control exercise, and 10-fold during heated exercise ($p < 0.0001$). There were no differences in limb haemodynamics between cohorts, other than the elderly group exhibiting a 16 ± 6 % larger arterial diameter, a 51 ± 6 % lower blood velocity, and a 73 ± 7 % lower shear rate following heating ($p < 0.0001$). **Conclusion:** Local hyperthermia increased leg blood flow over 3-fold and had an additive effect during knee-extensor exercise with no differences in leg perfusion between the healthy, exercise-trained elderly and the young groups. Hence, against our hypothesis, age *per se* does not compromise local hyperthermia-induced

or small-muscle group functional hyperaemia despite evident aged-related structural and functional differences in the leg conduit arteries. The novel finding that leg hyperthermia induced a 2–3-fold higher shear rate—an important stimulus for vascular adaptation (Green *et al.*, 2017)—during rest and exercise may have important implications for elderly, sedentary populations who are not able to participate in sustained exercise. The combination of lower limb heating with low-intensity exercise may be a suitable intervention to enhance the stimulus for vascular adaptation without inducing systemic thermal discomfort and physiological strain.