THERMAL AND NON-THERMAL FACTORS AFFECTING REGIONAL BLOOD FLOW REGULATION

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Doctoral Dissertation
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VPLIVI TERMALNIH IN NETERMALNIH DEJAVNIKOV NA REGIONALNO REGULACIJO PRETOKA KRVI

Doktorska disertacija

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To Mum and Dad,

Who have always supported me in the pursuit of my wild dreams and ambitions.

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Abstract

Whilst the interaction of multiple vascular mechanisms under various stressors is a well-documented topic, how this interaction effects the regional regulation of blood flow is less well known. In order to assess this phenomenon, a series of successive studies were conducted:

I. Assessing the regional blood flow and haemodynamic response to separate and combined effects of an acute orthostatic test during prolonged heat exposure in a simulated 3-day heatwave.

II. Assessing the regional blood flow and haemodynamic responses to separate and combined effects of two postures and a transient thermoregulatory stressor with ambient temperatures ranging from 15 – 40 °C. Mechanisms of autonomic control were assessed by heart rate variability and wavelet transform analyses.

III. Assessing the regional blood flow and haemodynamic responses to the separate and combined effects of acute artificial gravity (1 and 2 Gz), high and low ambient temperatures, and differing partial pressures of oxygen.

IV. Regional blood flow regulation and haemodynamic responses to artificial gravity and differing partial pressures of oxygen between females and males. The implications of similarities or differences between sexes are considered in the context of orthostatic intolerance.

The results of these four studies have led to following conclusions:

1. Skin blood flow of the arm is predominantly controlled via thermoregulatory vascular mechanisms, particularly when no interaction of mechanisms is required (i.e., when temperature is the only stressor). However, the influence of dual stressors (temperature and orthostatic) cause an interaction of multiple mechanisms (thermoregulation and baroreflex) related to the regulation of deep body temperature and blood pressure concurrently.
2. Skin blood flow of the leg, despite substantial levels of heating, appears to be unaffected by thermoregulatory mechanisms and is almost entirely controlled by the baroreflex for maintenance of stable blood pressure.
3. No significant difference in vascular mechanisms exist between females and males, in response to artificial gravity, high ambient temperatures and partial pressure of oxygen.

Povzetek

Medtem ko je interakcija žilnih mehanizmov pod vplivom različnih stresorjev dobro dokumentirana, pa je interakcija vpliva na regionalno regulacijo pretoka krvi manj znana. Da bi ocenili ta pojav, je bilo izvedenih več zaporednih študij:

i. Ocenjevanje regionalnega krvnega pretoka in hemodinamičnega odziva na ločene in kombinirane učinke akutnega ortostatskega testa med dolgotrajno izpostavljenostjo vročini v simuliranem 3-dnevnem vročinskem valu.
ii. Ocenjevanje regionalnega krvnega pretoka in hemodinamičnih odzivov na ločene in kombinirane učinke dveh telesnih položajev in spreminjajoče se temperature okolja v razponu od 15 do 40 °C. Mehanizmi avtonomnega nadzora so bili ocenjeni z analizo variabilnosti srčnega utripa in valovne transformacije.
iii. Ocenjevanje regionalnega pretoka krvi in hemodinamičnih odzivov na ločene in kombinirane učinke akutne umetne gravitacije (1 in 2 Gz), visokih in nizkih temperatur okolja ter različnih parcialnih tlakov kisika v zraku.
iv. Regionalna regulacija krvnega pretoka in hemodinamični odzivi na umetno gravitacijo in različne parcialne tlake kisika pri ženskah in moških. Podobnosti in razlike med spoloma so obravnavane v kontekstu ortostatske intolerance.

Rezultati teh štirih študij so privedli do naslednjih zaključkov:

1. Pretok krvi v koži roke je pretežno nadzorovan preko termoregulacijskih vaskularnih mehanizmov, zlasti kadar interakcija mehanizmov ni potrebna (tj. ko je temperatura edini stresor). Dodaten stresor (temperaturni in ortostatski) privede do interakcije več kot enega mehanizma.
2. Zdi se, da kljub izpostavljenosti visokim temperaturam termoregulacijski mehanizmi ne vplivajo na pretok krvi v koži noge, ki ga skoraj v celoti nadzoruje barorefleks za vzdrževanje stabilnega krvnega tlaka.
3. Umetna gravitacija in različni parcialni tlaki kisika v zraku pri ženskah in moških izzovejo podobne vaskularne odzive.

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Abbreviations

AG	Artificial Gravity
ANOVA	Analysis of Variance
BF_M	Microvascular Blood Flow
C	Convection
CH	Cold Hypoxia
CIVD	Cold Induced Vasodilation
CN	Cold Normoxia
CNS	Central Nervous System
CO	Cardiac Output
CVC	Cutaneous Vascular Conductance
DBP	Diastolic Blood Pressure
E	Evaporation
ECG	Echocardiography
Eres	Evaporation from Lung Surface Area
ESA	European Space Agency
EVA	Extra-Vehicular Activity
FFT	Fast Fourier Transformation
GRF	Ground Reaction Force
Hb	Haemoglobin
Hct	Haematocrit
HF	High Frequency
HR	Heart Rate
HRV	Heart Rate Variability
HUT	Head Up Tilt
HW	Heatwave
IRT	Infrared Thermography
ISS	International Space Station
K	Conduction
LBNP	Lower Body Negative Pressure
LCT	Lower Critical Temperature
LDF	Laser Doppler Flowmetry
LF	Low Frequency
M	Metabolism
MAP	Mean Arterial Pressure
MBP	Mean Blood Pressure
METS	Metabolic Equivalents
NG	Normal Gravity
NO	Nitric Oxide
OT	Orthostatic Tolerance
PCO_2	Partial Pressure of Carbon Dioxide

pH	Potential of Hydrogen
PNS	Parasympathetic Nervous System
PO/AH	Preoptic Anterior Hypothalamus
PO_2	Partial Pressure of Oxygen
PORH	Post Occlusion Reactive Hyperaemia
PV	Plasma Volume
R	Radiation
RH	Relative Humidity
RMSE	Root Mean Square Error
RMSSD	Root Mean Square of Successive Differences
RRi	RR Interval
S	Heat Storage
SAHC	Short Arm Human Centrifuge
SARS-COV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SBF	Skin Blood Flow
SBP	Systolic Blood Pressure
SD	Standard Deviation
SNS	Sympathetic Nervous System
STA	Standing
SUP	Supine
SV	Stroke Volume
T_A	Ambient Temperature
T_{core}	Core Temperature
T_{gi}	Gastrointestinal Temperature
TPR	Total Peripheral Resistance
T_{sk}	Skin Temperature
T_{sk}C-T	Calf-Toe Skin Temperature Gradient
T_{sk}F-F	Forearm-Fingertip Skin Temperature Gradient
T_{sk}P-D	Proximal-Distal Skin Temperature Gradient
T_{ty}	Tympanic Temperature
UCT	Upper Critical Temperature
VLF	Very Low Frequency
VOP	Venous Occlusion Plethysmography
W	External Work
WBGT	Wet Bulb Globe Temperature
WH	Warm Hypoxia
WN	Warm Normoxia
WTA	Wavelet Transform Analysis

Chapter 1

Introduction

1.1 Review of Literature

The following review of literature focusses on maintenance of thermal and cardiovascular homeostasis. It details the blood flow mechanisms which contribute to the maintenance of this internal homeostasis, and delves into the interaction between these two homeostatic mechanisms. Finally, a summary of the research relating to regional blood flow regulation is provided. The literature review will be concluded with a rational for the work described in the thesis, a description of the organisation of the thesis, the aims and hypotheses, and finally a list of publications resulting from the thesis.

1.1.1 Thermoregulation

The human body relies on homeostatic control mechanisms to maintain the internal environment of the body. Examples of such homeostatic mechanisms include the regulation of stomach acid (Holzer, 1998), concentration of ions in the blood (Yu et al., 2001), and blood glucose concentrations (Hers, 1990). Homeostasis in physiological systems is achieved by regulatory systems, which incorporate negative feedback loops. Namely, each regulatory system incorporates sensors and effectors to regulate a physiological variable. A displacement of the value of the regulated variable is sensed by specific sensors, and this will initiate a response by the effectors to minimise the displacement. For example, high blood glucose level is recognised in the pancreas by specialist cells, causing insulin to be released to reduce blood glucose levels (Hers, 1990). One of the key whole-body homeostatic mechanisms is the control of core temperature (T_{core}), which is altered as a result of both internal (exercise, fever), and external (ambient temperature, radiant heat exposure) stimuli. The thermoregulatory mechanism, often considered a 'physiological thermostat', is very efficient at sustaining an internal temperature of ~37 °C (36.1 – 37.5 °C) (Cabanac & Massonnet, 1977); beyond which thermoregulation must occur to return T_{core} to homeostatic values via the same feedback loop design. It is, however, disputed that this thermoregulatory ‘set-point’ is a didactic and convenient analogy regarding the control of T_{core}; an inherited term from a time when models of physiological responses were often explained via engineering models (Riggs, 1963). The suggestion of a single ‘set-point’ for the onset of mechanisms to both raise (shivering) and lower (sweating) the T_{core} has previously been deemed unsatisfying, and further research identified a ‘null zone’ whereby neither thermoeffector is producing a response (Mekjavic et al., 1991). A more appropriate and elegant terminology for this ‘null zone’ may be the ‘inter-threshold zone’ (Mekjavic & Eiken, 2006), or indeed the ’vasomotor zone’; whereby a vascular response is elicited to maintain suitable T_{core}.

The mechanism by which changes in temperature are detected and an appropriate response coordinated have been discussed throughout a centuries worth of research. Cooper et al. (2002) discuss this journey which is believed to have begun with Wunderlich (1871) initially attempting to define temperature changes in animals, followed by human studies. The next significant leap in knowledge occurred due to new methodological understandings of neurophysiological techniques which enabled researchers to explore the role of the CNS and hypothalamus in thermoregulation (Ranson & Ingram, 1935; Ranson & Magoun, 1939). The process of thermoregulation begins with stimuli detected via internal (deep body) or external (skin surface) thermoreceptors, which can detect both steady and rapid changes in temperature. Cold temperature receptors predominate in the skin, whereas warm receptors are more abundant centrally, such as in the spinal cord, viscera, and great veins (Tansey & Johnson, 2015). Information is then relayed via afferent neurones in the central nervous system (CNS) towards the central control of thermoregulation in the preoptic/anterior hypothalamus (PO/AH). Once detected, heat is removed from the body via the mechanisms of evaporative, radiant, conductive and convective heat loss, described by the heat balance formula (Equation 1.1). The dissipation of heat from the core has been described by both simple and complex versions of the core-shell model, first described by Aschoff and Wever (1958).

1.1.1.1 The Core-Shell Model

The 2nd Law of Thermodynamics proposes that thermal energy must always transfer from a region of higher temperature to a region of low temperature, and not in reverse in the absence of external work. For this reason, organisms must comply with maintenance of an energy balance whereby energy input is balanced with energy output; as described in the heat balance equation (Mekjavic & Tipton, 2021):

$$S = M \pm \mathrm{W} \pm \mathrm{R} \pm \mathrm{C} \pm \mathrm{K} - \mathrm{E} - E_{res}$$

Equation 1.1. Heat Balance Formula

Where:
S = heat storage (Wm^{-2}),
M = metabolism (Wm^{-2}),
W = external work (Wm^{-2}),
R = heat exchange by radiation (Wm^{-2}),
C = heat exchange by convection (Wm^{-2}),
K = heat exchange by conduction (Wm^{-2}),
E = heat loss by evaporation from the skin (Wm^{-2}),
Eres = respiratory evaporative heat loss (Wm^{-2}).

In a generalised core-shell model of heat transfer in an endotherm (Fig.1.1), the deep body temperature, or T_{core}, is maintained and stable. Heat is dissipated via the pre-described channels of heat removal from either the core or shell. In this model, the radius of the core (*r)* and diameter of the shell (*d*) are centred on the same point, and pre-defined heat loss qualities are exhibited by different tissues within the human body. In the absence of additional heat transfer via the more variable method of evaporation, the removal of heat through these tissues can be calculated with high efficiency (Speakman & Król, 2010).

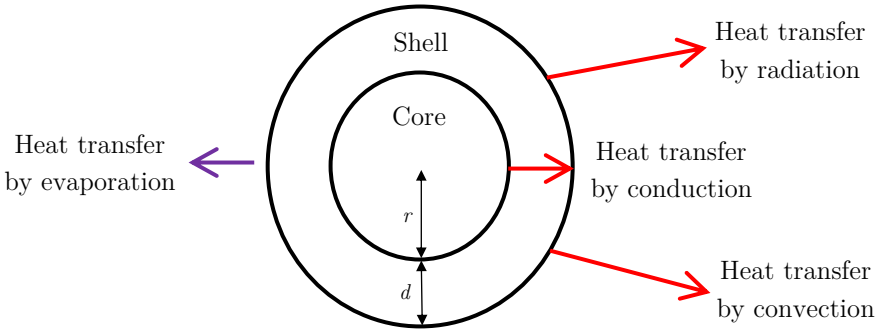

Figure 1.1: Generalised Core-Shell model of heat transfer in an endotherm. Red arrows indicate standardised heat transfer via described mechanisms, purple arrow indicates variable heat transfer via evaporation. "r" represents the radius of the core, and "d" represents the diameter of the shell. Adapted from Speakman and Król (2010).

However, while this model explains the transfer of heat to the external environment of an endotherm with calculable accuracy, it is not appropriate for the explanation of the regulation of T_{core}. Firstly, it does not account for the heterogeneity of core-shell ratios (i.e., r:d in Fig. 1.1), which alters surface-area-to-volume ratios, throughout the body; thus impacting the transfer of heat by methods of radiation, conduction, and convection. Secondly, it fails to consider the complexity of the regulation of the peripheral vasculature by cardiovascular system, and the thermoregulatory system to alter this core-shell ratio to benefit either heat gain or loss. By vasodilation and vasoconstriction of peripheral blood vessels in particular, the shell increases or decreases in diameter. Upon description of this principle by Aschoff and Wever (1958), it was considered that alterations in core-shell ratios are uniform throughout the body. As shown in Fig 1.2, a warm ambient temperature significantly increases what is considered the 'core' uniformly in the human, thus allowing appropriate heat dissipation throughout the body. This model of heat transfer may yet lack the specificity required to truly explain the thermoregulatory response of the human body.

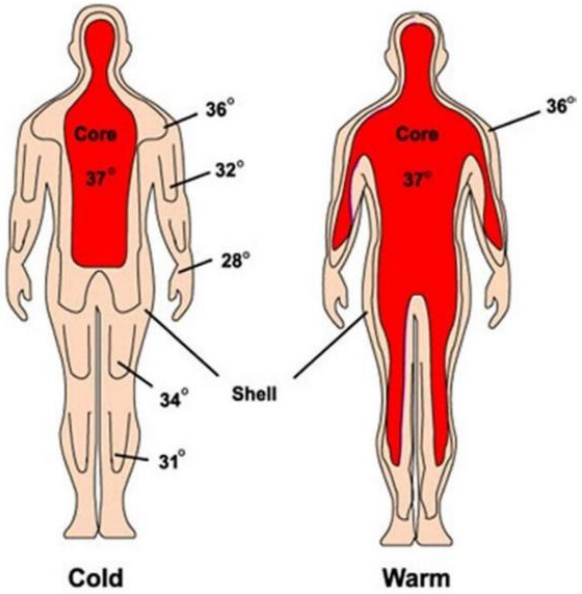

Figure 1.2: Simulated core and skin surface temperature distribution. Simulations displayed in cold and warm ambient environments. Figure c.f Tamura et al. (2018).

1.1.2 Blood Flow Mechanisms

The following section will present and categorise the research relating to the key contributors to the regulation of skin blood flow (SBF) in the human body, how they interact with one another under multiple external stressors, and finally how they may interact in specific regions of the body.

1.1.2.1 Thermoregulatory Control of Blood Flow

As a result of previously described thermoregulatory processes, shivering thermogenesis is initiated below a lower critical temperature (LCT) of ambient temperature (T_A) and sweating above an upper critical temperature (UCT) of T_A. Within the LCT and UCT exists a zone in which T_{core} is primarily controlled by changes in blood flow, particularly to the peripheral regions of the body; the vasomotor zone (Mekjavic et al., 1991). Thermoregulatory processes within the vasomotor zone are highly efficient, in thermoneutral environments the SBF is around 0.3 $L.min^{-1}$, yet during heat stress it can reach 6 - 8 $L.min^{-1}$ (Charkoudian, 2003). Recognising the role of thermoreceptors and the vasomotor zone is key in understanding how the thermoregulatory system maintains homeostasis through SBF. As previously stated, during severe heat stress SBF can increase to around 6-8 $L.min^{-1}$. This substantial increase is achieved via withdrawal of sympathetic noradrenergic vasoconstrictor and active cholinergic vasodilator pathways. However it is considered that the active vasodilator system is responsible for ~80 - 90 % of cutaneous vasodilation in the body (Charkoudian, 2003). Edholm et al. (1957) further explained this by providing a nerve blocker to one arm, inhibiting active vasodilation, to observe the effect of vasodilator withdrawal alone. This resulted in a decrease of ~10 mL per 100 $mL.min^{-1}$ of SBF to the nerve blocked arm during heating to a core temperature of 38 °C. The effects of raised SBF on heat removal were significant. Johnson (1986) proposed that with an increase in SBF from resting levels (~4 mL per 100 $mL.min^{-1}$) to as little as 8 mL per 100 $mL.min^{-1}$ will result in a doubling of heat transfer from core to shell to outside the body.

During cold stress the opposite effect occurs, though still mediated by the hypothalamus. Vasoconstriction occurs moving the blood away from the skin surface through the deep veins, which allows greater conservation of heat. This response is produced by sympathetic nerve activation of α-noradrenergic receptors to cause blood vessel smooth muscle contraction and vasoconstriction (Tansey & Johnson, 2015). Cold induced vasodilation, whilst effective at reducing heat loss, also creates a larger cardiac work demand which occurs as blood pressure and viscosity are increased whilst plasma volume decreases (Rintamaki, 2007).

1.1.2.2 Baroreflex Control of Blood Flow

The control of blood pressure, and thus blood flow, is of critical importance at rest and during heating to avoid significant cardiovascular consequences. Indeed, in his book Human Cardiovascular Control, Loring B. Rowell (1993) comments:

> *"If an engineering analysis of the aeronautical features of the bumblebee could lead to the conclusion that these insects cannot fly, then a haemodynamic analysis of the human circulation could also lead to the conclusion that human beings cannot stand up."*

Wehrwein and Joyner (2013) describe these negative consequences in terms of low and high blood pressure. Low pressure causes inadequate blood flow to vital organs, syncope,

and shock; high pressure, alternatively, creates an increased oxygen demand on the heart, ventricular remodelling, vascular injury, end organ damage, and stroke. Therefore, blood pressure regulation is essential, and such regulation is achieved via sympathetic vasoconstriction of peripheral vasculature.

Research regarding circulatory control of blood flow, was initiated by (Hering, 1927), identifying the role of the carotid sinus in baroreceptor function. In 1932, investigations into sympathetic discharge in mammals revealed the underlying and persistent regulation of blood pressure under differing manipulations (Adrian et al., 1932). Ernsting & Parry (1957) discovered that localised increase in pressure to the carotid arterial stretch receptors caused a sustained decrease in blood pressure and decreased blood flow to the forearm, hand and calf. More recently, the understanding of the integrative baroreflex system has become more complex with several important component parts; afferent pathways, central neural circuits, and vascular networks (Wehrwein & Joyner, 2013). Changes in pressure are detected in the carotid artery and aortic arch (high pressure arterial baroreceptors), and in large veins and atria of the heart (low pressure cardiopulmonary baroreceptors). These signals are sent via afferent nerves to the brain which initiates sympathetic (blood vessels and heart) and parasympathetic (pacemaker cells in sinoatrial node) efferent pathways. Work by Cowley Jr et al. (1973) proposed the novel idea that long term control of blood flow does not rely on the baroreflex. This was achieved by measuring 24-hour responses of blood pressure in dogs who had carotid sinus and aortic baroreceptors denervation. Alternative mechanisms may include a combination of salt loading and arterial baroreceptors (Osborn & Hornfeldt, 1998), and the chronic baroreceptor unloading model (Thrasher, 2004, 2005). Regardless of the long-term control of blood pressure, the baroreflex system is clearly a key driver in acute changes to blood flow in the peripheries, during significant increases or decreases in blood pressure often associated with situations in which changes in the hydrostatic gradient and exercise occur; to name a few.

Changes in the hydrostatic pressure gradient occur when there is a change in posture, known as postural stress. This stress, also known as gravitational or orthostatic stress, is caused by the effect of gravity acting on blood within the body; usually when moving from lying to sitting to standing. The effect of such stressors require immediate reaction from the baroreflex system to maintain blood pressure, and avoid pooling of blood in the lower limbs. In response to this, a positive correlation between increased levels of tilt and sympathetic cardiopulmonary baroreflex activity (Cooke et al., 1999) has been identified. Additionally, Westerhof et al. (2006) proposed that gravitational stress decreases the sensitivity to the arterial baroreflex and produces a rapid reaction. These studies agree that during gravitational stress, the baroreflex is activated and primarily activates the sympathetic system responsible for vasoconstriction in peripheral vascular vessels.

1.1.2.3 Chemoreflex Control of Blood Flow

Changes in blood pH, PCO_2, and PO_2 may occur as a result of changes in inspired gas concentration, exercise, illness, or hydration status; causing hypoxaemia or hypercapnia. In these instances, hypoxaemia is detected in carotid bodies located in the bifurcation of the common carotid artery (Biscoe, 1971) and hypercapnia is detected in the brainstem; however these are not exclusive to each other (Kara et al., 2003). Activation of either chemoreceptor area primarily elicits two responses, hyperventilation and sympathetic activation. The response to chemoreceptor activation, however, is complex, regional, and multifaceted.

Hypoxaemia elicits peripheral vasodilation and increases skeletal muscle blood flow despite an increase in sympathetic activity (Dinenno, 2016) originating from the stimulation of α-adrenergic receptors on vascular smooth muscle (Jacob et al., 2021). There

is a complex interaction between the sympathoadrenal system and locally derived vasodilatory substances (including nitric oxide) that ultimately determines the net peripheral vasodilatory response to systemic hypoxia in humans. Whereas the effect on muscular blood flow is well documented, the effect of hypoxia on SBF is more varied. It is known that in response to an acute hypoxic stimulus, regions of the body produce different vascular responses. For example, vasodilation of peripheral non-acral (Minson, 2003) and splanchnic (Halliwill & Minson, 2005; Rowell & Blackmon, 1986) vasculature, concomitant with vasoconstriction of acral (Jones et al., 2021) and pulmonary (Weir & Archer, 1995) vasculature. During exposure to hypoxia (~4500m altitude equivalent) vasodilation of cerebral arteries through cerebral autoregulation allows stable oxygenated blood supply to the brain (Ainslie et al., 2007).

1.1.3 Interaction of Blood Flow Mechanisms

The interaction of these mechanisms occurs when multiple stressors such as heating, orthostatic stress, or hypoxia are acting on the human body simultaneously. While the combination of heating and orthostatic stress occur commonly, the activation of the chemoreflex due to hypoxia is less common, and thus will not be described in such detail. Combined thermoregulatory and baroreflex mechanisms occur when sympathetic pathways required to maintain thermoregulatory homeostasis are activated, which produces a change in blood pressure sufficient to require activation of the baroreflex; or vice versa. For example, in situations where SBF is at or near maximum levels due to thermoregulation producing active vasodilation, there is a significant risk of syncope due to a lower blood pressure decreasing the flow of blood to vital organs (e.g., the brain and heart) and CNS. To mediate this, the baroreflex system coordinates an opposing response to maintain appropriate perfusion of the brain and other vital organs; described as the integrated cutaneous response (Kellogg Jr et al., 1990). Eiken et al. (1991) previously proposed that it was yet unknown in which manner cardiovascular reflexes (arterial baroreflex, muscle chemoreflex, and cardiopulmonary baroreflex) interacted in the control of circulation. Over the next thirty years, the mechanisms that control SBF have been assessed in various forms. Situations in which this balance (or competition) between differing vascular mechanisms include heating in the presence of a postural or gravitational stressor (section 1.1.3.1), during changes in leg pressure (1.1.3.2), and during exercise (section 1.1.3.3).

1.1.3.1 Heating and Gravitational Stress

At lower levels of SBF, observed in normothermia, cutaneous vasoconstriction is of little significance. It is during hyperthermia, however, that baroreflex regulation of blood pressure becomes vitally important. Raised body temperatures can produce SBF which accounts for well over 50% of cardiac output (Johnson, 1996); such an occasion requires an appropriate baroreflex response to mediate SBF and sustain blood pressure. Modification of the thermoregulatory control is understood to be a result of a change in the internal temperatures at which a significant shift towards vasodilation occurs, as a result of baroreceptor unloading (Johnson & Park, 1981; Roberts & Wenger, 1980). A seemingly small shift in the threshold, which may only be ~0.5°C, can have a significant impact on the SBF at a given time point, as depicted in Fig. 1.3 (Charkoudian, 2003). It has been reported that a 1 °C shift in skin temperature threshold is sufficient to reduce the slope of the SBF to T_{core} relationship by as much as 12 - 13 % (Wenger et al., 1985).

Under normothermic temperature conditions, both head-up tilting (Lind et al., 1968; Wilson et al., 2002) and lower body negative pressure (Johnson et al., 1973) initiate cutaneous vasoconstriction; preventing blood pooling. Consequently, in normal

temperatures this system is almost entirely controlled by the baroreflex. It is when gravitational stress is combined with heat stress that an interaction of the blood flow mechanisms must occur, and often pronounced reductions in orthostatic tolerance are observed. For example, Wilson et al. (2002) observed only four of nine participants were able to withstand orthostatic stress for 10 minutes after a 0.9°C increase in core temperature. Despite this, Crandall et al. (2003) proposes that whole body heating does not alter baroreflex sensitivity and consequent impairment of the baroreflex. Rather, it is suggested the progressive reductions in orthostatic tolerance during heat stress are the product of reductions in post-synaptic vasoconstrictor responsiveness in the skin and muscle (Crandall et al., 2003; Wilson et al., 2002). This is also likely to influence heart rate and blood pressure due to prevailing heart stress, causing a shift in the baroreflex curve.

Interestingly, whilst it is known that these mechanisms directly control SBF, the effect of sympathetic nerve activity that produces vasoconstriction or vasodilation varies across the skin's surface. Johnson and Proppe (Johnson, 1996) identify that while non-acral skin (limbs, torso) is controlled by both types of nerve activity, acral skin (fingers, toes, regions of face) is almost entirely controlled by vasoconstrictor nerves only. A number of other studies have also confirmed this presence of vasodilatory nerves in acral skin (Kellogg Jr et al., 1990; Roddie et al., 1957a, 1957b). For this reason, it must be considered that during heat stress whereby active vasodilation occurs, the primary areas for heat removal are in the limbs and torso. The baroreflex, likewise, also acts heavily in the limb regions of the human body; attempting to maintain blood pressure and regulate the majority of the blood in the centre of the body. The limbs, then, are the key arenas in which thermoregulation and the baroreflex compete for control of SBF during heat stress.

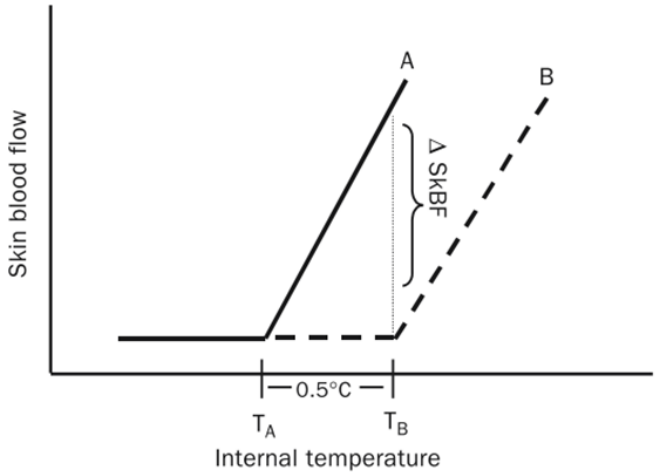

Figure 1.3: Skin blood flow responses to whole-body heating. T_A and T_B represent two internal thresholds 0.5°C apart. The vertical line (i.e., change in skin blood flow) emphasises the effect of this shift. Figure c.f Charkoudian (2003).

1.1.3.2 Positive and Negative Lower Body Pressures

Leg Positive Pressure (LPP) and Lower Body Negative Pressure (LBNP) stimulate baroreflex and chemoreflex action due to changes in blood flow resulting from alterations in hydrostatic pressure (Eiken et al., 1991). LPP is achieved by exposing the legs to a supra-atmospheric pressure, causing a restriction of blood flow to the muscles as subsequent chemoreflex activation. LBNP causes an increase in leg blood flow via exposure to sub-atmospheric pressure, creating an unloading of cardiopulmonary baroreceptors. When combining LPP and LBNP with exercise, an interaction of multiple baroreflex and chemoreflex mechanisms are required to maintain blood flow to working muscles and

subsequently exercise performance. Eiken et al. (1991) detected that sensitivity and operational range of the carotid baroreflex was higher during LPP with exercise than without exercise; likely to be caused by a resetting of the arterial baroreflex, and signifying significant modulation via the chemoreflex. Yet the unloading of cardiopulmonary baroreceptors via LBNP appeared to have no impact on the carotid baroreflex response. During subsequent studies designed to view the interaction of blood-volume changes via LBNP with carotid baroreflex stimulation and exercise, it was again confirmed that exercise is responsible for increases in carotid baroreflex sensitivity and buffering range, yet reduced central venous pressure and subsequent cardiopulmonary unloading had no impact on the carotid baroreflex (Eiken et al., 1994). Finally, the effect of ischemia during exercise, via LPP, on thermoeffector sweating responses was examined (Eiken & Mekjavic, 2004). In this study, reductions in muscle blood flow caused an increase in the sweating response of ~43 % (Fig. 1.4), which were recommended to be due to triggering of non-thermal stimuli. Eiken and Mekjavic (2004) also suggested an alternate theory whereby this response may also, in part, be a 'central command' baroreceptor-mediated response. It is concluded that the possibility of modulation in thermoregulation via baroreceptor activity is under-represented and somewhat ambiguous. With an absence of significant research since this study, the hypothesis of interaction between vascular mechanisms, therefore, remains open to interpretation.

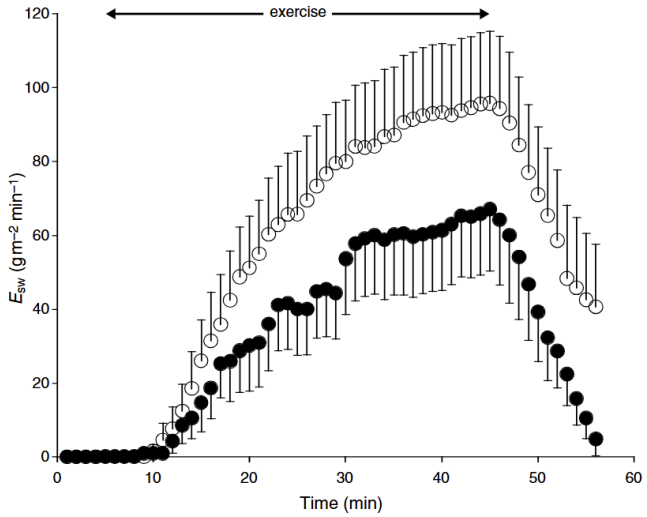

Figure 1.4: Effects of steady state exercise on evaporative sweat rate. ΔEsw = evaporative sweat rate. Ishaemic = clear dots, control = black dots. Displaying the modulation of a thermoeffector sweating response via LPP, which may be due to a muscle chemoreflex or a 'central command' initiating sympathetic drive. Figure c.f Eiken & Mekjavic (2004).

1.1.3.3 Exercise

During dynamic exercise, T_{core} rises as a result of exercise induced heat load which is turn causes an increase in vasodilation (Kenny & Journeay, 2010). However, the relationship between exercise and SBF is not as linear as first assumed. Dynamic exercise is responsible for an increase in the threshold for skin vasodilation (Johnson & Park, 1981), and the level of where this threshold exists also appears to be related to exercise intensity (Smolander et al., 1991; Taylor et al., 1988) and ambient temperature (Kenny et al., 1997). It is suggested that this increased threshold is a result of the active vasodilatory system, which is known due to studies identifying that skin either treated or untreated with noradrenaline

blockers (responsible for vasoconstriction) display identical changes in the thresholds (Kellogg Jr et al., 1991; Kenny et al., 1997).

However, a different theory for this shift in the vasodilatory threshold identifies the interaction of the baroreflex during exercise in the heat; proposing that baroreceptors have a prominent role in modulating the active cutaneous vasodilation (Mack et al., 1995). Kellogg et al. (1993) also recommends that the baroreflex system mediates a reflex adaptation to exercise by altering active vasoconstrictor activity, regardless of thermoregulatory status. This control appears to be observed most clearly during prolonged exercise. Following the discussed rise in core temperature and subsequent SBF, a plateau of SBF occurs despite this elevated state of hyperthermia (Brengelmann et al., 1977; González-Alonso et al., 2008; Kellogg Jr et al., 1993) which occurs at internal temperature of ~38°C. It would appear that this is due to mediation via the baroreflex to avoid significantly high levels of SBF. However, studies assessing this response using localised nerve blockers (Kellogg Jr et al., 1993; Kenny et al., 1997) show that control in the rise of SBF is primarily due to limiting of the active vasodilatory system, rather than an increase in the more baroreflex controlled vasoconstrictor activity.

1.1.4 Regional Variations in Skin Blood Flow

The previous sections have discussed the great body of research that already exists to understand thermoregulatory and baroreflex mechanisms, and their effect on SBF. These studies have also explained the interaction of these mechanisms and how one is able to mediate the other in differing situations, often under the influence of external stressors such as heat or exercise. More than 30 years since the proposal of an integrated cardiovascular response,

The following section will describe and critically evaluate the research that currently exists regarding the interaction of thermoregulatory and baroreflex mechanisms in specific regional areas of the human body.

The earliest identified observations of regional blood flow differences were completed by Nishiyasu and colleagues (1992). In this study thirteen participants conducted either single leg cycle ergometer in 85 % humidity, or intermittent supine cycling exercises in 30% humidity; both occurred at 35 °C temperature. Forearm and calf blood flow were measured using venous occlusion plethysmography (VOP). The results of the study propose some key findings; forearm and calf blood flow responses to increased core temperature are significantly different, and that in this case the forearm blood flow was considerably higher. It was suggested that when exercising in the heat, to resist blood pooling, the skin vasodilator responsiveness in the legs in attenuated. These results likely support a notion of higher thermoregulatory drive in the forearms; however, this study could not confirm this was due to the greater role of the baroreflex during exercise. This may have dampened the calf's ability to vasodilate as it may under resting heat stress.

Hales and colleagues (1994) continued with the notion of regional measurement of SBF, but focused on validating the use of the forearm as an index of whole body SBF, measuring at three novel sites. To achieve this, four elite endurance athletes exercised on a cycle ergometer at 60 % of their maximal oxygen uptake; in 40 - 42 °C ambient temperature. During exercise SBF was measured using photoplethysmography at the forearm, forehead, chest, and finger. The authors discovered that, particularly at rest, these four sites were highly different from each other. The forearm reached a blood flow double that of the chest upon onset of exercise, whereas the forehead and finger displayed resting blood flow 3-4 times higher than the other sites. Very little true explanation for these variations are described in this study, and the authors simply identify that interregional difference clearly

exist. Future studies are also recommended which should consider the SBF response to heat stress; whereby the vasodilator response is not maintained due to competition for maintenance of blood pressure. Although not mentioned, these variations may be due to the abundance of arterial anastomoses found in acral skin (forehead and fingertip).

Finally, Ciuha et al. (2019) observed differences in proximal-distal temperature gradients, an index of cutaneous perfusion, between the arm and leg. The arm displayed a greater acute reaction to increases in whole body heating, suggesting a greater sensitivity to temperature changes, whereas the blood perfusion of the leg was delayed and much smaller (Fig. 1.4). These results have also been noted by Gerrett et al. (2017) who observed a greater thermoregulatory sensitivity to sweating in the fingers. The authors suggest that the increased skin perfusion observed during heat stress is associated with differing sympathetic nerve responses to heat stress in the upper and lower extremities. However, they go on explaining that the major reflex changes in vascular resistance to thermal stressors is similar to those seen in Essandoh et al. (1988) during orthostatic stress. This seems unlikely as previous work has distinguished the differences in the mediation of response between thermoregulatory and baroreflex mechanisms.

Each of these previous studies take place in ambient conditions designed to raise the core temperature above the threshold for cutaneous vasodilation brought on by the thermoregulatory system. Experiments conducted by Nishiyasu et al., (1992) and Hales et al. (1994) also layered another additional stressor of exercise on top of heat stress, with each identifying the forearm as the region of highest blood flow. Despite both conducting lower body cycle ergometer exercise, these were performed in different postures and intensities which does not provide a full understanding of the underlying mechanics required for the human body to function under exercise and heat stress. Ciuha et al. (2019) focused solely on the effects of acute and prolonged increases in heat stress, which confirms that the forearms are more sensitive to changes in temperature (Fig. 1.4).

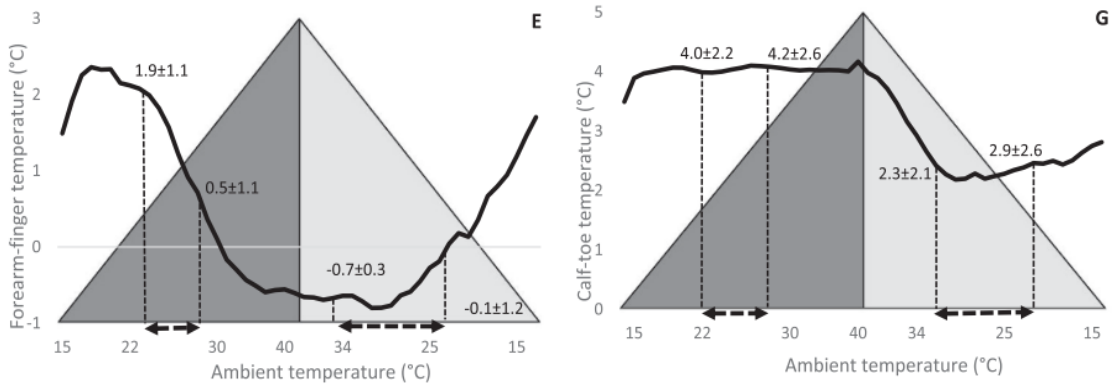

Figure 1.5: Acute response to continuous changes in ambient temperature. Measurements collected between 15 - 40°C. Proximal-distal measurements (y-axis) display vasodilation <0°C and vasoconstriction >2°C. Figure c.f. Ciuha et al. (2019).

1.2 Thesis Rationale and Application

To the author's knowledge, no study has considered the integrated roles of thermoregulation and the baroreflex in regional areas of the human body whilst experiencing a range of external stressors at rest; including temperature, postural and gravitational stress, and hypoxia. The knowledge and understanding gained during the presented body of research will add valuable information to the current body of knowledge surrounding the separate and combined effects of multiple vascular mechanisms;

particularly with reference to regional alterations in the core-shell ratio. Moreover, this information may also assist health and performance in multiple facets of life. In particular, the understanding of thermoregulatory control on a more regional basis can aid development of more effective and efficient advisory information, cooling strategies, and heat acclimation protocols in both industrial and sport performance spheres. Additionally, knowledge of regional blood flow and cardiovascular response to artificial- and hyper-gravity will aid development of training countermeasures for spaceflight in the pursuit of orthostatic intolerance prevention. The following sections will describe these benefits in greater detail.

1.2.1 Excessive Ambient Temperatures

Knowledge of regional thermo- and baro-regulatory control may be of benefit in occupational and public health settings. Frequency, duration, and intensity of heatwaves (HWs) have steadily increased in the 21st century, generating excessive cardiovascular and thermoregulatory strain and potential for fatality (Bell, 1981; Brücker, 2005; Ciuha, Pogačar, et al., 2019). When combined with the orthostatic challenges presented via occupational work, and clinical conditions such as orthostatic hypotension (Freeman et al., 2018), the possibility of conditions such as heat strain and/syncope rapidly increases. Better knowledge of regional vascular mechanisms may allow better workplace advisory information, cooling strategies, and healthcare for those enduring regular HW conditions. In contrast, understanding of regional blood flow in response to cold environments may also aid our understanding of the treatment and pathophysiology of freezing and non-freezing cold injuries (Eglin et al., 2023).

1.2.2 Sports Performance

In sport and exercise environments, the use of cooling strategies before, during, and after exercise have been proven to improve performance in thermally challenging environments. In a review of cooling methods anticipated to be used during the Tokyo Olympics (2020), pre-exercise cooling methods provided a 2.0 – 7.1 % improvement in exercise performance, and cooling-methods used during exercise offered a 3.2 – 11.9 % benefit (Bongers et al., 2021). The greatest improvements in exercise performance were achieved by cold-water immersion and cooling vests, for pre- and during- exercise respectively, which both may be adapted with more specific thermoregulatory input. It is possible that with a more specific understanding of the regional thermoregulatory control mechanisms, the ergonomics and efficacy these cooling strategies may be improved even further.

1.2.3 Diagnosis of Fever

Current methods of diagnosing illness and fever quickly include the use of infrared thermography to calculate T_{core} via skin temperature (T_{sk}). However, it is commonly reported that these devices are inefficient at establishing absolute T_{core} via estimations from a single site (Mekjavic & Tipton, 2021). It is suggested that alternative sites, such as the inner canthus of the eye, or simple measures of skin perfusion, may be more effective in detecting fever or other properties of illness. It is likely that areas of the body more sensitive to changes from thermoregulation, such as the arm as suggested by previous literature, may be the most successful at estimating T_{core}. Appendix A details whether the use of contact thermography and forearm skin perfusion can successfully estimate T_{core}.

1.2.4 Spaceflight Countermeasures

In both terrestrial and spaceflight examples, orthostatic intolerance is a frequently observed event relating to the loss of blood pressure, circulating blood volume, and resultant cerebral oxygenation (Stewart, 2012). In spaceflight, reductions in orthostatic tolerance are an issue upon return to earth and a hydrostatic column is re-established, whilst deconditioning of the cardiovascular system means this gravitational/orthostatic stress cannot be resisted and syncope occurs. One such method of training the cardiovascular system to resist deconditioning is the use of a short-arm human centrifuge, and knowledge of the regional variations in thermo- and baro-regulatory control may be beneficial in optimising the countermeasure's efficacy via individualisation (Goswami, Evans, et al., 2015). Once known and understood, we may be able to successfully integrate these mechanisms in response to multiple stressors (temperature and artificial gravity), further improving the cardiovascular load during exercise and subsequent improvements in orthostatic tolerance upon return to Earth.

1.3 Organisation of the Thesis

This thesis presents four consecutive studies which identified the occurrence of regional SBF, and examined possible mechanistic factors related to this phenomenon. Each of the studies received appropriate ethical approval (appendices B, C, and D) and conformed to the Declaration of Helsinki. The studies are as follows:

1. Study I: Cardiovascular Responses to Orthostasis During a Simulated 3-Day Heatwave.
2. Study II: The Combined Effects of Temperature and Posture on Regional Blood Flow and Haemodynamics.
3. Study III: The Combined Effects of Artificial Gravity, Temperature and Hypoxia on Haemodynamic Responses and Limb Blood Flow.
4. Study IV: Implications of Sex Differences in Orthostatic Tolerance During Exposure to Acute Artificial Gravity.

The introduction (Chapter 1) presents the background of research in the field of blood flow regulation in humans, followed by the more specific themes of integrated SBF regulation, and finally the regional variation in the control of SBF. The chapter concludes with a rationale and application of the research to more generalised use cases.

Study I (Chapter 2) utilised an orthostatic tolerance test via a head-up tilt test during a nine day study which included a simulated three-day HW. During this test, an index of blood perfusion, proximal-distal skin temperature gradient, was employed to assess the regional variation in SBF to temperature and postural stressors. In addition, the validity of proximal-distal skin temperature gradient is discussed.

Study II (Chapter 3) evaluated regional SBF responses, measured by laser-Doppler flowmetry, in two separate postures during a transient change in T_A from 15 - 40 °C. Using mechanistic analyses, the central and peripheral autonomic analyses are also examined. This study aimed to discover how thermoregulation is modulated by the baroreflex.

Study III (Chapter 4) assessed regional SBF responses during higher gravitational stress than previous studies via artificial gravity (AG), achieved using a short-arm human centrifuge (SAHC). By also varying T_A (18 and 29 °C) and partial pressures of oxygen (92 and 133 mmHg), the regional interaction of SBF responses were assessed, and their effect of overall cardiovascular strain observed. This study attempted to identify how the baroreflex may be modulated by both thermoregulation and the chemoreflex.

Study IV (Chapter 5) evaluated regional SBF and haemodynamic responses of females and males to AG and varying partial pressures of oxygen (92 and 133 mmHg), whilst experiencing high T_A only. These responses are considered in their implications for sex differences in tolerance to an orthostatic stressor.

Conclusions (Chapter 6) of the work conducted during the previously described studies under the framework of the overall thesis are summarised.

1.4 Aims and Hypotheses

The aim of this thesis is to identify and document the regional SBF responses to thermal and non-thermal factors, including the examination of autonomic control via mechanistic analyses. In each of the four studies a null hypothesis was tested, and in the case of rejection a set of alternative hypotheses are provided. The aims, null hypotheses, and alternative hypotheses are provided below:

Study I (Chapter 2): Assess the haemodynamic and regional perfusion responses to an orthostatic test during simulated 3-day HW.

- ***Null Hypothesis 1 ($H_0$1):*** There will be no effect of a 3-day heat wave on the cardiovascular responses to orthostasis of young male subjects.
- *Alternative Hypothesis 1 (H_a1):* High ambient temperature conditions will result in increased peripheral perfusion augmenting heat loss.
- *H_a2:* Orthostatic stress will cause a reduction in blood flow to maintain arterial blood pressure.
- *H_a3:* The interaction of heat and orthostatic stress will produce regional differences (i.e., arms and legs) in the regulation of blood flow balancing the need to maintain arterial pressure and heat loss.

Study II (Chapter 3): Assess the regional microvascular blood flow, neural control, and haemodynamic responses to postural and transient thermoregulatory stress.

- ***$H_0$2:*** There will be no differences in regional blood flow of the arm and leg, in either resting or demanding ambient conditions.
- *H_a4:* Thermal stress alone will increase SBF, with a greater increase observed in the arm.
- *H_a5:* Postural stress alone will decrease SBF, with a greater decrease observed in the leg.
- *H_a6:* Combinations of thermal and gravitational/postural load will cause the blood flow to diverge in different regions, with the arm displaying a greater predisposition towards thermoregulation and the leg towards the baroreflex.
- *H_a7:* Central and peripheral mechanisms of autonomic control will differ in the arm and leg, under thermal and gravitational stressors.

Study III (Chapter 4): Assess the haemodynamic and microvascular blood flow responses to stressors resulting from acute artificial gravity, ambient temperature, and partial pressure of oxygen.

- ***$H_0$3:*** Separate and combined effects of ambient temperature, oxygen concentration, and artificial gravity will have no effect of regional blood flow response.

- *H_a8:* Thermal stress alone will increase SBF, with a greater increase observed in the arm.
- *H_a9:* Gravitational/postural stress alone will decrease SBF, with a greater decrease observed in the leg.
- *H_a10:* Hypergravity alone will increase blood pooling in the lower limbs, which will be observed in the leg blood flow.
- *H_a11:* Hypoxia alone will increase skin blood in both the arm and leg.
- *H_a12:* Combined effects of ambient temperature, oxygen concentration, and artificial gravity will differentiate regional blood flow with the arm displaying a greater predisposition towards thermoregulation and the leg towards the baroreflex.

Study IV (Chapter 5): Assess the differences in haemodynamic and regional blood flow responses between sexes, and its impact of orthostatic tolerance in differing ambient conditions.

- ***$H_0$4:*** There will be no differences in the regional blood flow response between females and males, to differing and demanding conditions.
- *H_a13:* Females and males will exhibit a difference in the blood flow response to various stressors, with males exhibiting greater vasoconstriction that females.
- *H_a14:* The cardiovascular response will be unable to prevent participants exhibiting pre-syncopal symptoms in hypergravity.

1.5 Publications Resulting from the Thesis

Fisher, J. T., Ciuha, U., Ioannou, L. G., Simpson, L. L., Possnig, C., Lawley, J., & Mekjavic, I. B. (2022). Cardiovascular responses to orthostasis during a simulated 3-day heatwave. *Scientific Reports, 12*(1), 19998.

Fisher, J. T., Ciuha, U., & Mekjavic, I. B. (2024). The Combined Effects of Temperature and Posture on Regional Blood Flow and Haemodynamics. *Journal of Thermal Biology, 123,* 103937.

Fisher, J.T., Ciuha, U., Denise, P., McDonnell, A.C., Normand, H. & Mekjavic, I.B. (Submitted). The Combined Effects of Artificial Gravity, Temperature and Hypoxia on Haemodynamic Responses and Limb Blood Flow. *NPJ Microgravity.*

Fisher, J.T., Ciuha, U., & Mekjavic, I.B. (Submitted) Implications of Sex Differences in Orthostatic Tolerance During Exposure to Acute Artificial Gravity. *European Journal of Applied Physiology.*

Fisher, J. T., Ciuha, U., Tipton, M. J., Ioannou, L. G., & Mekjavic, I. B. (2022). Predicting deep body temperature (Tb) from forehead skin temperature: Tb or not Tb? *Sensors, 22*(3), 826.

Chapter 2

Cardiovascular Responses to Orthostasis during a Simulated 3-Day Heatwave

2.1 Foreword

While some of the most recent work detecting regional variations in blood flow regulation identified clear differences between the upper and lower limbs in the body (Ciuha, Tobita, et al., 2019), this phenomena was only detected as a secondary observation. The true aim of the study was to assess the effects of a transient thermal environment on the perception of thermal comfort, and participants were in a seated position throughout the entire protocol whilst the ambient temperature varied. Whilst the seated position creates an orthostatic challenge, in non-orthostatic hypotensive adults this position vs. a standing position is negligible in terms of blood pressure changes (Juraschek et al., 2022). Therefore, the lack of a significant orthostatic challenge or comparative orthostatic variable mean that little baroreflex related conclusions may be drawn from this study. The results, then, largely indicate the differences between arms and legs in response to a thermoregulatory challenge alone.

In order to validate and build on this previous research, a study with a specific focus on the regional blood flow response was formulated and conducted within the context of a larger study. This larger study assessed the effects of a simulated heat wave (HW) on physiological strain and labour productivity (Ioannou et al., (2021). The study that will be discussed in the present chapter in the context of the thesis assessed skin perfusion and haemodynamic responses to separate and combined effects of thermoregulatory and baroreflex stressors. The results of this study helped to validate the observations made by Ciuha et al. (2019) and provide an understanding of the regional interaction of blood flow mechanisms.

The findings of this study were published in Scientific Reports under the following citation: Fisher, J. T., Ciuha, U., Ioannou, L. G., Simpson, L. L., Possnig, C., Lawley, J., & Mekjavic, I. B. (2022). Cardiovascular responses to orthostasis during a simulated 3-day heatwave. *Scientific Reports, 12*(1), 19998.

2.2 Introduction

Due, in part, to the ever-increasing concentration of greenhouse gases in the 20th and 21st century, global temperatures have continuously risen, increasing the risk to health and wellbeing for billions of people worldwide. As global temperatures continue to increase, heatwaves (HW) have become a regular occurrence; defined as a minimum of three consecutive days where the daily mean temperature exceeds 95% of the seasonal average (Nairn & Fawcett, 2011). The occurrence of these HWs has increased, as has their duration, and intensity. HWs can cause serious health consequences and risk of fatality to those experiencing them, due to the prolonged and excessive strain on the human body's cardiovascular and thermoregulatory systems. Exceptionally severe HWs, such as the 2003 pan-European HW, can be responsible for tens of thousands of deaths: particularly in the elderly and vulnerable populations (Brücker, 2005).

The effects of HWs on humans can range from thermal discomfort and decrements in cognitive performance during complex tasks (Ciuha, Pogačar, et al., 2019; Hancock & Vasmatzidis, 2003), to more serious complications such as heat exhaustion/stroke/cramp, risk of syncope, and fatality (Bell, 1981; Crandall et al., 2010; Lye & Kamal, 1977). Complications may, in part, be the result of excessive demand on the cardiovascular system, which needs to maintain both blood pressure and heat exchange (Charkoudian, 2003). These health issues may also vary due to a multitude of factors, including age, acclimatization, physical fitness, medical conditions, and environmental stress experienced (Ioannou, Gkikas, et al., 2021). Additionally, those working in a HW will likely experience orthostatic stress, the combination of which with heat stress is likely to be detrimental. During sustained heat stress and orthostatic challenge, ventricular filling and stroke volume are reduced due to blood pooling. This leads to sympathetically mediated adjustments for increases in heart rate (HR), cardiac contractibility and vascular resistance (Schlader et al., 2016). Lind et al. (1968) reported a decrease in forearm blood flow whilst HR increased, however, these responses precede syncope, which is characterised by a significant fall in HR concomitant with a significant decrease in blood pressure. However, individuals can aid their thermoregulatory systems via changes in behaviour. These include seeking shelter, using cooling items such as water or fans, or wearing light-coloured clothing to reflect direct sunlight; the primary aim of which is the reduction of metabolic rate, consequently reducing the metabolic heat production (Ioannou, Mantzios, Tsoutsoubi, Nintou, et al., 2021). Some occupations, however, limit individuals from behaviourally assisting thermoregulation. Occupational heat strain in jobs that require intense physical activity, protective clothing, and exposure to extreme ambient temperatures, such as the military, construction, agriculture, and tourism, are well documented (Brake & Bates, 2002; Hunt et al., 2016; Ioannou, Mantzios, Tsoutsoubi, Nintou, et al., 2021). Indoor workers though, who are also unable to control their external environment, have received far less scrutiny. Ciuha, Pogačar, et al. (2019) observed the impact of elevated ambient workplace temperatures, during a summer HW, which produced significant drops in manufacturing productivity (Odelo Slovenija d.o.o, Prebold, Slovenia). A systematic review by Flouris et al. (2018) suggested that an estimated 35% of individuals working in hot conditions experience occupational heat strain, and 30% of workers report productivity losses. Understanding the cardiovascular and thermoregulatory challenges associated with indoor workers during a HW is essential for developing appropriate detection, prevention, and treatment techniques for workers.

The principal aim of the present study was to assess the cardiovascular and regional blood flow responses to HW conditions using a novel protocol: confining participants to laboratory-controlled ambient conditions for nine consecutive days, during which the first

and last three days were normal temperature days, whereas days 4 to 6 simulated heat wave conditions. It was hypothesized that during a simulated 3-day HW:

1. Both gastrointestinal (T_{gi}) and skin (T_{sk}) temperatures would significantly increase and thus indicate the onset of heat strain.
2. High ambient temperature conditions will result in increased peripheral perfusion augmenting heat loss.
3. Orthostatic stress will cause a reduction in blood flow to maintain arterial blood pressure.
4. The interaction of heat and orthostatic stress will produce regional differences (i.e., arms and legs) in the regulation of blood flow balancing the need to maintain arterial pressure and heat loss.

2.3 Materials and Methods

The present study was part of a larger project (European Commission Horizon 2020 project "Heat Shield") investigating the effect of HWs on the health, well-being, and labour productivity of workers in industry. The aim was to simulate a schedule of work (0900 – 1800 hrs) and rest (1800 – 0900 hrs), as would be experienced by workers in the manufacturing industry, but under controlled conditions. Participants were confined to three areas (living quarters, work area, and cafeteria) at the Olympic Sport Centre Planica (Rateče, Slovenia).

2.3.1 Experimental Design

The study investigated the effect of a laboratory-controlled 3-day HW and orthostatic stress on cardiovascular measures of heart rate (HR, $b \cdot min^{-1}$), stroke volume (SV, mL), cardiac output (CO, $L \cdot min^{-1}$), systolic (SBP) and diastolic (DBP) blood pressure (mmHg), total peripheral resistance (TPR, $mmHg \cdot min \cdot L^{-1}$), and SBF, as reflected by proximal-distal skin temperature gradient (ΔT_{sk}P-D). Thermal status was assessed by gastrointestinal (T_{gi}) and skin (T_{sk}) temperatures. Both cardiovascular and thermal variables were collected during a 10-minute supine rest, and 10-minute 60 ° head-up tilt (HUT) designed to introduce orthostatic stress. The 9-day experimental period commenced with a 3-day pre-HW period at neutral temperature (work: 25.4 ± 0.3 °C; rest: 22.3 ± 0.5 °C). A 3-day HW followed during which the diurnal and nocturnal ambient temperature increased in work and living areas (work: 35.5 ± 0.3 °C; rest: 26.3 ± 0.8 °C). The study concluded with a 3-day post-HW period at neutral temperature conditions. Ambient temperatures in the work areas were changed at 0700hrs of days four and seven, so the participants arrived in the work area at the correct temperature. Similarly, the living areas were changed at 1200hrs on days four and seven, so the participants returned to their living areas at the correct temperature. Relative humidity remained constant at 45 % throughout the study in all areas, which is more commonly associated with dry HWs such as those observed in Europe and the Western US (Russo et al., 2017). To avoid habitual acclimatization prior to the study, experiments took place in ambient conditions of 19.8 ± 1.8 °C Wet-Bulb Globe Temperature (WBGT; from www.wunderground.com; accessed on 28 July 2021).

The ambient conditions throughout the whole study were simulated using data from a 3-day HW of similar intensity in Slovenia (Ciuha, Pogačar, et al., 2019). The 24-hr ambient temperature within the facility did not mimic the sinusoidal change in ambient temperature observed in the mentioned HW, but maintained the diurnal and nocturnal temperatures in

the work and rest areas at average temperatures. Each day during working hours, the participants conducted low-intensity exercise sessions, comprising a stepping test (metabolic requirement of approximately 2.8 METS) in two 40-minute sessions, and simulated work tasks (metabolic requirement of approximately 1.5 METS) for 2 hours each day to represent a normal working day in industry; alongside cognitive testing. During the HW, participants took a 1-hour lunchbreak in normothermic conditions (25 °C), similar to industrial workers taking a break in a cooled canteen. The participants wore identical work overalls throughout the work period. The full global protocol used in the present study has been described previously Ioannou, Mantzios, Tsoutsoubi, Panagiotaki, et al. (2021) .

2.3.2 Participant Information

A sample size of seven participants was deemed to provide sufficient power to detect a statistical significance, assuming an α of 0.001 and β of 0.99 (G*Power Version 3.1.9.6, Germany). These results were calculated from the results of a previous study (Ioannou et al., 2017) which described a 0.8 % increase in labour loss for every degree increase in air temperature ($R^2 = 0.47$). Ioannou, Mantzios, Tsoutsoubi, Panagiotaki, et al. (2021) further discuss the sample size calculation used in the present study. Thus, seven healthy young male participants were recruited to take part in the study; age: 21.5 ± 1.2 years; body stature: 180.0 ± 5.6 cm; body mass: 81.5 ± 14.5 kg; body mass index: 25.1 ± 4.0 kg.m^2; fat mass: 22.5 ± 7.9 %. All were non-smokers, engaged in regular physical activity recreationally, and were free from known cardiovascular, respiratory, and autonomic disease. The protocol was approved by the National Committee for Medical Ethics at the Ministry of Health of the Republic of Slovenia (appendix B, approval no. 0120-402/2020/4) and conformed to the guidelines of the Declaration of Helsinki. Prior to the start of the study, participants were familiarized with the study protocol and procedures, and gave their written consent for participation. Participants refrained from heavy exercise, alcohol, and caffeine throughout the entirety of the study. The participants' diet was controlled and closely monitored throughout the study (Open Platform for Clinical Nutrition, www.opkp.si); set menus ensured the participants ate identical 3-day meal plans during each ambient condition.

Seven additional male participants (age: 27.7 ± 6.4 years; height: 178.7 ± 7.7 cm; body mass: 79.1 ± 9.2 kg; body mass index: 24.7 ± 2.0 kg.m2) were recruited for the validation of the ΔT_{sk}P-D measurement, as an index of SBF, against Laser Doppler Flowmetry.

2.3.3 60° Head-up Tilt (HUT) Protocol

At the same time each day during the work period, participants underwent a 60° HUT test protocol. This was done in order to expose participants to stressors (ambient temperature, orthostatic stress) that would manipulate the baroreflex and thermoregulatory systems and produce a conflict in SBF regulation. The HUT protocol was conducted 1.5-hours after the first set of stepping exercise and simulated work task, and before the second set. All tests were conducted between 1300hrs and 1630hr, the order of participants remained the same on each day. The tests occurred at least 45 minutes after eating, not including time for instrumentation. Upon arrival, participants were instructed to lie in the supine position on the tilt table with their feet resting on a footplate. Following instrumentation, participants rested quietly for a period of 10 minutes to allow stabilization of all measured variables and to ensure an appropriate fluid stabilization of total body water (Gibson et al., 2015). Following this baseline period (BL), participants were passively tilted to a 60° HUT position, where they remained for a further 10 minutes. While the test protocol would have been terminated early at the participants' request or signs of presyncope based on ECG

and beat-by-beat blood pressure recordings (Schroeder et al., 2002), however none of the participants requested premature cessation during HUT or showed haemodynamic signs of presyncope.

2.3.4 Measurements

2.3.4.1 Haemodynamics

HR was determined from Lead II electrocardiogram. Arterial pressure was measured, in duplicate, via electro-sphygmomanometry (Tango, SunTechMedical Instruments Inc., USA) with a microphone placed over the brachial artery at the heart level to detect Korotkoff sounds, while the arm was parallel to the body. Beat-by-beat SBP and DBP was recorded via the volume clamp method (Finapres Nova; Finapres Medical Systems BV, Amsterdam, The Netherlands) and used to estimate beat-by-beat changes in SV and CO using the Model Flow algorithm. Finometer values were calibrated against the average electro-sphygmomanometry brachial artery blood pressure measurements and corrected for the height of the participant. TPR was estimated as the ration of MAP to CO.

Hydration levels were monitored daily with dipstick measurements, which measured urine specific gravity (USG) using the colorimetric method. Finger capillary (15 µL) blood samples were taken prior to the onset of the simulated HW, and upon its conclusion. All blood samples were analysed using an ABL80 FLEX CO-OX Blood gas analyser (Radiometer Medical, Brønshøj, Denmark), measuring haematocrit (Hct, %) and haemoglobin concentration (Hb, $g \cdot 100mL^{-1}$).

2.3.4.2 Deep Body Temperatures

The temperature of the gastrointestinal tract (T_{gi}) was continuously measured using ingestible telemetric pills (Bodycap, Caen, France). These were ingested each morning upon being woken at a standardized time of 0700hrs; measurement started at 0900 hrs. This allows appropriate time for the ingested pill to reach the stomach. For the purposes of the present study, deep body temperature measurements during the HUT protocol only were utilised.

2.3.4.3 Skin Temperatures

The temperature of the skin (T_{sk}) was measured at four sites continuously throughout each day, using thermistors (iButtons type DS1921H, Maxim/Dallas Semiconductor Corp. USA). For the purposes of the present study, skin temperature measurements during the HUT protocol only were utilised. Measurement sites were located at the chest, bicep, thigh, and calf; all on the right side of the body. An average skin temperature was calculated (Ramanathan, 1964):

$$Mean\ T_{sk} = 0.3\left(T_{chest} + T_{Bicep}\right) + 0.2(T_{thigh} + T_{calf})$$

Equation 2.1. Mean Skin Temperature

2.3.4.4 Proximal-distal Temperature Gradient (ΔTskP-D)

ΔTskP-D, an index of localised SBF, was measured at 1-minute intervals, between the mid-point of medial aspect of the radius of the forearm and the pad of middle finger (ΔT_{sk}F-F), and the muscle-belly of the medial gastrocnemius on the calf and pad of the great toe (ΔT_{sk}C-T) (MSR145, MSR Electronics GmbH, Switzerland) on the left side of the body; areas considered to be heavily influenced by thermoregulatory and baroreflex mechanisms.

Wireless thermistors (iButtons type DS1921H, Maxim/Dallas Semiconductor Corp. USA) at the forearm and fingertip also allowed continuous measurement of arm SBF throughout each day. ΔT_{sk}P-D was validated as a method of measuring peripheral SBF against venous occlusion plethysmography in thermoneutral conditions ($r^2 = 0.98$) (Rubinstein & Sessler, 1990). Validation with Laser Doppler Flowmetry (LDF) also displays a strong correlation under anaesthesia ($r^2 = 0.63$) (Sessler et al., 1988), and during steady-state exercise ($r^2 = 0.68$) (Keramidas et al., 2013). House and Tipton (2002) also suggested it is a suitable index, and recommended an adjusted scale, which was used in the present study (vasoconstriction: ≥ 2 °C; vasodilation: ≤ 0 °C). Since this indirect method of monitoring peripheral SBF during an orthostatic tolerance test in normal and hot temperature conditions has not been validated previously, a separate validation was conducted to confirm ΔT_{sk}P-D as an index of SBF in the fingers and toes.

2.3.5 Validation of ΔTskP-D

ΔT_{sk}P-D was validated as an index of SBF by comparing the measurements with LDF (MoorVMS-LDF, Moors instruments, UK) in both the arm and leg on a separate session. ΔT_{sk}P-D, measured as described previously, and LDF of the fingertip and great toe were measured during supine and 60°HUT position, while exposed to stable ambient temperature of 25 °C and 35 °C in a climatic chamber. Immediately upon entry the participants entered the supine position, which was measured for 10 minutes, following which they were tilted into the 60°HUT position. The protocol was identical to the HUT protocol used in the 9-day HW simulation study. Spearman's Rank correlation was calculated for the absolute measurements of ΔT_{sk}P-D and LDF recorded during the final 2-minutes in each condition; considered a stable period.

2.3.6 Data Analysis

Cardiovascular data (HR, SV, CO, SBP, DBP, AND TPR) were collected on a beat-by-beat basis over the course of the 10-minute supine and 10-minute HUT postures. T_{gi} and T_{sk} data were measured every minute for 22 hours (Tgi: 0900-0700hrs; Tsk: 2300-2100hrs). T_{gi} and T_{sk} data were processed to produce minute averages over the course of the 10-minute BL and 10-minute HUT. In each of these measures, the final 2 minutes of each condition used as a stable period Minute averages of ΔT_{sk}F-F and ΔT_{sk}C-T were collected for the duration of BL and HUT. Analysis of drawn blood via a radiometer provided values of Hct and Hb. These values were used to calculate plasma volumes (PV) before and after the HW, thus providing an indication of any HW-induced change (ΔPV).

Two-way repeated-measures analysis of variance (ANOVA) were used to assess the effects of two independent variables (ambient temperature, orthostasis) on the measurements recorded (HR, SYS, DIA, SV, CO, TPR; T_{gi}; T_{sk}; ΔT_{sk}P-D). Simple main effects of these comparisons were also conducted. Partial Eta Squared was used to define the effect size in ANOVA tests. In each ANOVA, Mauchly's Test of Sphericity was run for relevant variables, and the assumption of sphericity was met for each interaction if $p > 0.05$. Paired samples t-tests assessed the differences in the ΔT_{sk}P-D responses of the arm and leg, in each of the four conditions produced by ambient temperature and posture (non-HW BL, non-HW HUT, HW BL, HW HUT); followed by Cohen's D effect sizes. All statistical tests were completed using an alpha value of $p < 0.05$, with IBM SPSS Statistics (Version 26, IL, USA). Finally, Paired T Tests and Pearson Correlation Coefficient Tests were carried out to determine the significance of difference and relationship that exists between all pre-HW to post-HW measures, indicative of a HW related residual strain or adaptation.

2.4 Results

All participants successfully completed the 9-day confinement study. As reported previously (Ioannou, Mantzios, Tsoutsoubi, Panagiotaki, et al., 2021), all experienced a varying degree of thermal discomfort, considerable physiological strain and drop in productivity during simulated work and exercise tasks. There was no significant effect of HW on hydration status as reflected in the USG measured during the non-HW (1.0239 ± 0.0043) and HW conditions (1.0224 ± 0.0036) (p = 0.156), suggesting appropriate maintenance of euhydration.

2.4.1 Deep Body and Skin Temperatures

In the non-HW, T_{sk} stabilised at 33.5 ± 0.5 °C, whilst in the HW ambient conditions T_{sk} increased to 35.9 ± 0.3 °C. Similarly, there was an increase in the core temperature from the non-HW (37.2 ± 0.2 °C) to the HW ambient condition (37.4 ± 0.2 °C), though to a lesser extent. Thus, between the non-HW and HW, there was a significant increase in T_{sk} (t = 24.06, $p < 0.001$), and also in the T_{gi} (t = 2.368, $p = 0.029$). In addition, there was a significant effect of participants posture (BL, HUT) on T_{sk} (F = 12.494, $p = 0.002$, ηp^2= 0.103), but no significant effect on T_{gi}. Skin and core temperatures in each time period over the full 24-hours are presented in Table 1.

Table 2.1: Measured mean ± SD and range of core and skin temperatures. Measured over a 24-hour period during work, rest, and sleep periods. Temperature is presented as a mean of both 3-day non-HW and HW conditions.

		Gastrointestinal Temperature (°C)		Skin Temperature (°C)	
		Mean ±SD	Range	Mean ± SD	Range
Work Period	Pre-HW	37.1 ± 0.3	36.4 – 37.8	33.3 ± 0.4	31.8 – 34.3
	HW	37.3 ± 0.3	36.6 – 38.0	35.7 ± 0.3	34.0 – 36.3
Rest Period	Pre-HW	37.3 ± 0.1	37.0 – 37.6	33.2 ± 0.5	31.9 – 34.1
	HW	37.5 ± 0.1	37.2 – 37.8	34.2 ± 0.6	33.0 – 35.8
Sleep Period	Pre-HW	36.5 ± 0.3	36.2 – 37.7	34.1 ± 0.4	32.3 – 34.8
	HW	36.6 ± 0.4	36.1 – 37.7	34.3 ± 0.3	33.7 – 34.9

Work period: 09.00-18.00. Rest period: 18.00-23.00. Sleep period: 23.00-07.00. Pre-HW: Average of testing days 1-3. HW: Average of testing days 4-6. Ambient temperature

2.4.2 Haemodynamic Measures

The combined effects of changes in ambient temperature and posture did not produce a significant interaction effect of any of the haemodynamic measures. The simple main effects, however, did produce significant differences in certain cardiovascular variables, as described below. Figure 1 displays the cardiovascular responses during BL and HUT, in both the non-HW and HW; significant differences between postures are denoted with an asterisk (*). PV did not differ significantly between the non-HW and HW, with an average ΔPV of -0.03 % (d = -0.1).

The HW condition caused a significant increase in in both HR (F = 9.104, $p = 0.008$, $\eta p^2 = 0.349$) and CO (F = 5.246, $p = 0.035$, $\eta p^2 = 0.236$). HR increased from a mean pre-

HW value of 67.1 ± 11.2 to 73.1 ± 12.2 b · min^{-1} during the HW, whilst CO increased from a pre-HW value of 4.6 ± 0.9 to 5.5 ± 1.8 L · min^{-1} during the HW. Notably, TPR decreased from pre-HW value of 20.2 ± 5.5 to 17.9 ± 7.9 mmHg · L · min^{-1} during the HW with a medium to strong effect size ($\eta p^2 = 0.127$) (Lakens, 2013), however, this change was deemed to be non-significant.

HUT caused significant increases in HR (F = 130.137, $p < 0.001$, $\eta p^2 = 0.884$), SBP (F = 13.167, $p = 0.002$, $\eta p^2 = 0.927$), DBP (F = 24.419, $p < 0.001$, $\eta p^2 = 0.996$), and TPR (F = 7.874, p = 0.012, $\eta p^2 = 0.317$). Conversely, SV decreased significantly (F = 43.717, $p < 0.001$, $\eta p^2 = 0.720$), whilst CO displayed no significant change during HUT. HR increased from 70.2 ± 1 to 96.2 ± 13.3 b · min^{-1}, SYS increased from 127.8 ± 11.5 to 135.8 ± 11.8 mmHg, DIA increased from 68.1 ± 11.7 to 81.7 ± 11.2 mmHg, and TPR increased from 19.0 ± 6.9 to 23.9 ± 8.0 mmHg · L · min^{-1}. For SV, the effect of HUT caused a decrease from 74.2 ± 21.5 to 49.3 ± 14.3 mL.

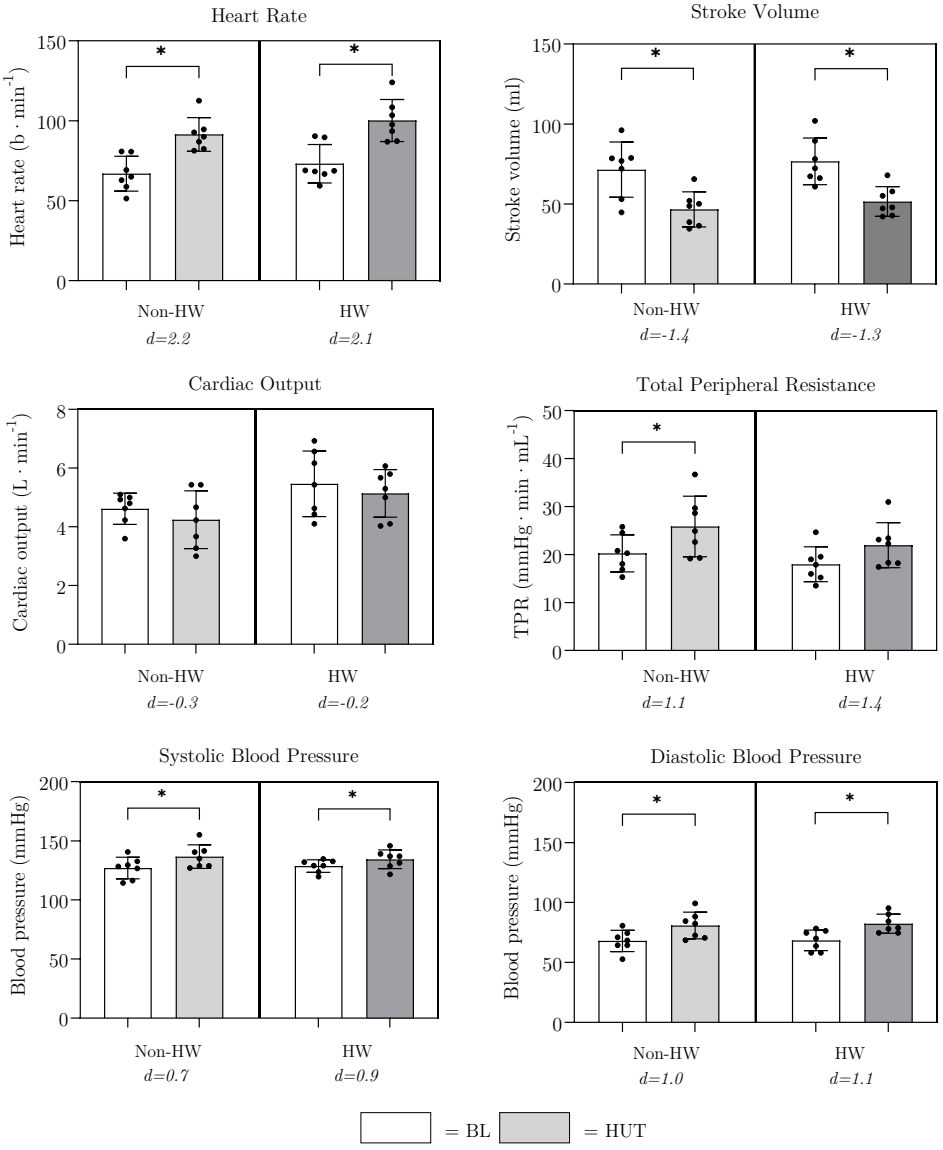

Figure 2.1: Mean ± SD and individual cardiovascular responses to 60° HUT. Measurements displayed during non-HW and HW. Sig. differences between postures denoted with an asterisk (*). Cohen's D effect sizes represents difference between supine and HUT in each ambient condition.

2.4.3 T_{sk}P-D (Index of Blood Flow)

The validation of ΔT_{sk}P-D as an index of SBF revealed a significant negative correlation (Fig. 2) between ΔT_{sk}P-D and LDF measurements (r = -0.776, $p < 0.001$). The measurements were correlated using absolute values (LDF: Laser doppler units; ΔT_{sk}P-D: °C); median and range values were also calculated for LDF (median: 277.3; range: 477.8) and ΔT_{sk}P-D (median: 0.0; range: 9.0).

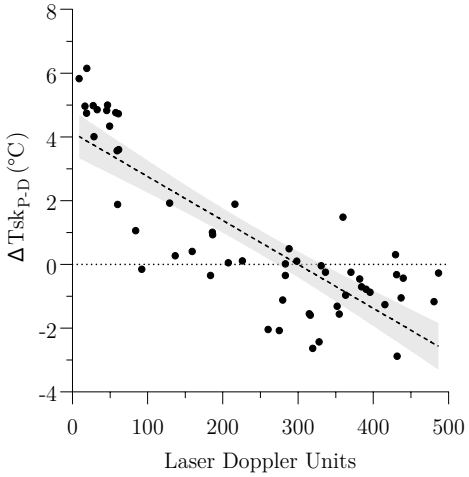

Figure 2.2: Relationship between ΔTskP-D and LDF measurements. Associated regression line is displayed. Measurements recorded during the final 2-minutes in each condition (supine or HUT).

The interaction of change in ambient temperature (non-HW, HW) and posture (BL, HUT) revealed a significant combined effect on ΔT_{sk}P-D in the arm (F = 8.069, $p = 0.014$, $\eta p^2 = 0.383$) but not in the leg. Further analysis identified that the ΔT_{sk}F-F was significantly different in the non-HW but not in the HW. Figure 3 displays the ΔT_{sk}P-D responses of the arm and leg during BL and HUT, in both the non-HW and HW.

The simple main effect of change in ambient temperature caused a significant decrease in the ΔT_{sk}C-T, decreasing from 2.2 ± 0.9 °C in the non-HW to 0.0 ± 0.6 °C in the HW (F = 9.174, $p = 0.010$, $\eta p^2 = 0.414$); indicating a shift towards vasodilation. The ΔT_{sk}F-F, despite crossing the suggested threshold for vasodilation (House & Tipton, 2002) from 0.0 ± 0.9 to -0.6 ± 0.4 °C, did not produce a significant response as a result of ambient temperature.

In response to HUT, the second simple main effect, both the ΔT_{sk}F-F and ΔT_{sk}C-T displayed a significant decrease, indicating a shift towards vasodilation. The ΔT_{sk}F-F decreased from -0.3 ± 0.9 to -1.0 ± 0.8 °C (F = 5.918, $p = 0.030$, $\eta p^2 = 0.313$), whilst the ΔT_{sk}C-T decreased from 1.0 ± 1.6 to 0.3 ± 1.4 °C (F = 9.009, $p = 0.010$, $\eta p^2 = 0.409$).

As described above, differences lay in the blood flow of the arms and legs, as indexed by the ΔT_{sk}P-D. When comparing between the arm and leg response in each condition, significant differences lay between each of the four conditions as produced by combining ambient conditions and postural changes: non-HW BL (t = -5.001, $p < 0.001$, d = 1.1), non-HW HUT (t = -4.934, $p < 0.001$, d = 1.2), HW BL (t = -3.822, $p = 0.001$, d = 0.9), HW HUT (t = -2.244, $p = 0.036$, d = 0.6).

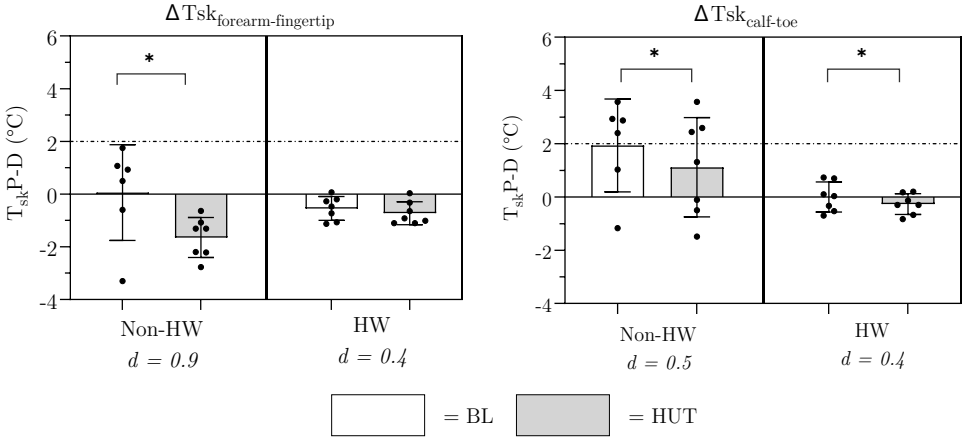

Figure 2.3: Mean ± SD and individual SBF responses to 60° head-up tilt. Measurements taken during non-HW and HW. Sig. differences between postures in each condition denoted with an asterisk (*). Dotted line = threshold for vasodilation, solid line = threshold for vasoconstriction. Cohen's D effect sizes represents the difference between supine and HUT in each ambient condition.

2.4.4 Adaptation to the Simulated HW

Pre-HW to post-HW significant differences were identified in ΔT_{sk}F-F (t = 13.40, $p < 0.001$), ΔT_{sk}C-T (t = 6.017, $p < 0.001$), CO (t = 2.739, $p = 0.034$), and T_{sk} (t = 3.418, $p = 0.014$). Significant pre-HW to post-HW correlations were only observed in ΔT_{sk}C-T (r = -0.5685, $p = 0.04$), and in mean blood pressure (MAP; r = 0.7633, $p = 0.023$). Figure 4 displays the mean ± SD responses of all variables on individual days, measured in the BL position.

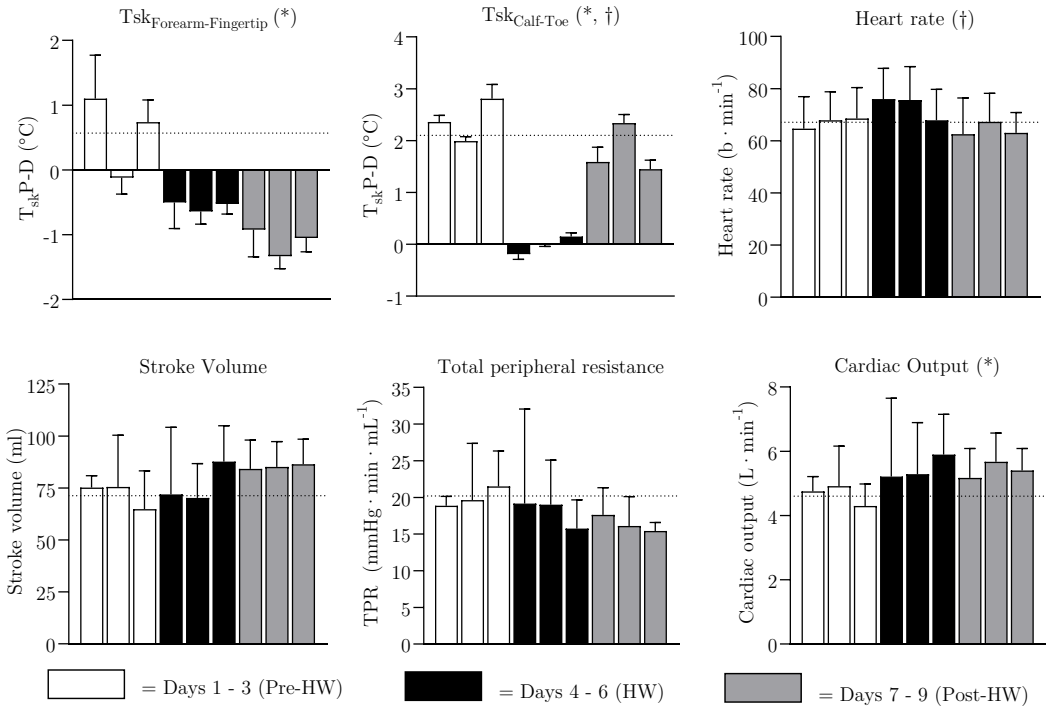

Figure 2.4: Mean ± SD of cardiovascular responses in baseline position. Measurements displayed as average from each individual day. White bars = pre-HW (days 1-3), black bars = HW (days 4-6), grey bars = post-HW (days 7-9). Dotted line = average value of the pre-HW period. † = sig. correlation between pre- and post-HW, * = sig. difference between pre- and post-HW.

2.5 Discussion

The present study utilized a novel method of confining participants to simulated HW conditions, to assess the cardiovascular strain experienced by those working in industry during a HW. By simulating ambient conditions measured in an industrial environment during a HW in Slovenia (Ciuha, Pogačar, et al., 2019), an understanding of the effects of a HW could be measured in controlled conditions. Perhaps unsurprisingly, the effect of increased ambient temperatures caused a significant increase in both T_{gi} and T_{sk}, confirming hypothesis one. As a result, the main findings of the study were that prolonged exposure to HW conditions and orthostasis, independently, significantly affected cardiovascular and SBF responses; thus, confirming hypotheses two and three. Indeed, while the interaction of higher ambient heating and orthostatic stress produced no significant effect on haemodynamic variables, a significant regional effect on SBF was observed. The results, and the physiological mechanisms that drive them, indicate a higher propensity for heat strain in occupational workers during a HW.

2.5.1 Deep Body and Skin Temperatures

As expected, when exposed to elevated ambient temperatures T_{gi} rose passively as heat production outweighed heat loss; though this was deemed to be a sustainable increase rather than an Uncompensable gain. The T_{sk}, which is much more responsive to external changes in temperature, varied considerably between ambient conditions. The changes observed are likely to reflect the T_{gi} measured, albeit not precisely due to large fluctuations that are seen during lunch breaks (12.00-13.00). However, it is worth nothing that the T_{gi} and T_{sk} described in the present study relate specifically to the HUT protocol. For these reasons, fluctuations observed in the data do not affect the results described. Table 1, however, displays a greater T_{gi} in the rest conditions than the work conditions, which represent a lower ambient temperature. This trend is observed regardless of simulated-HW conditions and is likely the result of circadian rhythm causing a peak core temperature at ~2100hrs (Krauchi & Wirz-Justice, 1994). Overall, the responses of the T_{gi} and T_{sk} reveal that a degree of thermal strain was experienced by the participants, which was suitable to elicit cardiovascular and SBF responses.

2.5.2 Haemodynamic Variables

High ambient temperatures passively raise T_{gi} and T_{sk} to levels that initiate heat adaptation, causing several significant physiological improvements such as reduced resting T_{gi}, enhanced sweat capacity, and lower resting/exercising heart rate (Heathcote et al., 2018). Conversely, individuals experiencing HW conditions, without prior acclimatization, appear to suffer adverse physiological effects. On HW days, as expected, there was a significant increase in HR which enabled a raised CO to sustain a greater level of SBF required for heat dissipation. These findings reflect those of Crandall et al. (2000) who identified an affected vagal modulation of HR during heat stress, leading to a decrease in cardiac vagal activity whilst simultaneously increasing sympathetic activity; possibly contributing to orthostatic intolerance. The haemodynamic responses to orthostatic stress

are well known, and the present study further consolidates these responses. Increases in HR, SBP, DBP, and TPR are the result of peripheral vasoconstriction in lower limbs, largely mediated by the baroreflex system and the venoarteriolar reflex. These responses are produced when an increase in arterial pressure is detected, reducing baroreceptor afferent discharge, and triggering reflex increases in HR, cardiac contractility, and vascular resistance to reduce blood pooling and promote venous return for vital organs (Lanfranchi & Somers, 2002). Conversely, a decrease in SV was observed, occurring when venous pooling and capillary filtration reduce the circulating blood volume by 500-1000 mL (Shaw et al., 2014), the result of which is lower venous return and subsequent outgoing stroke volume. In the present study, CO was not significantly higher during orthostatic stress compared to the supine position. This is unusual, particularly considering the relatively young age of the participant sample. Work by Hainsworth and Al-Shamma (1988) assessing the cardiovascular response to orthostasis in different age groups also observed a decreasing cardiac response to orthostasis with age, which is concurrent with additional research (Frey et al., 1994).

2.5.3 T_{sk}P-D (Index of Blood Flow)

The method of measuring SBF in the present study was ΔT_{sk}P-D, a non-invasive and robust method. A validation test using seven different participants ensured that these measurements were valid and reliable in the ambient conditions and postures used in the present study, to aid those results already established by previous studies (Keramidas et al., 2013; Rubinstein & Sessler, 1990; Sessler et al., 1988). Each of these previous studies proposed varied correlation coefficients in differing conditions, ambient temperatures, and using different measurement techniques with which ΔT_{sk}P-D was compared against. None of these previous validations have considered the changes in blood flow that occur with changes in ambient temperature from 25 °C to 35 °C, changes in posture from supine to HUT, and in different regions of the body; simultaneously. The present study considered the interaction of all external variables on ΔT_{sk}P-D, allowing a more conclusive relationship between measurement techniques to be drawn. The evidence obtained provides support that ΔT_{sk}P-D may be used as a suitable index of finger and toe SBF in place of more expensive, less robust, and less invasive alternatives. Additionally, the results of this validation also displayed regional differences in the SBF of individuals, when experiencing changes in posture and ambient temperature. These results further support the hypotheses outlined in the present study.

Both ambient temperature and posture (BL, HUT) independently appeared to significantly affect the ΔT_{sk}P-D, however, their combined influence was only observed in the ΔT_{sk}F-F. Though further analyses identified that this was largely due to the differences in the non-HW; indeed, during the HW conditions there is little influence of the HUT which therefore suggests thermoregulation is the predominant driver. This partially agrees with Crossley et al. (1966) who explains that during LBNP, vasoconstriction in the forearm occurs with whole-body heating, as a result of the baroreflex reducing the SBF; implying that the baroreceptor reflex can override thermoregulation. Additionally, Lind et al. (1968) suggest that during local or whole-body heating, the baroreceptor-mediated regulation of SBF is still maintained.

The effects of ambient temperature and orthostasis independently were also significant, and were also noted to differ regionally. The substantial regional differences in ΔT_{sk}P-D occurred in both normothermia (non-HW) and HW conditions, with consistently higher cutaneous vasodilation observed in the hands than the feet. The results agree with previous speculation (Ciuha, Tobita, et al., 2019) that the vasomotor response of the arms is driven predominantly by thermoregulatory systems, whereas the legs are influenced

predominantly by baroreflex actions to maintain blood pressure, as indexed by vasodilation and vasoconstriction. The greater vasodilation observed in the fingers in all measured conditions potentially suggests a predominant influence of thermoregulatory drive, reflected in greater sensitivity to changes in temperature (Gerrett et al., 2017) in the finger than in the toe. ΔT_{sk}C-T in the non-HW was significantly higher indicating vasoconstriction of the cutaneous vasculature, and during the HW both regions demonstrated a decrease in ΔT_{sk}P-D, indicative of greater SBF; suggesting an integrated reflex to promote vasodilation and withdraw vasoconstrictor drive, occurring as a 'thermal threshold' is met. This integrated regulation is described by Heistad et al. (Heistad et al., 1973) whereby the forearm, containing both cutaneous and muscular vasculature, displayed increased vasodilator reflex responses to heating. During the HW, the shift in blood flow to the skin may indicate an active vasodilator drive accounting for 80-90% of cutaneous vasodilation (Charkoudian, 2003). Though, the present study cannot confirm this theory as sympathetic nerve activity was not measured.

Previous research also agrees with the regional differences observed when gravitational stress is applied. Stewart (2002) noted a stronger inverse relationship between blood pressure and SBF in the leg than in the arm, implying a greater importance of leg circulation in blood pressure maintenance. Based on their findings, Kitano et al. (2005) described complementary evidence, observing a greater vasoconstrictor response in the lower body during HUT. Possible reasons cited include differing sensitivity of sympathetic nerve activity between the arm and leg, and modulation of vascular responses via local mechanisms in the leg. Those working in industry during a HW are at direct risk of orthostatic intolerance, as venous pooling in the legs may cause syncope if integrated vascular balance is not achieved.

2.5.4 Adaptation to the Simulated-HW

While it is clear there is an acute cardiovascular response during a simulated-HW, it is also important to consider the prolonging and residual effects of said HW upon return to normothermic conditions. During the 2003 European HW, Pirard et al. (2005) indicate that in France alone the daily number of excess deaths reached as high as 2197 on the final day of the HW. Upon return to non-HW temperatures, these daily numbers decrease day-by day as a result of the reduce due to, in part, a reduction in the cardiovascular strain. However, while a reduction is observed each day, it takes up to seven days for the number of deaths to return to normal levels; indicating a prolonged effect of the HW. This trend is also observed during other HWs, such as the 1995 Chicago HW (Kaiser et al., 2007) and various English summer HWs (Thompson et al., 2022).

The results of the present study indicated a severe increase in the forearm SBF post-HW, as indexed by T_{sk}F-F, rising to levels higher than that of the HW. In addition, there is also an increase in leg blood flow during this time. These changes are enabled by a significantly higher CO observed, which consequently result in an increase in the T_{sk} in the post-HW when compared to the pre-HW. These results certainly indicate a prolonged effect of the HW, though it appears these effects are a positive adaptation occurring in the body to respond to heightened ambient temperatures. Results such as these are not dissimilar to those observed in active heat acclimation studies (Fujii et al., 2012; Fujii et al., 2015; Roberts et al., 1977; Yamazaki & Hamasaki, 2003), which observed an increase in SBF at a given deep body temperature, indicating a reduction in the thermal threshold required for vasodilation. However, heat acclimation protocols normally incorporate high levels of exercise and repeat exposures to heat, to maintain an elevated core temperature (Otani et al., 2017; Sotiridis et al., 2020; Tyler et al., 2016); which are inconsistent with the present study. Studies assessing thermal therapy over a longer duration have described

improvements in brachial artery endothelial function (Brunt et al., 2016; Carter et al., 2014), similar to that of moderate-intensity exercise. Thus, adaptation may occur in place of negative cardiovascular responses, congruent with an increased risk of morbidity. The younger age and physical health of the participants in the present study may have exaggerated these results, and it is possible that elderly and more vulnerable populations, who are at greater risk from extreme HWs, may not exhibit the same positive adaptations (Hansen et al., 2011; Pandolf, 1997). It is therefore of importance that the future research studying this adaptation (or lack thereof) should seek to develop an understanding of the responses of elderly and vulnerable populations, to these increasingly regular yet adverse ambient conditions.

2.5.5 Limitations

The median age of manufacturing workers is 44.5 years (available from: www.statista.com; accessed 2 September 2021), and females represent 46.2 % of the labour force (available from: www.appsso.eurostat.ec.europa.eu; accessed on 11 August 2021). To fully appreciate the risk of a HW to those in industry, these populations should also be considered, as the young male sample group in the present study may not reflect the responses of the workforce in the manufacturing industry. A Heat Shield Technical report (Pogačar et al., 2018) of workers during a HW details 37 % and 47 % of males experiencing headaches or exhaustion, respectively, compared to 73 % and 64 % of females, respectively; additionally, 33 % of females experienced vomiting or nausea. During orthostasis, ΔT_{sk}F-F and ΔT_{sk}C-T will have been influenced partially by the venoarteriolar reflex (Low, 2004). However, measurements of neural pathways were not conducted in the present study, so its influence is hard to quantify; future research should endeavour to understand the venoarteriolar reflex's role in regional variation of SBF.

2.6 Conclusions

The present study used a protocol simulating HW conditions and application of an orthostatic stress to identify cardiovascular and thermoregulatory strain in workers. The novelty of the study design revealed that workers exposed to prolonged and high ambient temperatures, concomitant with orthostatic stress, does not produce significant changes in the cardiovascular responses, though individually these stressors have significant impacts. In addition, these combined influences produced significant changes in T_{gi}, T_{sk}, and SBF; early indicators of heat stress. Additionally, the study further detected regional variations in SBF, as represented by ΔT_{sk}P-D. These responses represent the apparent cardiovascular strain experienced by industrial workers during HWs, which may lead to heat illness, syncope, or potential fatality.

Chapter 3

The Combined Effects of Temperature and Posture on Regional Blood Flow and Haemodynamics

3.1 Foreword

The results of chapter two laid the groundwork for further developments in the area of regional blood flow responses to external stressors, however, it was limited in areas specific to the thesis aims. Firstly, while the method of measuring SBF has been validated by a number of previous studies, and indeed the study in chapter two, it is still an index of the perfusion of blood rather than a direct measure. Secondly, similarly to previous studies assessing regional blood flow responses, there is a lack of analysis which may reveal the mechanisms controlling the observed, systems-based changes in regional blood flow. Despite these limitations, the results of the first study were in agreement with the results of Ciuha et al. (2019) that in response to either separate or combined thermoregulatory and baroreflex stressors, there is a differing regional blood flow response. In particular, the arms appeared to be predominantly driven by thermoregulatory action, whilst the legs appear to be predominantly driven by baroreflex action for the maintenance of blood pressure.

The next study, then, had to address the limitations of study one (Chapter 2) by utilising a more direct method of blood flow measurement, and attempt to assess the mechanisms involved in the regional response. Study two achieved these by using LDF as a measure of SBF, whilst HRV and WTA were used to assess the mechanisms of regional blood flow. Therefore, this chapter will again discuss the regional blood flow response to thermoregulatory and baroreflex stressors produced by a transient ambient temperature in controlled ambient environment (i.e., climatic chamber) and two postures (supine and standing). It will also continue to build the knowledge area by presenting the response of autonomic indices of HRV and WTA.

The findings of study were published in the Journal of Thermal Biology under the following citation: Fisher, J. T., Ciuha, U., & Mekjavic, I. B. (2024). The Combined Effects of Temperature and Posture on Regional Blood Flow and Haemodynamics. *Journal of Thermal Biology, 123,* 103937.

3.2 Introduction

In humans, homeothermy is maintained over a wide range of ambient temperatures (T_A) by the autonomic responses of heat production and heat loss. The former being a by-product of metabolism, and the latter achieved via processes of evaporation, conduction, convection, and radiation. In this thermoregulatory zone of ambient temperatures, shivering thermogenesis is initiated below a lower critical temperature (LCT) of T_A and sweating above an upper critical temperature (UCT) of T_A. The variability of subcutaneous insulation is achieved by varying the degree of perfusion of this layer (Mekjavic & Bligh, 1989). The responses of shivering and sweating may also be described as a function of internal body temperature, whereby the inter-threshold zone of core temperatures for shivering and sweating has been defined as the vasomotor zone (Mekjavic et al., 1991). The peripheral vasomotor zone is defined by maximal vasoconstriction at LCT and by maximal vasodilation at UCT, whereby SBF can range from ~ 0.3 $L \cdot min^{-1}$ at LCT to as much as ~ 6-8 $L \cdot min^{-1}$ at UCT (Charkoudian, 2003). The regulation of SBF is achieved by the interaction of sympathetic neural control mechanisms, sympathetic vasodilator, and adrenergic vasoconstrictor nerves. This sympathetic/vasomotor regulation of the microvasculature ensures optimal thermal balance during small variations in ambient temperature. It is now well documented that in the skin vasomotor zone, SBF is regulated by skin temperature (Savage & Brengelmann, 1996; Shepherd, 1966), and modulated by the baroreceptor reflex (Crossley et al., 1966). The interaction between the pressure and temperature regulating systems is most apparent during the transition of posture from supine to upright (Nielsen et al., 1939) and during heating in the upright posture (Rowell et al., 1970).

These systems are exceptionally efficient at regulating homeostasis when in response to an imbalance from one stressor, such as high ambient temperatures or gravity-induced hydrostatic pressure shifts. It is when these systems are required to work simultaneously that responses must become integrated (Heistad et al., 1973). For example, Crossley and colleagues (1966) suggested that during acute exposure to lower body negative pressure (LBNP), the baroreflex supersedes thermoregulatory control. These results are in contrast, however, to reports indicating a decreased resistance to orthostatic stress under heating (Schlader et al., 2016). For example, the study by Wilson and colleagues (2002) reported that only four of nine participants were able to withstand orthostatic stress for 10 minutes after a 0.9°C increase in core temperature. It is therefore clear that the combination of gravitational and heat stressors establishes a need for interaction of the blood flow mechanisms. What is less clear, is how this interaction of central neural mechanisms affects efferent autonomic vascular control and consequently, microvascular blood flow.

Previous research has alluded to regional disparities in microvascular blood flow, when experiencing external stressor. Early research by Nishiyasu and colleagues (1992) noted that in different scenarios designed to elevate core temperature using ambient temperature and exercise, there were significant differences in upper and lower body blood flow with considerably higher forearm flow observed in all conditions. Hales et al. (1994) also utilized exercise and high ambient temperatures, identifying significant variations in blood flow at rest and during exercise measured at the forearm, forehead, chest, and finger. Regional responses have also been reported in reference to orthostatic/postural stress, with Essandoh and colleagues (1988) testing a number of different postural conditions. They summarized that marked changes in forearm blood flow were not matched by the calf, and that both high- and low-pressure baroreceptors mediate forearm vasoconstriction in response to postural changes. Ciuha and colleagues (2019) reported that during exposure to transient ambient temperature in a seated position, the perfusion characteristics of the arm and leg

differ; with different sympathetic nerve responses provided as a possible reason. Finally, previous work by the same group identified that there was a substantial regional difference in skin perfusion to the fingers and toes, with the higher fingertip perfusion being attributed to greater sensitivity to changes in temperature (Fisher et al., 2022).

The aforementioned research detected regional variations in microvascular blood flow yet suggested few possible reasons for these variations. Therefore, the aim of the present study was to compare the responses of microvascular blood flow to thermoregulatory and postural stress, in the upper and lower limbs. It is also anticipated that the use of central and peripheral frequency analyses may allude to differing autonomic control of vasomotor tone which may reveal the relative contributions of thermoregulatory and baroreflex drive in different regions. The hypotheses tested were that i) thermal stress alone (i.e., changes in ambient temperature in supine) increases SBF (predominant thermoregulatory control), with a greater response identified in the forearm microvascular blood flow response, ii) postural stress alone (i.e., supine vs. standing) will cause a decrease in the SBF (predominant baroreflex control), with a greater response identified in the calf microvascular blood flow response, and iii) a combination of thermal and postural stressors will cause differences in the regional (arm vs. leg) SBF responses (thermoregulation/baroreflex interaction), altering cardiovascular responses to the transient temperature.

3.3 Materials and Methods

A total of eleven male participants were recruited for the study. Their mean (SD) and range physical characteristics are displayed in Table 1. The protocol was approved by the National Committee for Medical Ethics at the Ministry of Health of the Republic of Slovenia (appendix C, approval no. 0120-180/2023/7) and conformed to the guidelines of the Declaration of Helsinki. Prior to the start of the study, participants were familiarized with the study protocol and procedures, and gave their written consent for participation. Participants were asked to refrain from caffeine, alcohol, smoking, and intense physical exercise in the 24-hours leading up to the study.

Table 3.1: Mean ± SD and range of participant characteristics.

	Mean ± SD	Range
Age (years)	29.5 ± 6.7	23.0 – 44.0
Body Mass (kg)	81.1 ± 10.3	64.4 – 100.2
Body Stature (cm)	181.5 ± 6.2	172.0 – 193.0
Body Mass Index ($kg \cdot m^{-2}$)	24.6 ± 3.2	20.3 – 31.6
Body Surface Area (m^2)	2.0 ± 0.1	1.8 – 2.2
Blood volume (L)	5.4 ± 0.5	4.7 – 6.1

Body surface area (Mosteller, 1987) and blood volume (Nadler et al., 1962) were calculated using recorded body mass and body stature values.

3.3.1 Participant Information

The minimum required sample size for investigating "repeated measures, within-between factors" was calculated using the results of a previous study (Ciuha, Tobita, et al., 2019). This study identified that during heating there was a difference of 4.9°C in proximal-distal temperature gradient ($\Delta Tsk_{p\text{-}d}$), an index of skin perfusion, and a difference of 3.0°C during cooling. Using these data, an effect size (d) between 1.58 to 3.38 for the association between

temperature and regional blood flow was computed. Assuming an α of 0.05 and β of 0.99, eight participants would provide sufficient power to detect a statistical difference of a similar magnitude (G*Power Version 3.1.9.2). To account for any potential participant drop-out, a total of eleven participants were screened and recruited for the study. Inclusion/exclusion criteria for participation in the study included: smokers, physically inactive, extreme exposure to hot or cold ambient conditions in one month prior to the onset of the study, a history of freezing or non-freezing cold injuries, a history of microvascular peripheral disease, a history of high or low blood pressure, and diabetes.

3.3.2 Experimental Protocol

Participants attended two separate testing sessions, in which they were positioned in either a supine (SUP) or standing (STA) position for the full duration of a transient ambient temperature protocol described below. Each session was scheduled at the same time of day and conducted on different days with at least 24 hours separating the two sessions. Upon arrival at the laboratory participants had their height and naked weight recorded, and were then instrumented in thermoneutral conditions, at an ambient temperature (T_A) of 23.6 ± 1.1 °C and relative humidity (RH) of 32.3 ± 4.3 %. Participants, wearing shorts only, rested in this thermoneutral environment for a further 20-min baseline period, in order to reduce day-to-day variability. Thereafter, the participants were transferred to a climatic chamber (IZR d.o.o., Škofja Loka, Slovenia) in which T_A was maintained at 15.7 ± 0.6 °C. Once inside the chamber, the participants assumed the designated posture, either SUP or STA. During the SUP trial participants led on a bed at a height of 75 cm. The order of the STA and SUP trials was randomized. The participants were then exposed to a 60-min transient thermal exposure. This exposure comprised an initial 30-min linear increase in T_A to 38.9 ± 0.6 °C, followed by a 30-min linear decrease in T_A to 15.7 ± 0.6 °C. The rate of change in T_A was 0.77 °C · min^{-1}. RH within the chamber was maintained at 25.9 ± 6.6 %, and laminar airflow at 0.1 ± 0.0 m · s^{-1} throughout the trials. A weather station (Kestrel 5400FW, Nielsen-Kellerman, PA, USA) recording T_A and RH was located at a height of 1.5 m. Uniformity of T_A was confirmed within the climatic chamber (3.9 x 2.3 x 2.3 m; 20.6 m^3) at 0.6 m, 1.1 m, and 1.7 m to conform with ISO 7726.

3.3.3 Measurements

3.3.3.1 Microvascular Blood Flow (BF_M)

Laser Doppler flowmetry (LDF; MoorVMS-LDF, Moor Instruments, UK) was used to non-invasively determine BF_M of the mid-point of medial aspect of the radius of the right forearm (SBF_{arm}) and muscle-belly of the medial gastrocnemius on the right calf (SBF_{calf}). The device utilizes probes producing a near-infra-red laser with a power of 1.0 mW at a wavelength of 780 nm, sampling at 40Hz. The device was calibrated using a fluid undergoing Brownian motion before each testing session. Both measurement sites on the forearm and calf were located on non-glabrous skin, to avoid highly significant and variable effects of arterio-venous anastomoses located within glabrous skin (Walløe, 2016). A permanent marker was used to define locations for replication of the probe placement between sessions. Arbitrary laser doppler flux units were converted into cutaneous vascular conductance (CVC, flux · $mmHg^{-1}$) as a ratio of LDU flux units to mean arterial pressure (mmHg).

3.3.3.2 Haemodynamics

Heart rate (HR, min^{-1}) was derived from a five-lead ECG (Finapres NOVA, Finapres Medical Systems B.V., Netherlands). Stroke volume (SV, mL), cardiac output (CO, $L \cdot min^{-1}$), systemic vascular resistance (SVR, $mmHg \cdot min \cdot mL^{-1}$), systolic blood pressure (SBP, mmHg), diastolic blood pressure (DBP, mmHg), mean arterial pressure (MAP, mmHg), and rate pressure product (RPP, $mmHg \cdot min^{-1}$) were obtained continuously throughout the protocol period (Finapres NOVA, Finapres Medical Systems B.V., Netherlands). Haemodynamic responses were recorded non-invasively with a finger cuff and calculated using the model flow algorithm (Wesseling et al., 1993) utilizing the finger volume-clamp method and a five-lead electrocardiogram (ECG). Reconstructed systolic and diastolic blood pressures were calculated via direct finger pressure measurements using waveform filtering and level correction (Westerhof et al., 2002), and normalized to the heart level via a height correction unit measuring the hydrostatic pressure difference between the heart and finger. Additionally, participants held their arm in a sling with the measured finger at the heart level.

3.3.3.3 Skin Temperature (T_{sk})

Measured at minute intervals throughout the protocol using wireless iButton thermistors (type DS1921H, Maxim/Dallas Semiconductor Corp., USA) located at four sites (mid-belly of the bicep brachii, pectoralis major at mid-clavicular level, rectus femoris at femur midpoint, gastrocnemius on medial aspect) on the right side of the body. Weighed T_{sk} was then determined using Ramanathan (1964) equation ($T_{sk} = 0.3chest + 0.3arm + 0.2thigh + 0.2$ calf). A permanent marker was used to define locations for replication of the thermistor placement between sessions. Wireless iButton thermistors have an accuracy of ± 1.0 °C, a resolution of 0.125 °C and a range of 15 – 46 °C.

3.3.3.4 Tympanic Temperature (T_{ty})

In a previous study (Ciuha, Tobita, et al., 2019) it was established that no significant change in deep body temperature occurred during a similar protocol. As a consequence, the maintenance of deep body temperature during the thermal transient exposure was verified with a non-invasive indicator of deep body temperature; infrared tympanometry. Specifically, tympanic temperature (T_{ty}) of the left ear was measured in triplicate at 5-minute intervals throughout the protocol, with the average value used as an indicator of deep body temperature. T_{ty} has been found to have good agreement with esophageal temperature and pulmonary artery temperature in resting humans, with a mean difference between methods of 0.1 °C and 0.85 – 0. 94 °C, respectively (Brinnel & Cabanac, 1989; Fulbrook, 1997).

3.3.3.5 Subjective Measures

At 5-min intervals throughout the thermal exposure, participants provided subjective ratings (ASHRAE, 2017) of thermal sensation on a 7-point scale (ranging from cold to hot), and thermal comfort on a 4-point scale (ranging from comfortable to very uncomfortable).

3.3.4 Mechanistic Analyses

3.3.4.1 Heart Rate Variability (HRV)

HRV analysis was used to provide insight into the central autonomic nervous system control. Beat-to-beat heart rate was measured via a 5-lead ECG (described under *haemodynamics*) and the resultant R-R interval (RRi) analyzed using Kubios HRV Software (Version 3.5.0, Kubios Oy, Finland) based on the guidelines produced by Camm et al. (1996). Fast-Fourier transformation (FFT) frequency analysis of the RR-i signal was conducted at 10-minute intervals over the full 60-minute protocol; with equidistantly sampled data obtained from the RR interval series via a cubic spline interpolation method. A 5-minute selection was used at each interval and corrected via threshold-based beat and automatic beat correction algorithms (Lipponen & Tarvainen, 2019). The three main spectral frequencies used were high frequency (HF; 015 – 0.40 Hz), low frequency (LF; 0.04 – 0.15 Hz), and very low frequency (VLF; 0.03 – 0.04 Hz). The parasympathetic nervous system (PNS) index is calculated using mean RR interval, RMSSD, and Poincaré plot index SD1; sympathetic nervous system (SNS) is calculated using mean HR interval, Baevsky's stress index (Baevsky & Berseneva, 2008), and Poincaré plot index SD2. In both indexes, a value of 0 indicates the values resting population averages, whereas positive or negative values indicate the index is above or below population averages; as a result of stress, high intensity exercise, etc. The relative power of each spectral band of frequencies was calculated in proportion to the total power (Sun et al., 1993), and from these the ratio of low frequency (LF) to high frequency (HF) derived in an attempt to derive a quantitative indication of sympathetic/parasympathetic balance.

3.3.4.2 LDF Wavelet Transform Analysis (WTA)

WTA provides information regarding the regulation of skin perfusion occurring via interactions of autonomic stimulation, endothelial control, and myogenic activities (Bagno & Martini, 2015; Stefanovska et al., 1999). Prior spectral investigations of LDF signals using Wavelet Transform Analysis (WTA) have identified six frequency bands which relate to differing vascular control mechanisms: Band I (cardiac; 0.6 – 2.0 Hz), Band II (respiratory; 0.145 – 0.6 Hz), Band III (myogenic; 0.052 – 0.145 Hz), Band IV (Neurogenic; 0.021 – 0.045 Hz), Band V (endothelial NO-dependent; 0.0095 – 0.021 Hz), and Band VI (endothelial NO-independent; 0.005 – 0.0095 Hz). In the present study, WTA analysis was conducted at three time points, using a 2-minute stable window at each interval. The three chosen intervals were minute 0 (15.7 ± 0.6 °C), minute 30 (38.9 ± 0.6 °C), and minute 60 (15.7 ± 0.6 °C).

3.3.5 Statistical Analyses

All data was averaged to provide minute values and is reported as mean (SD) throughout. A two-way repeated measures ANOVA compared the effect of two independent variables (the relative change in temperature over time (ΔTemp) and body position) on the dependent variables (SBF_{arm}, SBF_{calf}, HR, SV, CO, SVR, RPP, SBP, DBP, MAP, T_{sk}, T_{ty}). Effect sizes (ES) were calculated using Hedge's G. Three-way ANOVAs at each WTA time-point were conducted, comparing the effect of limb, posture, and frequency interval on WTA results. Finally, segmental linear regressions of T_{sk} (x) and mean CVC (y) were calculated to identify the threshold for increases in BF_M (i.e., upper vasomotor zone); linear regressions post-threshold detected the slope and fit (RMSE) of these data. Data was analyzed using IBM SPSS statistics (Version 26, IL, USA) and Graphpad (Graphpad Prism 9, Version 9.1.2, USA); using an α value of $p < 0.05$.

3.4 Results

All participants completed the supine protocol condition. However, one participant displayed pre-syncopal symptoms resulting in premature termination of their standing protocol condition. These data were removed from the standing dataset to avoid anomalous results. There was no difference ($p > 0.05$) in haemodynamic and blood flow variables recorded during the final ten minutes of the baseline period, ensuring low day-to-day variability.

3.4.1 Skin and Tympanic Temperatures

T_{sk} during the SUP condition (32.6 ± 1.5 °C) was significantly higher than the STA condition (32.2 ± 1.7 °C) ($p < 0.001$). The difference between the postures was more distinct at lower ambient temperatures (Fig.1). Thus, the effect of ΔTemp also significantly affected T_{sk} ($p < 0.001$). There was no difference in mean T_{ty} between the two conditions, with an average temperature of 36.9 ± 0.1 °C in SUP and 36.9 ± 0.2 °C in the STA condition ($p > 0.05$). Figure 1 displays the mean (SD) skin temperature in both conditions.

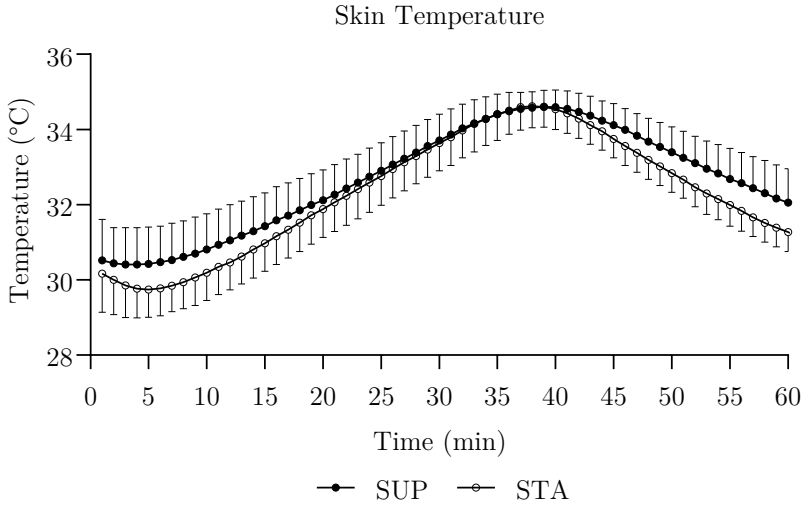

Figure 3.1: Mean ± SD weighted skin temperature (Tsk) during full protocol, in both supine (SUP) and standing (STA) trials.

3.4.2 Subjective Ratings

At the onset of the trial where the ambient temperature was lowest, participants rated their thermal sensation as cool/slightly cool (-1.3 ± 1.0), and at the highest ambient temperature they rated the exposure as warm/hot (2.4 ± 0.6). Upon returning to the low ambient temperatures at the end of the study, participants rated their thermal perception similarly to the start of the protocol (-1.4 ± 1.0).

Participants reported overall higher thermal comfort during the SUP condition compared to the STA condition ($p = 0.04$). In addition, there was a significant effect of ΔTemp throughout the protocol ($p < 0.001$). At the start of the test (15.7 ± 0.6 °C), participants' thermal comfort was equal between the two postural conditions and described as 'slightly uncomfortable' (0.8 ± 0.7). Participants' comfort then changed to 'comfortable' at ~25 °C (SUP = 0.1 ± 0.3, STA = 0.3 ± 0.3), before transitioning back to 'slightly uncomfortable' at 38.9 ± 0.6 °C (SUP = 0.7 ± 0.6, STA = 1.1 ± 0.4).

3.4.3 Microvascular Blood Flow

Figure 2 presents the temporal variation in mean CVC observed in the calf and forearm, in the supine and standing postures. Forearm CVC displayed a significant response to both ΔTemp ($p = 0.003$, $F = 1.599$) and Posture ($p < 0.001$, $F = 275.9$). The lowest forearm CVC during supine was 0.13 flux · mmHg-1 whilst in the standing condition it was 0.07 flux · mmHg-1; both conditions observed an increase with temperature up to a maximum mean CVC of 0.24 flux · mmHg-1 in SUP and 0.14 flux · mmHg-1 in STA. Across the whole protocol, mean forearm CVC in SUP, 0.18 ± 0.03 flux · mmHg-1 was double that of the STA condition, 0.09 ± 0.02 flux · mmHg-1; indicating the large, significant effect of posture in the arm. Calf CVC was only significantly affected by posture ($p < 0.001$, $F = 144.4$), with a large difference in mean CVC between postures across the whole protocol (SUP = 0.15 ± 0.02 flux · mmHg-1; STA = 0.09 ± 0.01 flux · mmHg-1). In neither the arm nor leg, the interaction of both stressors had no effect on CVC, which may be due to little effect of temperature between limbs in the first half of the test.

In addition, the timepoints at which the increase in mean CVC occurs varies between the differing conditions. In both the forearm and calf, BF_M begins to substantially increase at 19 min in the SUP condition, and 25 mins in the STA condition. However, the time to peak and peak value (i.e., the magnitude) vary considerably between limb region and postural condition. In the forearm, peak BF_M occurs at 31 mins in the SUP condition, and 38 mins in the STA condition, and in the calf, this happens at 32 mins in the SUP condition and 44 mins in the STA condition. Therefore, the magnitude of response, during ambient temperature increase, in the forearm was 0.008 flux · mmHg · min^{-1} in SUP and 0.008 flux · mmHg · min^{-1} in STA; while in the calf it was 0.003 flux · mmHg · min^{-1} in SUP and 0.002 flux · mmHg · min^{-1} in STA. For the decrease in temperature, the CVC values reached pre-heating levels, however, the magnitude of response was significantly reduced in the forearm (SUP = 0.004 flux · mmHg · min^{-1}; STA = 0.003 flux · mmHg · min^{-1}), but not in the calf (SUP = 0.004 flux · mmHg · min^{-1}; STA = 0.001 flux · mmHg · min^{-1}).

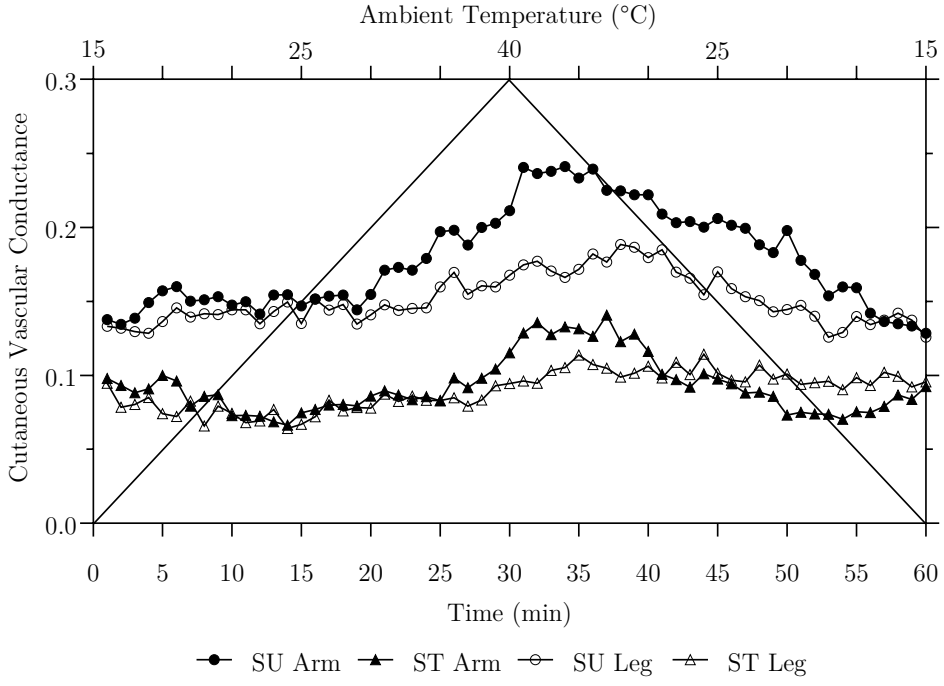

Figure 3.2: Temporal CVC response to transient changes in temperature, and postural conditions.

3.4.4 Vasomotor Threshold

The correlation between T_{sk} and mean CVC (Fig. 3) demonstrates that the threshold for vasodilation occurred at a slightly lower skin temperature in the forearm than in the calf, in both the SUP (forearm = 32.1 °C, calf = 33.0 °C) and STA conditions (forearm = 32.5 °C, calf = 33.3 °C). No observable threshold for vasoconstriction was reached at lower skin temperatures, meaning no data was available for vasoconstriction onset. Regression analyses established that the gain of the response (blood flow (arbitrary units, AU)/CVC (mmHg)) of the forearm in the SUP condition (0.020 flux.mmHg $\cdot$ °C^{-1}) was more than double that of the foot or postural condition (SUP Leg = 0.009 flux $\cdot$ mmHg $\cdot$ °C^{-1}, STA Arm = 0.008 flux $\cdot$ mmHg $\cdot$ °C^{-1}, STA Leg = 0.006 flux $\cdot$ mmHg $\cdot$ °C^{-1}); matching the temporal results observed in Fig. 2. Additionally, RMSE analysis of the variability of individual responses from the best fit identified that the STA condition produced considerably lower values (STA Arm = 0.045 flux $\cdot$ mmHg^{-1}, STA Leg = 0.047 flux $\cdot$ mmHg^{-1}) than the SUP condition (SUP Arm = 0.098 flux $\cdot$ mmHg^{-1}, SUP Leg = 0.096 flux $\cdot$ mmHg^{-1}).

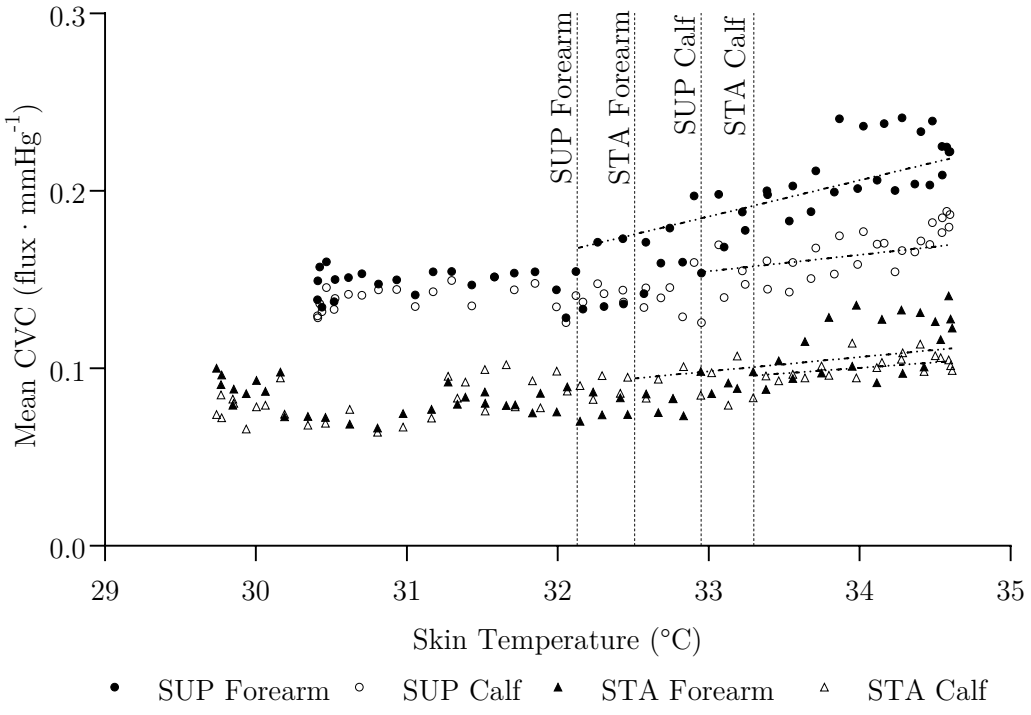

Figure 3.3: Mean CVC response as a function of the forearm and calf skin temperature, during both SUP and STA conditions. Dashed lines indicate the thresholds of vasodilation.

3.4.5 Haemodynamics

Table 1 provides mean ± SD cardiovascular values at the lowest and highest ambient temperatures observed, in both standing and supine conditions. A lack of hysteresis in haemodynamic variables means no differences were seen between minute-0 and minute-60; hence, only minute-0 is displayed in Table 1. In response to the main effect of ΔTemp, there was a significant increase in HR from 63.2 ± 10.6 min−1 in the cold to 69.5 ± 13.2 b $\cdot$ min−1 in the hot period (p = 0.008, F = 1.516, d = 0.5). In contrast, there was significant decrease in SV (cold = 93.0 ± 20.3 mL, hot = 81.3 ± 20.0 mL, $p < 0.001$, F

= 3.309, d = -0.6), SBP (cold =142.0 ± 14.0 mmHg, hot = 132.6 ± 14.7 mmHg, p < 0.001 , F = 4.226, d = -0.6), DBP (cold = 109.5 ± 12.6 mmHg, hot = 105.0 ± 12.5 mmHg, p = 0.018, F = 2.753, d = -0.4), and MAP (cold = 89.7 ± 12.4) mmHg, hot = 88.5 ± 11.8) mmHg, p = 0.006, F = 3.271, d = -0.1), as a result of the change in Ta from cold to hot conditions.

There were also significant differences as a result of the two postural conditions. HR was significantly higher with 78.6 ± 9.4 $\cdot$ min^{-1} in the STA condition compared to 59.0 ± 7.3 min^{-1} the SUP condition ($p < 0.001$, F = 1679, $d = 1.9$); this also occurred in RPP (SUP = 8292.1 ± 1506.2 mmHg $\cdot$ min^{-1}, STA = 11007.4 ± 2081.3 mmHg $\cdot$ min^{-1}, $p < 0.001$, F = 15.94, $d = 1.9$), DBP (SUP = 82.8 ± 10.1 mmHg, STA = 95.6 ± 10.0 mmHg, p = 0.002, F = 13.21, $d = 1.8$), and MAP (SUP = 101.0 ± 9.9 mmHg, STA = 112.6 ± 13.2 mmHg, $p = 0.013$, F = 7.655, $d = 1.7$). On the contrary, higher responses were observed in the SUP condition compared to the STA condition in SV only (SUP = 96.1 ± 18.6 mL, STA = 70.7 ± 11.1 mL, $p < 0.001$, F = 16.99, d = -0.6).

Finally, the interaction of both independent variables (ΔTemp and Posture) as stressors caused a significant effect in RPP ($p < 0.001$, F = 4.048), DBP (p =0.003, F = 1.613), and MAP ($p = 0.019$, F = 1.434). In each of these variables the Hot STA condition elicited the highest recorded values, and the Hot SUP produced the lowest values (Table 2). Multiple comparisons analysis of RPP and DBP indicate the crossover between Cold SUP and Hot STA, and Cold STA and Hot SUP, were significantly different from each other.

Table 3.2: Mean ± SD cardiovascular values at absolute lowest (16 °C) and highest (38.6 °C) ambient temperatures, in both supine and standing conditions.

Cardiovascular Parameter	Sig.	Postural condition	Cold Conditions 15.7°C (Min 0)	Hot Conditions 38.6°C (Min 30)
Heart Rate	†,*	SUP	57.8 ± 8.0	60.0 ± 7.3
(min^{-1})		STA	70.4 ± 8.9	81.5 ± 8.3
Stroke Volume	†,*	SUP	96.6 ± 22.6	92.1 ± 20.2
(mL)		STA	85.2 ± 16.1	65.0 ± 6.1
Cardiac Output		SUP	5.6 ± 1.2	5.5 ± 1.0
($L \cdot min^{-1}$)		STA	5.8 ± 0.8	5.3 ± 0.6
Rate Pressure Product	*,#	SUP	8957.2 ± 1651.5	8018.4 ± 1539.8
($mmHg \cdot min^{-1}$)		STA	10612.9 ± 2065.8	11654.9 ± 2127.8
Systemic Vascular Resistance		SUP	1.1 ± 0.5	1.0 ± 0.3
($mmHg \cdot min \cdot mL^{-1}$)		STA	1.1 ± 0.2	1.2 ± 0.2
Systolic Arterial Pressure	†	SUP	138.9 ± 11.5	128.8 ± 12.1
(mmHg)		STA	143.4 ± 16.8	137.3 ± 16.7
Diastolic Arterial Pressure	†,*,#	SUP	105.9 ± 11.5	99.3 ± 9.2
(mmHg)		STA	112.2 ± 13.1	112.3 ± 12.7
Mean Arterial Pressure	†,*,#	SUP	86.3 ± 11.7	82.4 ± 9.0
(mmHg)		STA	92.7 ± 12.0	96.5 ± 10.7

† = significant effect of ΔTemp, * = significant effect of posture, # = significant interaction of both independent variables, $p < 0.05$.

3.4.6 Mechanistic Analyses

3.4.6.1 Heart Rate Variability

Figure 4 displays effects of ΔTemp (indicated by time) and postural condition on a number of key autonomic indices. In response to ΔTemp, significant effects were observed in multiple indices indicating an overall shift towards greater sympathetic activation.

PNS at the lowest temperature (i.e., 0-min) was at its peak (SUP = 1.5 ± 0.2, STA = -0.1 ± 0.5), before significantly decreasing to lowest values at minute-40 (SUP = 0.8 ± 0.2, STA = -1.5 ± 0.5; $p < 0.001$, F = 13.93). In direct contrast, SNS index at the lowest temperature (0-min) was at its lowest value (SUP = -1.0 ± 0.2, STA = 0.1 ± 0.5), before significantly increasing to a peak at 40-minutes (SUP = -0.5 ± 0.2, STA = 1.6 ± 0.5; $p < 0.001$, F = 12.42). RRi significantly decreased as a result of ΔTemp, with an average decrease of 115.9 ± 41.5 ms ($p < 0.001$, F = 12.88) from minute 0 – 30. Finally, the LF/HF ratio significantly increased as a result of ΔTemp ($p < 0.001$, F = 5.821), though this is likely to be solely the influence of the STA condition which increased by from 6.6 ± 1.8 to 20.1 ± 4.7 from minute 0 - 30; compared to an increase from 2.9 ± 0.2 to 4.0 ± 0.4 in SUP. This is reflected in the significant interaction of ΔTemp and posture in the LF/HF ratio ($p = 0.003$, F = 4.113).

The change in posture condition also caused significant changes in multiple indices. PNS index was significantly lower in the STA condition (-1.0 ± 0.5) compared to the SUP condition (1.1 ± 0.2; $p < 0.001$, F = 24.54, ES = -1.35), whereas the SNS index was significantly higher in the STA condition (SUP = -0.7 ± 0.2, STA = 1.0 ± 0.5, $p < 0.001$, F = 24.16, ES = 1.34). The changes in these two indexes between the two conditions signify an increase in sympathetic autonomic activity. Finally, the LF/HF ratio, an indicator of sympho-vagal balance, was significantly higher in the STA condition versus the SUP (SUP = 3.5 ± 0.4, STA = 14.1 ± 4.7, $p < 0.001$, F = 52.84, ES = 6.7). In addition, analysis of the spectral frequency distribution identified there was no difference in relative VLF between SUP and STA, however both relative LF (SUP = 60.9 ± 2.6 %, STA = 85.0 ± 5.9 %, $p < 0.001$, F = 21.93, ES = 1.8) and relative HF (SUP = 31.9 ± 2.9 %, STA = 8.0 ± 3.5 %, $p = 0.002$, F = 17.40, ES = -1.7) were significantly affected.

Finally, the interaction of both ΔTemp and posture caused a significant effect in SNS index ($p = 0.003$, F = 4.031), RRi ($p = 0.013$, F = 5.963), and LF/HF ratio as described above.

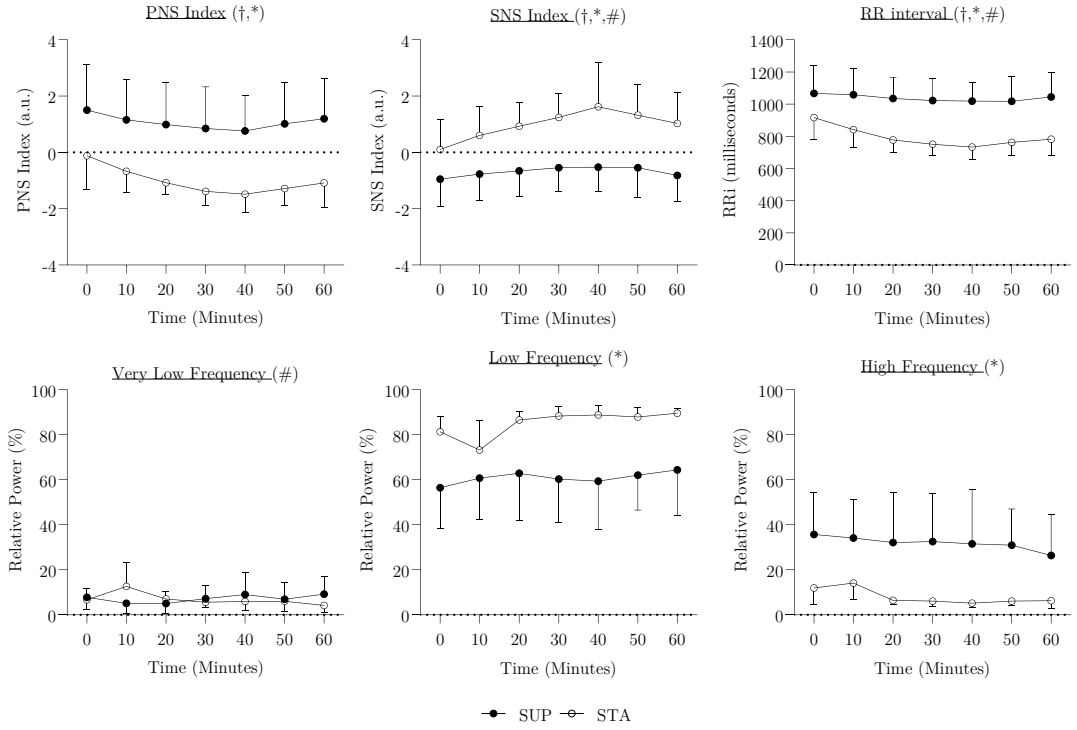

Figure 3.4: Impacts of ΔTemp (indicated by time) and postural condition on heart rate variability indices. PNS = parasympathetic nervous system, SNS = sympathetic nervous system, † = significant effect of ΔTemp, * = significant effect of posture, # = significant interaction of both independent variables, $p < 0.05$.

3.4.6.2 Wavelet Transform Analysis (WTA)

WTA was conducted on LDF signals recorded at both the forearm and calf, at discreet regions of interest (ROI) at 0, 30, and 60 minutes (Fig. 5). Three-way ANOVAs conducted at both 0- and 60-minutes only identified the limb (i.e., forearm or calf) as a significant source of variation in WTA amplitude (0-min: $p < 0.001$, F = 18.36; 60-min: $p = 0.001$, F = 10.53). Multiple comparisons analysis identified that in the 0-min group, STA calf was significantly higher than forearm SUP ($p = 0.039$) or STA ($p = 0.042$) in band 3 (myogenic pathways). In the 30-min ROI, however, limb was again identified as a significant source of variation ($p < 0.001$, F = 30.86) alongside the interaction of limb and posture ($p = 0.015$, F = 5.981); signifying that posture significantly affected vascular control during high ambient temperatures. As in the 0- min analysis, multiple comparisons analysis also noted that STA calf was significantly higher than forearm SUP ($p = 0.039$) or STA ($p = 0.014$) in band 3 (myogenic pathways).

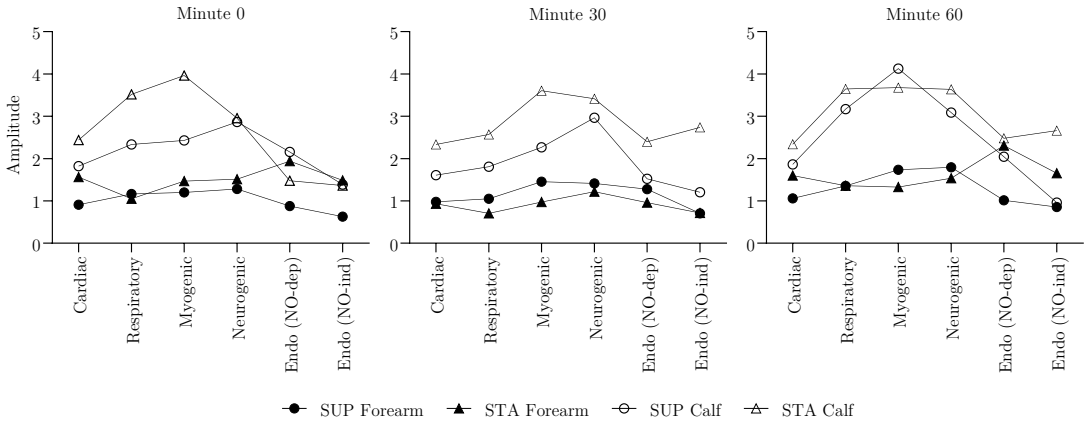

Figure 3.5: Relative contributions of vascular activities (denoted by frequency bands on x-axis) calculated by WTA of LDF signals. Analyses conducted at 0-, 30-, and 60-min. Lines between data points do not represent continuous data, only to provide visual aid.

3.5 Discussion

In the present study, the role of central (HRV) and peripheral (WTA) mechanisms of microvascular blood flow was assessed in relation to regional vascular responses of the forearm and calf, and its impact on the vasomotor zone. The main findings of this study are that, as previously suggested by Ciuha, Tobita, et al. (2019) and Fisher et al. (2022), there are regional differences in the microvascular response to one or more stressors. The results of the present study suggest, both mechanistically and functionally, that while the microvasculature of the legs remains predominantly baroreflex-controlled, the microvasculature of the arms receives input (and thus interaction) from both (skin temperature and arterial pressure) vascular regulatory mechanisms. Calf CVC minimally responded to the significant increase in ambient temperature during the heating phase of the protocol, and thus was solely affected by the change in posture from supine to standing; thus, confirming that postural stress alone causes a decrease in the SBF (predominant baroreflex control), with a greater response identified in the calf microvascular blood flow response. Forearm CVC, however, responded to both thermal and postural stressors. There was a 95.1 % increase in CVC during hot conditions when compared to cold conditions, which subsequently decreased by the same magnitude albeit at a slower rate during cooling; a 46.4 % increase in forearm CVC during the standing condition was also observed. This confirms that thermal stress alone will increase SBF (predominant thermoregulatory control), with a greater response identified in the forearm microvascular blood flow response, and that a combination of thermal and postural stressors will cause differences in the regional (arm vs. leg) SBF responses (thermoregulation/baroreflex interaction), altering cardiovascular responses to the transient temperature.

3.5.1 Functional LDF and Haemodynamic Response

The effect of posture (or gravity) is observed in the differences between the SUP and STA conditions in a cool ambient. In response to a change in posture, there is a reduction in overall blood flow to the microvascular circulatory system due to a redistribution of blood to different regions of the body. In the STA condition, the influence of gravity redistributes 70% of circulating blood volume below the heart level, creating a cascade of haemodynamic

responses to maintain the flow of oxygenated blood to the brain and other vital organs. These responses, including increases in HR, SBP, and DBP, whilst SV decreases, have been commonly observed; thus, were not unexpected in the present study. In the absence of physical and physiological countermeasures to increase orthostatic tolerance such as muscle tensing/pumping (Wieling et al., 2015), symptomatic or episodic orthostatic hypotension and syncope may be likely.

Concurrently, responses to heat stress as a lone stressor are commonly understood and are observed in the present study when comparing the difference in responses at 15.7 (0.6) °C and 38.9 (0.6) °C. In situations of greater heat gain via higher temperatures or longer exposure, it is possible that thermoregulatory control of leg vasculature may override the observed baroreflex control. It is also possible that the lessened thermoregulatory input, observed in the present study, in the leg may be a precursor to accelerated damage in certain situations including cold exposure. It has been observed in regional cold-induced vasodilation (CIVD) that a reduced response occurs in the leg regions, which may leave these regions open to greater risk of freezing and non-freezing cold injuries during prolonged cold exposure (Cheung & Mekjavic, 2007; Tsoutsoubi et al., 2022). The combination of changes in ambient temperature and the STA condition produced the requirement for interaction of both thermoregulatory and baroreflex mechanisms. Indeed, this condition produced a lower microvascular flow as circulating blood volume was required for other vital roles. Nonetheless, forearm microvascular flow increased, albeit to a lower magnitude, during the standing trial in response to higher ambient temperatures; indicative of a thermoregulatory drive. This indicates that the interaction of thermal and baroreceptor pathways, while initiated at a central level (Heistad et al., 1973), create differing functional regional responses. This finely balanced interaction is essential in the maintenance of suitable internal body temperatures and appropriate oxygenation of the brain to avoid syncope. One participant in the present study displayed an inability of sustaining this balance, thus experiencing pre-syncopal symptoms. The failure of this balance under heat and orthostatic stress is also observed in literature; Schlader et al. (2016) show a significant leftward shift in a Kaplan-Meier survival probability curve. Similarly, Lind et al. (1968) stated that no participants experienced syncope during 70°C head-up tilt for 5-minutes in normothermia, however 25% experienced syncope in a hot ambient climate.

3.5.2 Vasomotor Zone

Upper and lower thresholds for sweating (UCT) and shivering (LCT) define the vasomotor zone (Mekjavic et al., 1991), which may be defined on the basis of either core (Mekjavic et al., 1991) or peripheral (Kakitsuba et al., 2007) temperature thresholds. The present study clearly displays the relationship between T_{sk} and BF_M, in differing postural conditions and regions of the body, which reveal the upper threshold of the vasomotor zone (UCT); denoting vasodilation of BF_M. In agreement with our observations of the forearm exhibiting predominant thermoregulatory control, though still interactive with the baroreflex, the 'vasomotor threshold' occurred earlier in this limb regardless of posture. In fact, the onset of vasomotor activity occurred at a skin temperature 0.9 °C lower in the forearm than in the calf, and the slope of the response (indicating magnitude of BF_M response) was more than double that of the leg. While the UCT was detected in the present study, it appears that skin cooling at the lower ambient temperatures was not sufficient to elicit vasoconstrictive action; thus, the LCT remained undetected. Taniguchi et al. (2011) proposed that the onset of vasoconstriction is reached after 53 minutes from a starting temperature of 35.5 °C, with a skin cooling rate of 2 °C/hour. Assuming a similar cooling rate and a peak starting T_{sk} of 34.6 °C, the LCT should have been reached in the present after ~35 minutes. However, differences in the cooling methods (i.e., ambient air vs. water

cooling) may have lengthened the time to threshold in the present study. In addition, whilst the postural condition appeared to have a smaller influence on vasodilation threshold, causing only an average difference regardless of region of 0.3 °C, it did appear to influence the individual variability of the response; RMSE analysis revealed that the variability in STA responses was half that of the SUP condition. It may therefore be considered that the thermoregulatory response alone (i.e., heating in SUP) produces a variety of responses which may be influenced by such inter- and intra-individual characteristics including anthropometry, acclimation, physical fitness, etc., (Ioannou et al., 2022). However, interaction of thermoregulatory and baroreflex responses (i.e., heating in STA) appears to produce a more specific response, which may be due to the preservation of carotid-cardiac modulation of heart rate rather than carotid-vascular responses (Crandall, 2008).

3.5.3 Central Autonomic Control

As discussed earlier, several studies have identified the existence of a regional response (Ciuha, Tobita, et al., 2019; Essandoh et al., 1988; Fisher et al., 2022; Hales et al., 1994; Nishiyasu et al., 1992), and alluded to possible reasons for the variation. None, however, have recorded the central and peripheral autonomic responses to differing environmental stressors. In the present study, HRV provided an understanding of the varying sympathetic and parasympathetic neural control at any given temperature, whilst also indicating the source of variation via spectral frequency analysis. Transient change in ambient temperature caused a response in the central sympathetic response, which also resulted in reduced RRi and an increase in HR. The HRV response to acute exposure to heat stress was anticipated, whereby SNS control is activated to regulate thermal balance. These responses have been observed in acute and continuous cooling protocols (Sawasaki et al., 2001), acute hot and cool air temperature exposure (Liu et al., 2008), and long duration hot air exposure (Carrillo et al., 2016); yet only the temporal aspect of heating is often considered, rather than the relationship between T_{sk} or deep body temperature and HRV.

It is also clear that the change in posture from SUP to STA represents an orthostatic stressor which activates the SNS regulation of blood flow. Fig. 4 illustrates that in the SUP condition, parasympathetic withdrawal occurs, whilst an increase in SNS activity shifts the sympathovagal balance (LF/HF ratio) towards 'sympathetic dominance'. These results match those of previous research relating to differences between different postures (Acharya et al., 2005), and to graded levels of orthostatic tilt (Montano et al., 1994). As with these previous studies, the observed HRV response of increasing LF (sympathetic) and decreasing HF (vagal) to changes in posture are a baroreceptor response to maintain blood flow to vital organs under diminished venous return caused by lower-limb pooling (>500mL). Redistribution of blood reduces stretch and unloads arterial baroreflexes, therefore inducing sympathetic adrenergic vasoconstriction of peripheral vasculature (Stewart, 2012) and splanchnic circulation (Fink & Osborn, 2023) to return central blood volume; among other passive blood pooling recoil responses. It is also well understood that slight tachycardia that occurs during standing is a result of SNS vagal activity withdrawal in sinoatrial node activity (Montano et al., 1994). The combination of these responses to increases in SNS activity during standing are major benefits for orthostatic tolerance. However, it is possible that when conflicting neural responses are required, such as during heating and orthostasis, peripheral efferent pathways may alleviate some of the stress on the autonomic system.

3.5.4 Efferent Autonomic Control

Peripheral efferent control refers to the interaction of endothelial and myogenic actions, and sympathetic nerves; which regulate flow of blood to the skin via changes in vessel diameters (Bagno & Martini, 2015). Peripheral vascular control is initiated via rostral ventrolateral medullary neurons, passing through intermediolateral cell columns in the spinal cord and activating synapses on sympathetic ganglia which result in vasoactive outputs in blood vessels via norepinephrine (Freeman et al., 2018). Spectral analyses of LDF signals via WTA are suggested to elucidate the activation of different areas due to this peripheral efferent response, by associating the response with a specific frequency interval. In the present study, WTA identified significant differences in the frequency intervals' amplitudes between limbs at all three measured temperatures, which are clearly related to higher myogenic and neurogenic activation in the legs, whilst the arms have clearly definable central control mechanism at either low or high temperatures. This is concurrent with our theory that the arms receive input from two (or more) regulatory mechanisms, whereas in the legs the dominant role is regulation of blood pressure. It was also noted that under peak heat stress there was a significant effect of posture on efferent control, and further analysis identified that the calf exhibited greater activation of myogenic pathways in both cool and hot conditions. This suggests that baroreflex control of blood flow occurs independently of thermoregulatory drive, even under heat stress up to ~40 °C ambient temperature. The ability of the baroreceptor system to override thermoregulation has also been observed during local- and whole-body heating, and LBNP (Crossley et al., 1966; Lind et al., 1968). Research by Pawelczyk and Levine (2002) provides a potential reason as to the greater sympathetic activity in the calf in the present study, postulating that vascular responsiveness of the arms and legs to α-receptor agonists is heterogeneous, with legs displaying larger sensitivity. Further, greater sympathetic tone is required in the lower limbs to maintain circulatory function at rest. After 60-minutes in the STA condition, the observed reduction in myogenic activities in the leg may be due to reductions in synaptic control responsible for baroreflex efferent activation. This response is similar to those seen in muscle fatigue (Sesboüé & Guincestre, 2006), overtraining syndrome (Carrard et al., 2022), and afferent synaptic pathways (Hay & Hasser, 1998). It is also possible that these changes in efferent baroreflex control are the product of transient outward K^+ current, caused by overuse of the Na^+-K^+ pump, during sustained carotid sinus baroreceptor activation which result in a decline in depolarization frequency, thus reducing vasoconstrictor action in vascular smooth muscle (Chapleau et al., 1995; Ko et al., 2008). It may also be speculated that chronic high-frequency stimulation of afferent baroreceptor pathways (i.e., sympathetic ganglion) may decrease discharge frequency of excitatory potentials (Hay & Hasser, 1998), reducing the requirement for an efferent response (Freeman et al., 2018). It is viable that these factors may contribute to the causes of orthostatic intolerance in the absence of mechanical movement.

3.5.5 Limitations and Future Perspectives

Deep body temperature was not directly recorded in the present study, as previous research indicates that the change in temperature and exposure time would not be sufficient to produce any significant change, as reported by Ciuha, Tobita, et al. (2019). Future studies may wish to lengthen the exposure times to compare the regional responses to shifts in deep body temperature. T_{sk} was monitored using thermistors with a high resolution of 0.125 °C but a relatively low accuracy of ± 1.0 °C. However, research conducted on iButton thermistors by Smith et al. (2009) report an accuracy closer to ± 0.5 with a mean bias from a verified mercury thermometer of + 0.121. In the present study, T_{sk} was recorded to

indicate the level of heat stress experienced by participants, however, further studies seeking to specifically determine vasomotor zone thresholds should utilize devices with higher accuracy. The two conditions used in the present study produce different levels of stress on the body's thermoregulatory and baroreflex control. The SUP condition focuses purely on thermoregulatory stress, whereas the STA condition creates a conflict between thermoregulatory and baroreflex mechanisms. An issue that remains unresolved is the interaction of constant ambient temperature and transient levels of gravitational stress in the head-to-foot direction in the regional regulation of blood flow.

3.6 Conclusions

We conclude that, mechanistically and functionally, the arm vasculature responds swiftly to modulation from multiple regulatory mechanisms including thermoregulation and baroreceptor activity. The legs, meanwhile, are more sensitive and strongly innervated by baroreflex regulatory mechanisms. These observed regional responses aid the haemodynamic response to multiple stressors, however if the interaction is unbalanced syncope or heat illness may occur.

Chapter 4

The Combined Effects of Artificial Gravity, Temperature and Hypoxia on Haemodynamic Responses and Limb Blood Flow

4.1 Foreword

By utilising direct measurement techniques and implementing mechanistic analyses of autonomic activity, the study presented in chapter 3 built upon the results of chapter 2 via more acute and controlled stressors. Previous research and chapter 2 had described clear differences between the control mechanisms associated with different regions of the body. Yet the central interaction of blood flow mechanisms is known to be considered a relationship (or competition) between two or more mechanisms acting simultaneously. Chapter 3 highlighted this simultaneous action, yet only in the forearm, whereby the combination of high temperatures and standing produced a modulation from the baroreflex system to reduce vasodilation despite a thermoregulatory response still occurring. However, high T_A appeared to have little to no effect on leg SBF, unlike the differences between postures which produced a large effect; hence greater baroreflex action.

Baroreflex modulation of thermoregulation occurred even at relatively low temperatures (~25-30 °C); yet the baroreflex response to postural stress did not reach a level at which modulation was required from thermoregulation. In addition, the chemoreflex has yet to be discussed as a potential mechanism for variation in regional blood flow. Therefore, the study in chapter 4 employed high levels of artificial gravity (AG) via a short arm human centrifuge (SAHC) that resulted in a stimulus (caudal displacement of blood) of significant strength, requiring regulation of blood pressure; whilst exposed to different levels of ambient temperature and partial pressure of oxygen.

The findings of study have been submitted to NPJ Microgravity under the following citation: Fisher, J.T., Ciuha, U., Denise, P., McDonnell, A.C., Normand, H. & Mekjavic, I.B. (Submitted). The Combined Effects of Artificial Gravity, Temperature and Hypoxia on Haemodynamic Responses and Limb Blood Flow. *NPJ Microgravity.*

4.2 Introduction

Changes in the hydrostatic pressure gradient within the circulation can compromise the regulation of arterial blood pressure. The ability to withstand these changes is defined as orthostatic tolerance (OT). A multitude of factors contribute to OT, including body (and ambient) temperature, acute or chronic hypotension, physical fitness, hydration, and anthropometrics (Brignole, 2007; Christou & Kiortsis, 2017; Levine et al., 1991; Stewart, 2013; Wilson et al., 2006). The role of the blood pressure regulatory system is to maintain appropriate perfusion of vital organs, particularly the central nervous system (CNS), by preventing pooling of blood in the lower extremities during exposure to natural gravity in the upright posture. The head-to-foot gravitational vector can be artificially elevated above 1 Gz, in which case the ability to maintain arterial pressure under such conditions is termed gravitational, or passive G-tolerance. Studies have shown that the baroreflex is capable of maintaining adequate blood pressure in individuals even at suitably high g-loads; known as G-tolerance. Goswami, Bruner, et al. (2015) proposed that centrifugation of subjects in a supine position on a short arm human centrifuge (SAHC) with 0.75 Gz at heart-level represents similar cerebrovascular and cardiovascular responses as observed in the upright standing position, at an ambient temperature of 22°C. Above these levels of G-load, the increased gravitational stress may lead to a detrimental decrease in cerebral perfusion pressure, and thus cerebral blood flow (Ogawa et al., 2016), eliciting syncope with increasing Gz or prolonged exposure. Despite the potential for syncopal symptoms, the use of SAHC as a potential training tool for astronauts to assist in the maintenance of a suitable arterial baroreflex operational range and subsequent OT (Adami et al., 2013), is of significant interest.

Concurrent with blood pressure control, regulation of homeothermy in the face of ranging ambient temperatures (Ta) is achieved via autonomic responses, in addition to behavioural responses. Above and below so-called 'critical temperatures' of Ta, mechanisms of heat loss (upper critical temperature) and production (lower critical temperature) initiate sweat secretion and shivering thermogenesis, respectively. However, within these upper and lower critical temperatures exists the 'vasomotor zone' (Mekjavic et al., 1991), whereby sympathetic neural control mechanisms produce vasodilatory and vasoconstrictor vascular control. Within this vasomotor zone, skin blood flow (SkBF) can vary from as little as 250 $mL \cdot min^{-1}$ to as much as 6-8 $L \cdot min^{-1}$ (Charkoudian, 2003).

The chemoreflex also influences regional vascular control, which is activated under hypoxic conditions, and may alter OT responses. Hypoxaemia elicits peripheral vasodilatation and increases skeletal muscle blood flow despite an increase in sympathetic activity (Dinenno, 2016) originating from the stimulation of α-adrenergic receptors on vascular smooth muscle (Jacob et al., 2021). There is a complex interaction between the sympathoadrenal system and locally derived vasodilatory substances (including NO) that ultimately determine the net peripheral vasodilatory response to systemic hypoxia in humans. Whereas the effect on muscular blood flow appears to be uniform throughout the body, the effect of hypoxia on SkBF is less uniform; seemingly with an increase in peripheral non-acral SkBF, and a decrease in peripheral acral SkBF and central SkBF (Golja & Mekjavić, 2002; Minson, 2003).

At lower G-loads it is clear that the baroreflex is capable of maintaining arterial pressure, with the magnitude of vascular response increasing with increasing G-load. It has previously been shown, however, that the influence of thermoregulation for the maintenance of homeothermy during gravitationally challenging situations creates interaction (or competition) in autonomic control (Crossley et al., 1966); which has been observed during transition from supine to upright posture (Nielsen et al., 1939), and during

heating in an upright posture (Rowell et al., 1970). In the absence of other factors, such as exercising muscle activity or behavioural thermoregulation, there may be an intolerable accumulation of heat or development of pre-syncopal symptoms. Previous research (Ciuha, Tobita, et al., 2019; Fisher et al., 2022) has identified a regional variation in the skin blood flow response to these external stressors, suggesting that the competition between regulatory vascular mechanisms exerts specific and altered control on different regions of the body. Specifically, blood flow in the arms is predominantly under thermoregulatory control, whereas the blood flow in the legs is predominantly regulated by the baroreflex. Further research conducted by our group has postulated that in response to combined postural and heat stress, arm vasculature responds swiftly to input from multiple mechanisms (i.e., thermoregulatory and baroreflex), whereas the legs are more strongly innervated by the baroreflex alone (Fisher et al., 2024).

The current study tested the hypothesis that the separate and combined effects of artificial gravity (AG), hypoxia and temperature will elicit differing regional responses in microvascular blood flow (M_{BF}), and significantly alter haemodynamic control. Specifically, that: 1) for the maintenance of blood pressure during AG, ambient temperature will most likely favour vasoconstriction of the peripheral circulation, particularly that of the lower limbs, 2) at higher ambient temperatures, the baroregulatory system will be somewhat diminished by the thermoregulatory response in the arms, via a withdrawal of vasoconstriction, 3) the combined vasodilatory effects of high ambient temperature and hypoxia, under a high gravitational gradient, will increase the rate of presyncopal symptoms.

4.3 Materials and Methods

The study was approved by the National Committee for Medical Ethics at the Ministry of Health of the Republic of Slovenia (appendix D, approval no. 0120-180/2023/7) and conformed to the guidelines of the Declaration of Helsinki.

4.3.1 Participant Information

The minimum required sample size for investigating "repeated measures, within-between factors" was calculated using the results of a previous study (Ciuha, Tobita, et al., 2019). A difference of 4.9°C in proximal-distal temperature gradient ($\Delta Tsk_{p\text{-}d}$) during heating, and a difference of 3.0°C during cooling, produced an effect size (d) of between 1.58 to 3.38 for the association between temperature and microvascular blood flow. Assuming an α of 0.05 and β of 0.99, eight participants provide sufficient power to detect a statistical difference. Therefore, to account for any potential subject dropout, a total of 10 male participants were recruited for the study. Their mean ± SD age was 27.9 ± 6.3 years, body mass was 78.2 ± 10.3 kg, body stature was 179.8 ± 6.3 cm, body mass index was 24.2 ± 2.8 $kg \cdot m^2$, body surface area was 2.0 ± 0.2 m^2 (Mosteller, 1987) and calculated blood volume was 5.3 ± 0.5 L (Nadler et al., 1962). Prior to the start of the study, participants were familiarised with the study protocol and procedures, and gave their written consent for participation. The following exclusion criteria were applied: smokers, physically inactive, diabetic, and/or a history of freezing or non-freezing cold injuries, cutaneous peripheral disease, or high or low blood pressure.

4.3.2 Experimental Protocol

Participants attended four testing sessions on different days separated by at least 24 hours, in a repeated-measures, crossover design. For each participant, the experimental trials were

conducted at the same time of day in the Gravitational Physiology Laboratory, a European Space Agency ground-based research facility maintained by the Jozef Stefan Institute at the Nordic Centre Planica (Planica Slovenia)in Jožef Stefan Institute's Gravitational Physiology Laboratory (located at the Nordic Centre Planica, Slovenia., at The facility is located at an altitude of approximately 940m (barometric pressure: 685mmHg). The experimental trials were conducted on a short arm human centrifuge (SAHC; Redwire, Belgium) developed by ESA. The SAHC has two nacelles, each aligned on either side of the rotation axis. On one of the nacelles, participants are positioned supine in a cradle, with their head towards the axis of rotation and their feet outwards and positioned on a force platform. The other nacelle serves as a counterweight. Participants on the SAHC experience elevated gravitational acceleration in the head-to-foot direction. At maximum SAHC angular velocity, the maximum acceleration is equivalent to 4 Gz at the footplate. While this type of gravitational stimulus does not exactly replicate standing in normal gravity, the footward hydrostatic shift is similar.

Figure 1 displays the protocol used in the present study, which was identical in each of the 4 testing sessions, shown in Table 1. As indicated in Table 1, ambient temperature and partial pressure of oxygen (PO_2, mmHg) were the key difference between trials. Relative humidity (%RH) remained constant at 49.5 ± 8.1 %. The order of sessions was randomised via a Latin square design.

Table 4.1: Ambient conditions experienced in each session.

	Warm	**Cool**
Normoxia	*Warm Normoxia (WN)* PO_2 = 133 mmHg Alt. = 940 m T_A = 29.1 ± 0.8 °C	*Cool Normoxia (CN)* PO_2 = 133 mmHg Alt. = 940 m T_A = 18.4 ± 0.8 °C
Hypoxia	*Warm Hypoxia (WH)* PO_2 = 92 mmHg Eqv. Alt. = 4000 m T_A = 29.1 ± 0.8 °C	*Cool Hypoxia (CH)* PO_2 = 92 mmHg Eqv. Alt. = 4000 m T_A = 18.4 ± 0.8 °C

PO_2 = partial pressure of oxygen, Eqv. Alt. = equivalent altitude, Ta = Ambient temperature

Upon arrival at the laboratory participants had their height and naked weight recorded, before donning shorts and a t-shirt without shoes/socks. Before entering the SAHC chamber, the participants rested in a thermoneutral ambient for 20 minutes. They then entered the SAHC room and were fully instrumented, a further 10 minutes of resting occurred, and maximum cutaneous blood flow responses were obtained (described in section 2.3). With the exception of the first condition, in which the subjects were standing in normal gravity (NG), the remaining five conditions were conducted with the subject supine on the nacelle of the SAHC. During the trials, the subjects were either resting supine (SUP), or were exposed to a gravitational vector in the head-to-foot direction, by adjusting the angular velocity of the centrifuge. The definition of gravitational stimuli experienced by the participants was defined by the ground reaction force (GRF) as measured with a force platform situated at the feet. Measuring GRF rather than Gz is required for the production of a consistent acceleration profile between participants of differing body anthropometrics; due to the non-uniform acceleration produced by the SAHC. Table 2

provides mean ± SD of value the distance the head, heart, COM, and feet, from both the force platform and the SAHC centre of rotation. Centrifugation at 1GRF relates to 100% of participants bodyweight measured at the force platform (equivalent to 1Gz at the heart level), whilst 2GRF relates to 200% of the participants bodyweight measured on the force platform (equivalent to 2.0 Gz at the heart level). In the present study, 1GRF centrifugation produced 99.9 ± 9.8 % of participants bodyweight (i.e., 1Gz) and 2GRF produced 187.0 ± 4.9 % (i.e., 1.9 Gz) of bodyweight. 2GRF did not achieve 200% bodyweight due to limitations in the SAHC motor speed, which are in place for safety purposes.
The six experimental conditions, depicted in Fig.1, were: i) standing in normal gravity (NG), ii) supine (SUP1), iii) supine with the angular velocity of the centrifuge adjusted to maintain 1GRF on the force platform (1GRF), iv) supine (SUP2), v) supine but with the angular velocity of the centrifuge adjusted to maintain 2GRF on the force platform (2GRF), vi) supine (SUP3). During the trials, participants were requested to remain still with no movement in their right (instrumented) arm and leg, and without crossing their legs and/or arms. Subjects were monitored with video cameras, and were in constant audio contact with the SAHC controller. The test would have been stopped if any of the following indicators of pre-syncope were observed: persistent decrease in systolic blood pressure >35 mmHg, or a decrease in heart rate > 15 $b \cdot min^{-1}$.

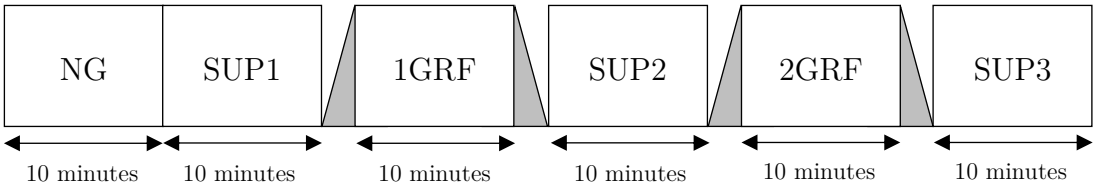

Figure 4.1. Session protocol displaying the order of conditions following time for acclimation and instrumentation (not shown in figure), identical in each of the four sessions. Grey areas represent the 90-second ramp up/down for centrifugation.

4.3.3 Data Collection Methods

4.3.3.1 Laser Doppler Flowmetry (LDF)

Measured at the forearm (ArmBF) and calf (LegBF), on the right side of the body, as a non-invasive measure of capillary, arteriole, and venule perfusion (Moors Instruments Laser doppler monitor, MoorVMS-LDF, Moor Instruments, UK). The device was calibrated using a fluid undergoing Brownian motion before each participant. Arbitrary units produced by the LDF (Laser doppler units: LDU) were converted into cutaneous vascular conductance (CVC) as a ratio of LDU to mean arterial pressure. They were then normalised against maximum blood flow for both the arm and leg in normothermic conditions in order to produce a relative measure of percentage maximum blood flow (%maxBF). Maximum blood flow values were obtained by locally heating the limb and producing a post-occlusion reactive hyperaemia (PORH) via occlusion then release the limb after 4 minutes.

4.3.3.2 Haemodynamics

Measurement of heart rate (HR, min−1), stroke volume (SV, mL), cardiac output (CO, L·min-1), systemic vascular resistance (SVR, mmHg·min·mL-1), rate pressure product (RPP, mmHg·min-1), systolic blood pressure (SBP, mmHg), diastolic blood pressure (DBP, mmHg), mean arterial pressure (MAP, mmHg), and oxygen saturation (SpO2, %) occurred

continuously throughout the protocol (Finapres NOVA, Finapres Medical Systems B.V., Netherlands). Haemodynamic responses were calculated using the model flow algorithm (Wesseling et al., 1993) utilising the finger volume-clamp method and a 5-lead electrocardiogram (ECG). Reconstructed systolic and diastolic blood pressures were calculated via direct finger pressure measurements using waveform filtering and level correction (Westerhof et al., 2002), and normalised to the heart level via a height correction unit measuring the hydrostatic pressure difference. Additionally, participants held their arm in a sling with the measured finger at the heart level throughout all conditions.

4.3.3.3 Skin Temperature (T_{sk})

Skin temperatures were measured at minute intervals using wireless iButton thermistors (type DS1921H, Maxim/Dallas Semiconductor Corp., USA) located at four sites (mid-belly of the bicep brachii, pectoralis major at mid-clavicular level, rectus femoris at femur midpoint, gastrocnemius on medial aspect) on the right side of the body. Weighed skin temperature (T_{sk}) was then determined using Ramanathan (1964) equation.

4.3.3.4 Gastrointestinal Temperature (T_{gi})

Deep body temperature was measured via ingested telemetric pills (BodyCap, eCelsius performance, Caen, France) measuring gastrointestinal temperature. The pills were ingested 60 minutes before start of the experimental protocol, and participants avoided drinking water after ingestion.

4.3.3.5 Subjective Measures

Participants were requested to verbally provide their thermal sensation and thermal comfort every 5-min (ASHRAE, 2017). Temperature sensation is measured from -3 (cold) through to 3 (hot), and thermal comfort is measured from 0 (comfortable) through to 3 (very uncomfortable). Finally, for participant safety, motion sickness was recorded during centrifugation using a 6-point scale ranging from 0 (no symptoms) to 6 (vomiting).

4.3.4 Mechanistic Analyses

4.3.4.1 Heart Rate Variability (HRV)

Heart rate was measured with a 5-lead ECG, and the resultant R-R interval (RRi) was analysed via Kubios HRV Software (Version 3.5.0, Kubios Oy, Finland). Fast-Fourier transformation (FFT) frequency analysis of the RRi signal was conducted for each of the 10-minute stages during the protocol, using a 5-minute selection ending one minute before the end of each interval and a medium beat correction threshold. The contributions of the parasympathetic (PNS) and sympathetic nervous systems (SNS) as an index, alongside a so-called 'stress index' were provided for each of the 10-minute stages. In addition, the relative powers of each spectral frequency were calculated to represent the relative power of each component in proportion to the total power (Karim et al., 2011). Finally, the ratio of the absolute powers of the LF and HF bands (LF/HF ratio) were calculated, which is an important indicator of sympatho-vagal balance.

4.3.4.2 LDF Wavelet Transform Analysis (WTA)

WTA has been shown to provide information regarding the regulation of skin perfusion occurring via interactions of autonomic stimulation, endothelial control, and myogenic activities (Bagno & Martini, 2015; Stefanovska et al., 1999). Prior spectral investigations

of LDF signals using WTA have identified six frequency bands which relate to differing vascular control mechanisms: Band I (cardiac; 0.6 – 2.0 Hz), Band II (respiratory; 0.145 – 0.6 Hz), Band III (myogenic; 0.052 – 0.145 Hz), Band IV (Neurogenic; 0.021 – 0.045 Hz), Band V (endothelial nitric oxide (NO)-dependent; 0.0095 – 0.021 Hz), and Band VI (endothelial NO-independent; 0.005 – 0.0095 Hz). In the present study, WTA analysis was conducted at each of the 6 protocol stages, using a 2-minute stable window at minutes three to five, to avoid unstable signals as a result of pre-syncopal symptoms.

4.3.5 Statistical Analyses

Following the conversion of raw LDF data into %maxBF data (described above), these data were averaged to produce minute values throughout the full protocol; presented as mean ± SD. Cardiovascular data, measured beat-by-beat, was also averaged to produce minute averages. Finally, Tsk and Tgi was averaged to produce minute values throughout the protocol. All data is presented as mean ± SD. Multiple three-way repeated measures ANOVA compared the effect of three dependent variables – level of gravity (GRAV), Temperature (TEMP), and PO2 (OXYGEN) – on the independent variables (ArmBF, LegBF, HR, SV, CO, SVR, RPP, SYS, DIA, MAP, Tsk, Tgi). Data was analysed using IBM SPSS statistics (Version 26, IL, USA) and Graphpad (Graphpad Prism 9, Version 9.1.2, USA). An alpha value of $p < 0.05$ was considered to represent statistical significance.

4.4 Results

All participants completed the NG and 1GRF conditions in all four conditions. No participant experienced full syncope under any test condition, however, during 2GRF, several participants exhibited pre-syncopal symptoms resulting in premature termination of the trial under some of the environmental conditions. Time to cessation was 10.0 min for the Cool Normoxia (CN) trial, 9.1 ± 1.3 min for the Cool Hypoxia (CH), 8.8 ± 1.7 min for the Warm Normoxia (WN) trial, and 8.4 ± 2.0 min for the Warm Hypoxia (WH) trial. Therefore, no participant experienced early cessation of the trial in CN, however early cessation was experienced in all other trials.

Participants reported being more thermally comfortable in NG (0.5 ± 0.2), compared to 1GRF (0.7 ± 0.4) and 2GRF (1.0 ± 0.1) across all conditions. Ratings of perceived exertion also increased with increasing G-load from 6.4 ± 0.3 during the NG trial to 7.0 ± 0.3 in the 1GRF and 8.7 ± 0.3 in the 2GRF trials.

4.4.1 Microvascular Blood Flow

Figure 2 presents responses of microvascular blood flow in the different ambient conditions and gravitational loads. ArmBF was significantly reduced as a result of GRAV ($p < 0.001$, $F = 21.11$). Multiple comparisons analysis identified that the significant effect of GRAV was a result of the differences between NG (20.7 %max) and 1GRF (14.9 %max, $p < 0.001$), and between NG and 2GRF (13.8 %max, $p < 0.001$). The difference between 1GRF and 2GRF was negligible (1.1 %max, $p = 0.624$). Additionally, TEMP caused a significant increase in armBF, regardless of GRAV and OXYGEN; increasing from 12.25 %max in cool conditions to 20.26 %max in warm conditions ($p < 0.001$, $F = 23.47$).

LegBF, however, was only significantly affected by GRAV ($p < 0.001$, $F = 13.64$). Unlike armBF, the multiple comparisons analyses identified that the difference between NG and 1GRF was non-significant, whereas the 2GRF condition (27.4 %max) produced significantly higher legBF than both NG (16.8 %max, $p < 0.001$) and 1GRF (19.7 %max, $p < 0.001$).

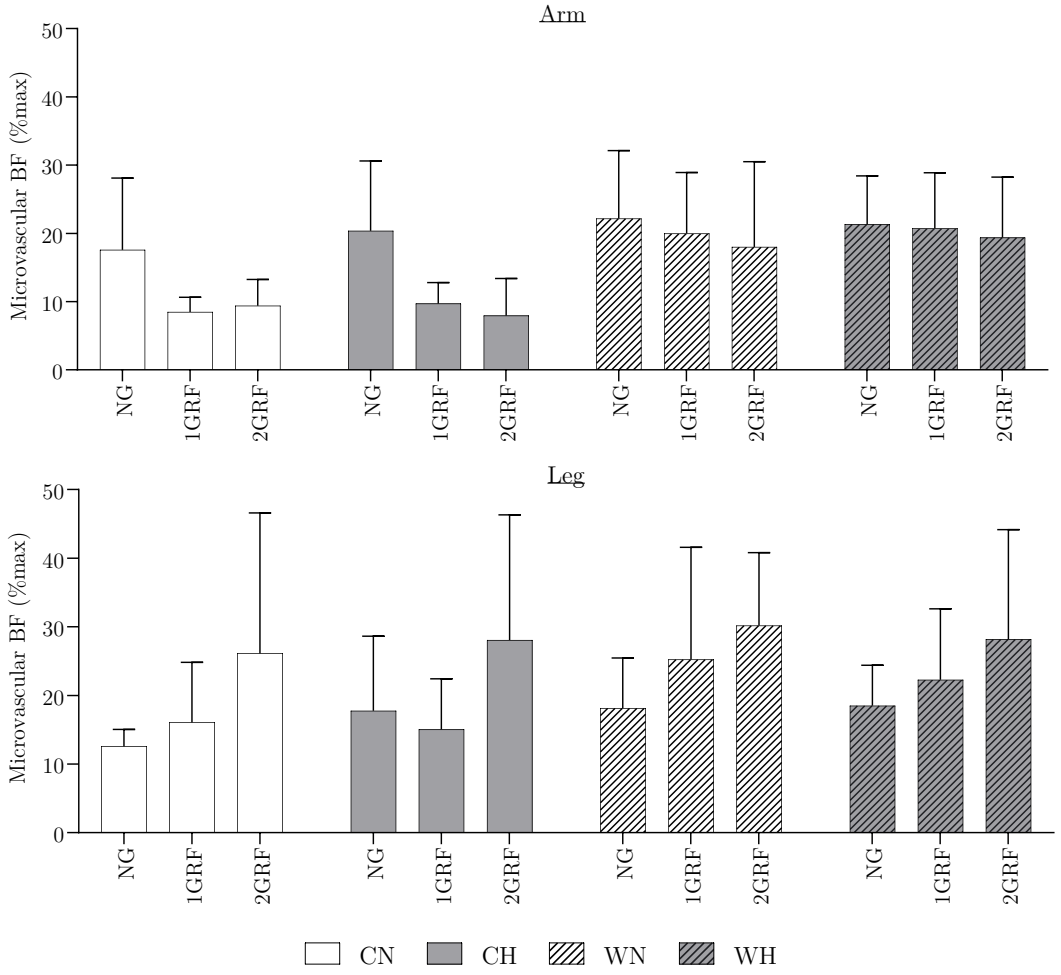

Figure 4.2: Mean ± SD microvascular blood flow in differing ambient conditions and gravitational loads. CN = cool normoxia, CH = cool hypoxia, WN = warm normoxia, WH = warm hypoxia.

4.4.2 Skin and Deep Body Temperature

Figure 3 presents mean ± SD skin temperature in all conditions. The NG condition produced the highest average T_{sk} (33.1 °C) compared to 1GRF and 2GRF, which were similar (1GRF = 31.7 °C, 2GRF = 31.1 °C; $p < 0.001$, F = 450.0). T_{sk} was considerably higher in the warm condition compared to the cool (cool = 29.9 °C, Warm = 34.1 °C; $p < 0.001$, F = 5481). Finally, the normoxic condition produced a higher T_{sk} than the hypoxic condition (Nor = 33.5 °C, Hyp = 32.1 °C; $p < 0.001$, F = 17.30). In addition, the interactions of TEMP+GRAV ($p < 0.001$, F = 133.0), and of TEMP+OXYGEN ($p = 0.047$, F = 4.029), on T_{sk} were significant. For the interaction of TEMP + GRAV, the drop in T_{sk} as a result of the GRAV was significantly larger in the cool conditions (-3.2 °C) than the warm conditions (- 0.9 °C) ($p < 0.001$). The difference between cool and warm conditions in hypoxia (4.4 °C) was significantly higher, than the difference in normoxic conditions (4.1 °C) ($p < 0.001$).

There was no difference in any of the measured deep body temperatures as a result of GRAV, TEMP, OR OXYGEN. The mean values were CN = 37.1 ± 0.2 °C, CH = 37.2 ± 0.2 °C, WN = 37.1 ± 0.2 °C, WH = 37.2 ± 0.2 °C.

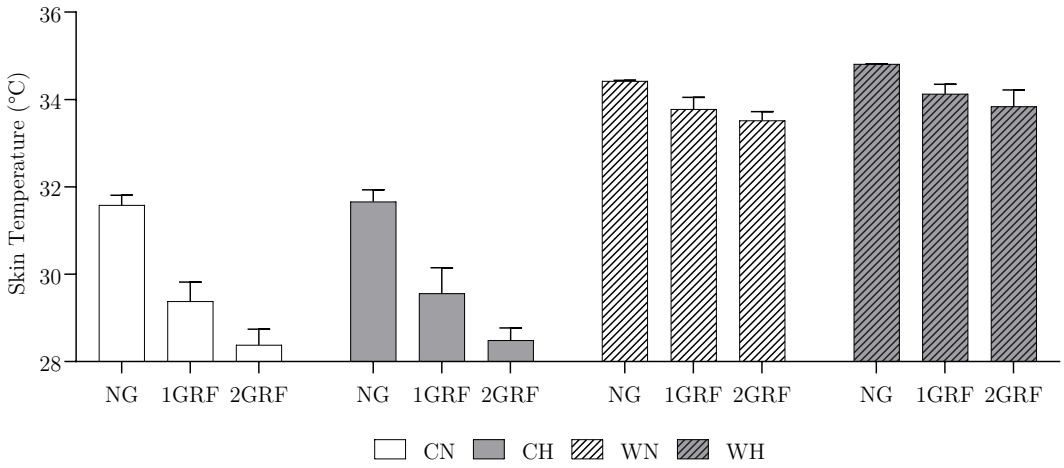

Figure 4.3: Mean ± SD skin temperatures under differing ambient conditions and gravitational loads. CN = cool normoxia, CH = cool hypoxia, WN = warm normoxia, WH = warm hypoxia.

4.4.3 Haemodynamics

Table 2 displays haemodynamic responses for each gravitational stimulus. Figure. 4 displays the same haemodynamic responses, but at each gravitational stimuli and in each of the ambient conditions.

HR significantly increased by ~25 min^{-1} from NG to 2GRF, as a result of GRAV ($p < 0.001$, F = 22.81). A significantly higher HR was observed in the warm condition (cool = 78.9 min^{-1}, warm = 88.4 min^{-1}; $p = 0.006$, F = 7.798), and in the hypoxic condition compared to normoxia (nor = 81.5 min^{-1}, hyp = 85.9 min^{-1}; $p = 0.02$, F = 5.613).

SV was significantly reduced by the increase in GRAV ($p < 0.001$, F = 11.91), with the multiple comparisons analysis identifying that SV was significantly lower in 2GRF compared to both NG (-15.4 mL, $p < 0.001$) and 1GRF (-14.8 mL, $p < 0.001$).

RPP significantly increased as a result of increases in GRAV ($p < 0.001$, F = 22.00), and by higher TEMP ($p = 0.021$, F = 5.458). Multiple comparisons analysis identified that RPP was significantly higher in 2GRF compared to both NG (+3459.0 mmHg $\cdot$ min^{-1}, $p < 0.001$) and 1GRF (+3519.0 mmHg $\cdot$ min^{-1}, $p < 0.001$). Additionally, the cool conditions (12426.3 mmHg $\cdot$ min^{-1}) produced higher RPP values than the warm conditions (11267.7 mmHg $\cdot$ min^{-1}).

SVR was significantly higher at greater GRAV ($p = 0.003$, F = 6.324), and lowered by the increase in TEMP (cool = 1.4 mmHg $\cdot$ min $\cdot$ mL^{-1}, warm = 1.1 mmHg $\cdot$ min $\cdot$ mL^{-1}; $p = 0.001$, F = 11.44). Multiple comparisons highlighted the difference between NG vs. 2GRF (+0.3 mmHg $\cdot$ min $\cdot$ mL^{-1}, p= 0.002) as a significant contributor to this difference.

SBP was lowest in NG (129.9 mmHg) compared to both 1GRF (+11.0 mmHg) and 2GRF (+16.4 mmHg) (p <0.001, F = 13.07). Additionally, the warm condition caused a significantly lowered SBP compared to the cool (cool = 144.2 mmHg, warm = 137.1 mmHg; p <0.001, F = 11.20).

DBP was also significantly elevated by GRAV ($p < 0.001$, F = 20.92). Multiple comparison analysis identified elevated DAP in the 2GRF condition (107.1 ± 14.9 mmHg), which displayed significantly higher values than either NG (-22.7 mmHg, $p < 0.001$) or 1GRF (-15.9 mmHg, $p < 0.001$).

SpO_2 was significantly higher in the 2GRF condition (91. 8 ± 6.5 %) compared to either NG (90.9 ± 5.2 %) or 1GRF (90.9 ± 6.2%) ($p = 0.022$, F = 3.970). The higher ambient TEMP caused a significantly lower SpO_2 (cool = 91.8 ± 1.0 %, warm = 90.5 ± 0.6; $p < 0.001$, F = 12.16). S_pO_2 in the normoxic conditions was 96.4 ± 1.1 % and in the hypoxic conditions, 86.1 ± 1.3 % ($p < 0.001$, F = 659.2).

Table 4.2: Mean ± SD haemodynamic values at each ambient condition and gravitational stimulus.

Haemodynamics	Sig.	Condition	NG	1GRF	2GRF
Heart Rate *(min^{-1})*	a,b,c	CN	73.1 ± 11.7	66.5 ± 12.0	84.8 ± 14.6
		CH	79.2 ± 8.2	74.3 ± 12.0	95.5 ± 14.7
		WN	80.7 ± 7.2	76.5 ± 7.1	107.1 ± 21.0
		WH	87.9 ± 10.8	80.6 ± 11.2	97.5 ± 22.6
Stroke Volume *(mL)*	a	CN	76.1 ± 11.3	83.6 ± 16.1	57.9 ± 15.9
		CH	77.2 ± 21	73.7 ± 16.9	61.1 ± 17.9
		WN	73.0 ± 10.4	71.6 ± 11.9	60.8 ± 13.1
		WH	71.5 ± 11	66.4 ± 10.8	61.7 ± 18.4
Cardiac Output *($L{\cdot}min^{-1}$)*		CN	5.5 ± 1.2	5.6 ± 1.6	5.1 ± 1.8
		CH	6.0 ± 1.4	5.6 ± 1.5	5.7 ± 1.9
		WN	5.8 ± 0.8	5.4 ± 0.8	6.5 ± 1.8
		WH	6.1 ± 0.9	5.3 ± 0.8	6.3 ± 1.7
Rate Pressure Product *($mmHg \cdot min^{-1}$)*	a,b	CN	10452.6 ± 1803.7	10382.6 ± 1557.7	15031.2 ± 2450.5
		CH	11613.3 ± 1959.3	12127.6 ± 2801.9	15871.6 ± 4289.4
		WN	10181.6 ± 960.2	9840.0 ± 1316.5	16160.3 ± 3572.9
		WH	10608.1 ± 2307.2	10266.6 ± 1233.6	14551.6 ± 3215.7
Systemic Vascular Resistance *($mmHg \cdot min \cdot mL^{-1}$)*	a,b	CN	1.1 ± 0.2	1.3 ± 0.4	1.7 ± 0.5
		CH	1.1 ± 0.3	1.3 ± 0.3	1.4 ± 0.6
		WN	1.0 ± 0.2	1.1 ± 0.3	1.1 ± 0.3
		WH	0.9 ± 0.2	1.1 ± 0.3	1.1 ± 0.4
Systolic Pressure *(mmHg)*	a,b	CN	132.9 ± 11.1	145.8 ± 11.8	161.3 ± 11.2
		CH	126.7 ± 8.4	148.6 ± 13	149.8 ± 16.1
		WN	131.3 ± 10.8	133.5 ± 14.9	150.2 ± 17.7
		WH	128.6 ± 17.3	135.7 ± 14.9	143.0 ± 15.0
Diastolic Pressure *(mmHg)*	a	CN	85.1 ± 10.2	92.6 ± 9.8	115.4 ± 10.7
		CH	80.6 ± 9.6	91.7 ± 11.9	102 ± 14.5
		WN	85.2 ± 10.2	87.7 ± 13.3	106.4 ± 15.5
		WH	86.7 ± 16.8	92.8 ± 13.1	103.5 ± 15.5
Mean Arterial Pressure *(mmHg)*	a	CN	103.8 ± 13.9	115.0 ± 15.0	133.7 ± 14.1
		CH	97.7 ± 9.1	112.8 ± 25.7	123.1 ± 21.0
		WN	103.0 ± 12.6	105.7 ± 17.3	123.3 ± 21.4
		WH	102.7 ± 18.8	109.5 ± 19.2	118.6 ± 16.1
Oxygen Saturation (%)	a,b,c	CN	95.9 ± 1.5	97.3 ± 1.1	98.1 ± 1.5
		CH	86.5 ± 1.8	85.4 ± 3.4	87.2 ± 4.9
		WN	95.5 ± 1.2	95.9 ± 1.0	96.3 ± 1.4
		WH	85.2 ± 2.2	84.9 ± 2.3	84.8 ± 3.3

CN = cool normoxia, CH = cool hypoxia, WN = warm normoxia, WH = warm hypoxia, a = significant main effect of gravity, b = significant main effect of temperature, c = significant main effect of oxygen, $p < 0.05$.

4.4.4 Mechanistic Analyses

4.4.4.1 Heart Rate Variability –

Figure 4 displays the HRV values averaged from each GRAV condition, however, analyses assessing the effects of TEMP and OXYGEN were also conducted. The single main effect of GRAV caused significant differences between conditions in PNS index ($p < 0.001$, F = 23.02), SNS index ($p < 0.001$, F = 14.49), and RRi ($p < 0.001$, F = 49.24). In all of these variables, the 1GRF condition exhibited the smallest deviation from SUP1 (i.e., rest period), however these differences were still significant in both PNS index (difference = 0.9 a.u.; $p < 0.001$) and RRi (difference = 109 ms; $p < 0.001$). 2GRF resulted in the greatest sympathetic autonomic strain and was thus significantly higher in SNS index (NG = 1.3, 1GRF = 0.8, 2GRF = 3.6; $p < 0.001$) and significantly lower in PNS index (NG = -1.2, 1GRF = -0.7, 2GRF = -2.1; $p < 0.001$) and RRi (NG = 765.8 ms, 1GRF = 818.3 ms, 2GRF = 628.5 ms; $p < 0.001$), in both other GRAV conditions.

The TEMP condition as a single main effect caused PNS index (cool = 1.0 ± 0.8, warm = -1.7 ± 0.7, $p < 0.001$, F = 14.49), RRi (cool = 777.9 ± 110.9 ms, warm = 700.5 ± 97.2 ms, $p < 0.001$, F = 14.49) and relative HF (cool = 1.0 ± 0.8, warm = -1.7 ± 0.7, $p < 0.001$, F = 14.49) to be significantly lower in the warm condition compared to cool. In contrast, SNS index (cool = 1.0 ± 0.8, warm = 1.7 ± 0.7, $p < 0.001$, F = 14.49) was significantly higher in warm ambient conditions, regardless of GRAV condition.

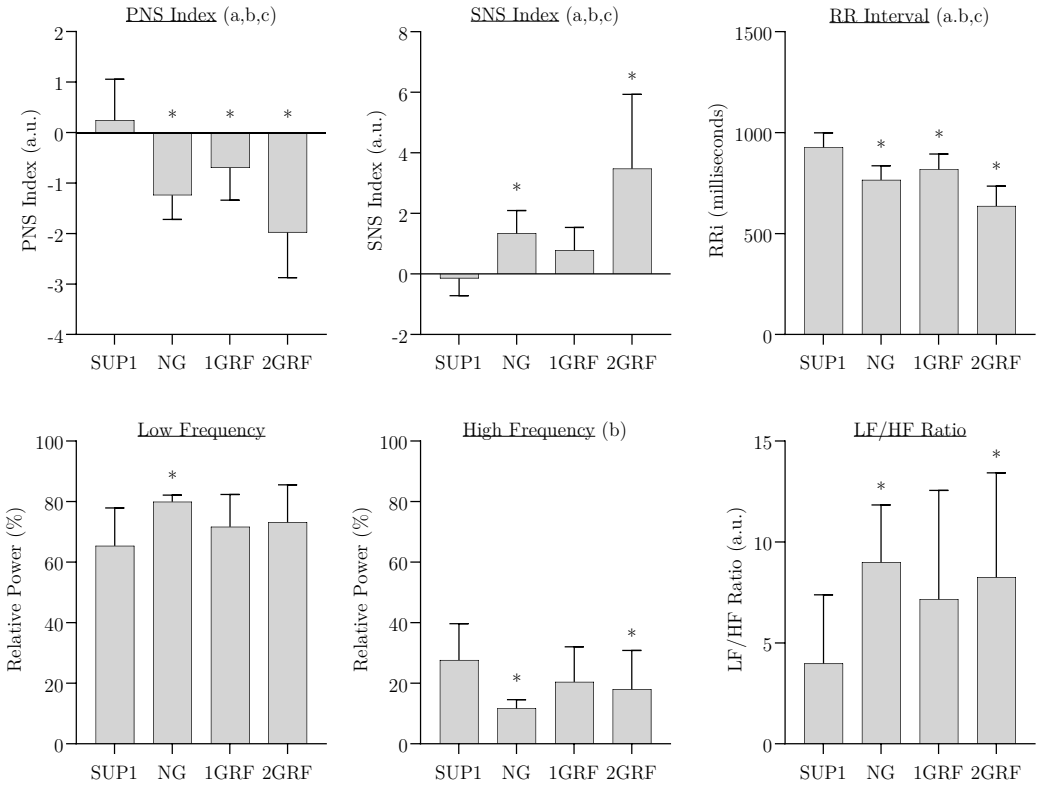

Figure 4.4: Heart rate variability indices as mean of all four conditions. PNS = parasympathetic nervous system, SNS = sympathetic nervous system, LF = low frequency, HF = high frequency, a = significant effect of gravity, b = significant effect of temperature, c = significant effect of oxygen, * = significantly differently to SUP1, p < 0.05.

Similarly to TEMP, OXYGEN also elicited higher sympathetic activity when under hypoxic conditions. There were significant decreases in PNS index (normoxia = 1.1 ± 0.6, hypoxia = 1.6 ± 1.5, $p < 0.001$, $F = 14.49$), and RRi (normoxia = 781.0 ± 89.4 ms, hypoxia = 697.4 ± 102.3, $p < 0.001$, $F = 14.49$); whilst SNS significantly increased (normoxia = 1.4 ± 0.7, hypoxia = 2.0 ± 1.2, $p < 0.001$, $F = 14.49$).

4.4.4.2 Wavelet Transform Analysis –

Initial analyses assessing the effects of ambient conditions (i.e., main effects of TEMP and OXYGEN) identified no differences in WTA amplitude at any frequency band or GRAV condition; therefore, further analyses only assessed differences between the mean amplitudes of all ambient conditions at each GRAV load (Fig. 5). These analyses identified significant effects of both frequency band ($p < 0.001$, $F = 70.31$) and GRAV ($p = 0.32$, $F = 3.673$), however, there was no interaction effect of these variables.

Follow-up multiple comparisons analyses were conducted, and three main observations were made. Firstly, frequency bands 4, 5, and 6 representing neurogenic, endothelial (NO-dependent and NO-independent) and vascular control were all significantly higher than bands 1, 2, and 3 (all: $p < 0.001$).

In the arm these frequency bands did not display amplitudes that were different from each other. However, in the legs, the endothelial (NO-dependent) frequency band was shown to be significantly higher than any other variable in each of the GRAV conditions ($p < 0.001$). Secondly, the frequency band representing respiratory control (band 2) displayed significantly lower amplitude (and therefore vascular control) than other frequency bands in both the arm and leg ($p < 0.001$). Finally, the cardiac and myogenic frequency bands (bands 1 and 3) displayed similar amplitudes and were therefore not statistically different ($p > 0.05$), in both the arm and leg regions; however, the amplitude of these bands was still significantly lower than bands 4, 5, and 6.

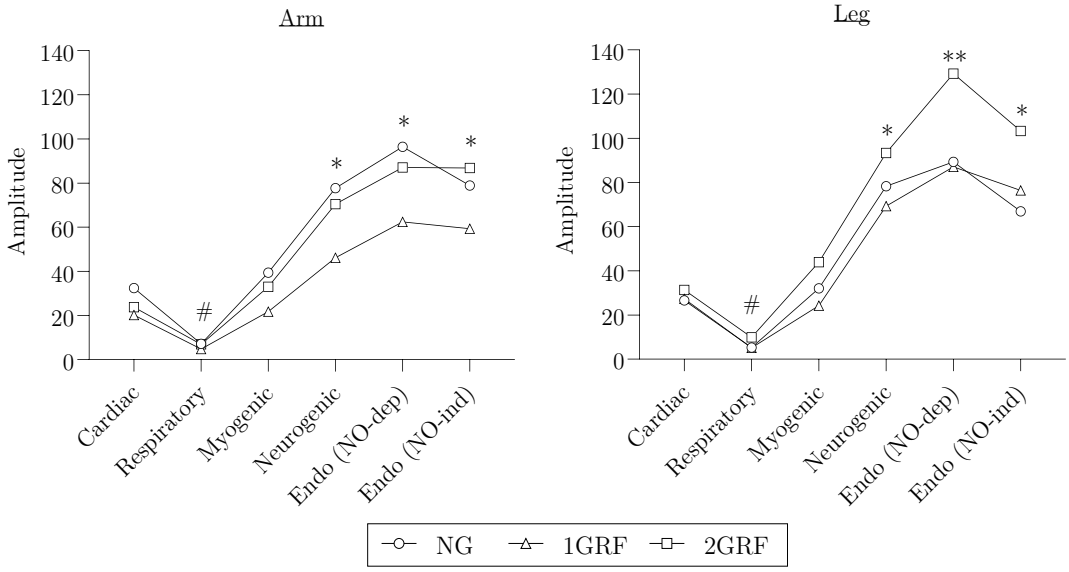

Figure 4.5: Wavelet Transform analyses averaged across all TEMP and GRAV conditions. * = frequency band value significantly higher than first three bands in all GRAV conditions, ** = frequency band value significantly higher than all other bands in all GRAV conditions, # = frequency band significantly lower than all other bands in all GRAV conditions.

4.5 Discussion

The main findings of the current study are that MBF in the leg (i.e., legBF) was unaffected by the varying ambient conditions, and only significantly responded to the change in GRAV. However, no difference was observed between NG and 1GRF, whilst it was clear that 2GRF centrifugation in the head-to-foot direction was sufficient to overcome the baroreflex and allow pooling of blood in the lower limbs. ArmBF, however, responded significantly to both the thermal and gravitational stressors. This is in line with previous observations (Fisher et al., 2024) purporting that the arms respond to the interaction of multiple regulatory vascular mechanisms while the legs do not. Despite the aforementioned vascular mechanisms, the effects of both ambient stressors (TEMP and OXYGEN) caused a 16% reduction in the time to test cessation from CN to WH conditions, based on indicators of pre-syncope, during the 2GRF condition. This is likely due, in part, to the significant pooling of blood observed in the legBF in the 2GRF condition. Interestingly, no effect of hypoxia was observed in either armBF nor legBF, but there was an increase in HR and an expected decrease in SpO_2; yet a significant decrease in the time to test cessation due to hypoxia occurred.

4.5.1 Orthostatic Tolerance (OT)

Time-to-test cessation at 2GRF represents the influence that ambient conditions place on the cardiovascular system's ability to continuously supply oxygenated blood to the brain and other vital organs; supported by the sudden drop in HR and MBP used as a primary indicator of syncope. Both TEMP and OXYGEN caused separate and combined reductions in OT, leading to frequent early cessation of the 2GRF phase due to pre-syncopal symptoms. In fact, all participants were able to complete the full 2GRF phase in the CN condition, whereas the overall decrease as a result of the high ambient temperatures and hypoxia was 9.5 % and 7.4 %, respectively. These results would indicate that cool temperatures are the most suitable operating temperatures for passive centrifugation. With the potential for exercise during centrifugation on a SAHC (Diaz et al., 2015; Duda et al., 2012; Katayama et al., 2004; Kramer, Kümmel, et al., 2020). Considerations should be made to define the most suitable operating temperature for different exercise types (i.e., aerobic, resistance, etc.,). Therefore, further research must extend the scope of SAHC utilisation beyond simple exercise modalities and consider the effect of ambient conditions and their subsequent role in exercise performance; similar to terrestrial research yet considering the unique aspects of a SAHC. The negative effects of temperature on OT are well documented (Schlader et al., 2016), by nature of individual and interactive mechanisms; including central cardiac responses, arterial and venous changes, redistribution of blood, and autonomic responses. In contrast, the literature regarding the effects of acute hypoxia on OT is sparse (Blaber et al., 2003; Halliwill & Minson, 2005) but alludes to altered autonomic activity, and vasodilatation of splanchnic circulation as triggers for pre-syncope. However, it is known that in response to an acute hypoxic stimulus, different regional vascular responses are initiated. There is vasodilation of peripheral non-acral (Minson, 2003) and splanchnic (Halliwill & Minson, 2005; Rowell & Blackmon, 1986) vasculature, concomitant with vasoconstriction of acral (Jones et al., 2021) and pulmonary (Weir & Archer, 1995) vasculature. Further, Ainslie et al., (2007) reported that during exposure to normobaric hypoxia equivalent to an air altitude of 4500m, vasodilation of cerebral arteries through cerebral autoregulation allows stable oxygenated blood supply to the brain. However, the frequent occurrence of pre-syncopal symptoms in the current study suggest the observations of Ainslie et al. (2007) at 4500m do not apply to the current study; if, in fact, the causes of pre-syncope is a result of unstable

oxygen delivery to the brain caused by competition for blood flow pooling in the lower extremities. The combination of these aforementioned factors, when combined with hypergravity causing lower limb blood pooling, would indicate reduced blood supply to the upper regions of the body and particularly the brain.

4.5.2 Microvascular Blood Flow Response

The orthostatic intolerance described in the previous section is the result of varied and profound cardiovascular responses to the temperature, gravity, and hypoxic stimuli. ArmBF was significantly affected by both the ambient temperature and head-to-foot gravitational force. As mentioned, these results match previous observations whereby the arm vasculature is controlled by interactions of multiple vasomotor mechanisms when under thermal and gravitational stressors. Interestingly, additional analyses identified that AG (i.e., 1GRF and 2GRF) produced significantly lowered armBF than in the NG condition, primarily in the cool conditions. . Conversely, there was an attenuated drop in armBF during the warm conditions, which may have contributed to the higher incidence in pro-syncopal symptoms. Increasing blood pooling in the skin vascular bed, combined with relaxation of cutaneous veins may be responsible for a drop in MAP of 5 – 10 mmHg (Crandall et al., 1999); in addition to the gravitational challenge this would certainly impact orthostatic tolerance. It is also possible that this response is due to the additional convective heat loss mechanism which would be more prominent during centrifugation. During the 2GRF trial, participants are spinning at an equivalent speed of 27.7 kilometres/hour, which induces a wind speed of 1.4 $m \cdot s^{-1}$ of the head and 4.2 $m \cdot s^{-1}$ at the foot. A convective mechanism of this magnitude will certainly induce reduced T_{sk} and consequently initiate vasoconstriction, which will reduce SkBF, when compared to standing in still air alone. LegBF, however, was only significantly affected at 2GRF. Also, in contrast to the armBF, similarities were observed between NG and 1GRF exposures, whereas the 2GRF condition produced the highest legBF (~7 – 10 %max higher). These results match those of Verma et al. (2018) and Goswami, Bruner, et al. (2015) who identified similarities in cardiovascular responses to 1 Gz (heart level) centrifugation and standing. Goswami, Bruner, et al. (2015) also identified increases in calf venous volume, particularly during 2 Gz at the foot level. During hypergravity exposure, lower body microvasculature experiences high transmural pressures from both the arterial and venous branches (Habazettl et al., 2016), which is largely mediated by autonomic control and myogenic vasoconstrictor activities. Beyond pressure limits of myogenic perfusion control at ~150 mmHg (Bullivant, 1978; Strandgaard et al., 1974), expected during centrifugation, resistance vessels may fail to overcome the transmural pressure leading to excess lower limb pooling of blood; as much as 700 - 800 mL (Bradley & Davis, 2003; Lipsitz, 1989). Interestingly, in contrast to Habazettl et al. (2016) who noted that vascular resistance was maintained even at 2 Gz (foot level), in the present study there was a significantly higher microvascular flow at 2GRF vs other conditions; these disparities may be due to the length of exposure between the two studies (4-min vs. 10-min). In contrast to the findings of the present study, Watenpaugh et al. (2004) noted altered responses of thigh and calf skin blood flow measured by LDF, proposing that decreases in blood flow occurred during transient changes in centrifugation up to 1 Gz at the heart level. It is possible that the differences in length (~40s) and magnitude (1.0 Gz foot level or 0.4 Gz heart level) of centrifugation by Watenpaugh et al. (2004) were sufficient to produce significantly different observations from the present study. As previously mentioned, there was no significant response of either armBF or legBF to the hypoxic condition, which is perhaps unusual as non-acral skin would be expected to observe an element of vasodilation (Minson, 2003).

4.5.3 Haemodynamic Response

During standing and 1GRF trials, haemodynamic responses did not significantly differ from each other. It is therefore likely that the 1GRF stimulus is suitable at recreating the hydrostatic and cardiovascular strain associated with standing. These results clearly match those of previous research, in relation to autonomic and blood pressure indices (Verma et al., 2018), and cardiovascular and cerebral responses (Goswami, Bruner, et al., 2015; Laing et al., 2020). This production of an equivalent AG stimulus to standing further promotes the use of centrifugation, at lower Gz, for the maintenance of cardiovascular health in situations where orthostatic stress cannot be regularly provided, such as the micro-gravity experienced during spaceflight. The 2GRF condition, however, produced a gravitational stimulus that, in certain conditions and for some participants, was intolerable for the full duration of the protocol phase (i.e., 10-minutes). In fact, across all ambient conditions the average tolerance was 91% of the full 10-min session (i.e., 9.1 $\pm$ 1.3 minute). The haemodynamic responses reveal that until the point at which pre-syncope or the end of the test was reached, participants exhibited higher HR, BP, and RPP, concomitant with a lower SV. Even at 2GRF, these values would somewhat match the type of response expected during low intensity exercise (Laughlin, 1999), except for the decrease in SV, despite the passive nature of centrifugation in the present experiment. In normal low-intensity exercise, SV would be expected to increase slightly rather than decrease, to compensate for the required increase in CO. The supine position would have limited diastolic filling (Warburton & Gledhill, 2008), despite the centrifugal stimulus. Due to the passive nature of the protocol used in the present study, there was no additional venous return stimulated via exercise; capable of increasing venous return from the lower limbs via compression of veins during skeletal muscle contraction, sympathetic stimulation of vein walls, and skeletal muscle arteriole dilation (Berlin & Bakker, 2014; Miller et al., 2005). Interestingly, while SpO_2 was predominantly influenced by the hypoxic environment (82 % of ANOVA variation), it was also significantly affected by TEMP and GRAV. SpO_2 appeared to be ~1 % higher in warm conditions, and in 2GRF, vs other conditions. Under these conditions the body is under greater physiological strain, and therefore produces a subsequent cardio-respiratory response; thereby improving oxygen delivery and subsequently SpO_2.

The effects of heat stress via passive heating alone on haemodynamic responses are well known, including elevated skin and core temperatures, elevations in SkBF, increased HR and CO, and little to minimal change in MBP (Crandall & Gonzalez-Alonso, 2010). Besides the additional artificial gravitational stress, the current study results are in line with the literature whereby the ambient temperature had a significant effect on a number of variables; in particular, increases in HR, RPP, SVR, and SYS.

4.5.4 Central and Peripheral Autonomic Control

Measures of central autonomic function were achieved via HRV frequency-based analyses for detection of para/sympathetic regulation. As expected, the gravitational stressor produced large deviations from a supine baseline value (i.e., SUP1 condition) in HRV variables of SNS index, PNS index, and RRi; though not in high or low frequency power bands. In agreement with the haemodynamic responses, NG and 1GRF produced similar responses in these variables (PNS, SNS, RRi), though 1GRF produced the lowest autonomic activity; in fact, SNS index during 1GRF was not significantly higher than the supine rest period. These similarities have been previously noted, yet increases in low/high frequency power distributions at 2 Gz at the foot in Verma et al. (2018) do not match those of the present study. The 2GRF centrifugation drew significantly lower PNS index

and RRi, whilst SNS was significantly higher, which reveals the level of sympathetic autonomic response required to tolerate double Earth's gravity for a sustained period. Indeed, while gravitational stress had a considerable effect on autonomic control, both ambient temperature and oxygen availability significantly impacted PNS index, SNS index, and RRi also. The interrelation of 2GRF, warm temperature, and hypoxia produced the greatest sympathetic drive, which was to be expected considering the singular effect each variable has on autonomic activity (Buchheit et al., 2004; Sollers et al., 2002; Stauss, 2003).

Whereas central autonomic mechanisms were significantly affected by the main independent variables of ambient temperature and oxygen concentration; these had no effect on peripheral mechanisms as indicated by spectral analyses of LDF signals using WTA. In fact, only the gravitational stimulus produced variation in the WTA results, as will be discussed with reference to each limb separately in at each gravitational load (i.e., NG, 1GRF, 2GRF). The main finding was significantly higher amplitudes identified in bands 4, 5, and 6 in both the arm and leg; representing neurogenic, endothelial (NO-dependent), and endothelial (NO-independent) (Bagno & Martini, 2015), respectively. Neurogenic activity represents the local influence of sympathetic control on the microvasculature. As described previously, the separate and combined effects of each independent variable in the present study caused increased central sympathetic drive, which is subsequently identified in the efferent pathways. Utilisation of endothelium-dependent and -independent vasodilators (acetylcholine and sodium nitroprusside, respectively) reveals the contribution of NO and prostaglandin E synthesis in band 5, whilst band 6 may be associated with other endothelial mechanisms such as endothelial derived hyperpolarisation factor (Kvandal et al., 2006). Each of these mechanisms are responsible for potent vasodilation, however the lack of a severe drop in MAP and syncope at the haemodynamic and WTA timepoint suggests this level of vasodilation is unlikely. It may therefore be possible that instead of detection of endothelial vasodilative mechanisms, the analysis may have detected the pooling of blood in peripheral regions due to excess transmural pressure. In addition, the leg in 2GRF produced an amplitude in band 5 (endothelial NO-dependent) that was higher than any other band and any other condition, further exhibiting the potential error. To the authors' knowledge, this is the first study which assesses WTA in individuals undertaking hypergravity procedures; more research is required to conclusively understand the potential error observed. It was also interesting that band 2 (respiratory) produced a negligible response in any condition, which may be due to the passive nature of the centrifugation; indeed, had aerobic or resistance exercise been conducted this may be different.

4.5.5 Limitations and Future Perspectives

Whilst the comparison between warm and cool ambient conditions provides a suitable understanding of the upper and lower limits of thermoregulation, it may have been beneficial to compare these responses by means of a variation from a normothermic control. Studies have assessed the cardiovascular responses to SAHC, in which a normothermic environment of 22 – 23 °C was reported (Goswami, Bruner, et al., 2015; Watenpaugh et al., 2004). The aim of the present study was not to repeat these data but build on the dataset by utilising adverse ambient conditions to elicit differing stress on the human body to further tease out the underlying mechanisms of thermoregulation and blood pressure control of the limbs.

Monitoring of microvascular circulation via LDF suitably presents responses of vasomotor mechanisms to alter peripheral vasculature. In addition, the transmural pressures generated by arterial and venous branches on capillaries during hypergravity may further indicate the stress elicited on the whole vascular system; rather than

microvasculature alone. However, to further understand the holistic influence of a SAHC on blood flow and haemodynamic responses, an assessment of whole-body fluid shifts would be beneficial. Studies assessing the effects of SAHC on fluid shift within the body have been previously conducted. Takhtobina et al. (2020) detected changes in impedance throughout the body, with an overall redistribution of blood towards the foot, citing it as an effective predictor of circulation changes. Both Vaitl et al. (2002) and Howarth et al. (2008) noted significant decreases in thoracic fluid volumes as a result of centrifugation; stating this would impact the perception of posture in the absence of vestibular influences, and detect venous return therefore predicting presyncope, respectively. It is clear that segmental impedance would be a useful tool in cardiovascular monitoring, and presyncope avoidance for SAHC operation.

Muscle activation during higher levels of AG may have altered the sympathetic response to ambient conditions; making the 2GRF condition, particularly, more tolerable by aiding the return of blood through muscle pump actions. Indeed, the AGBRESA study, which utilised continuous or intermittent 1 Gz centre of mass centrifugation for 30 mins/day over 60 days, found large individual variability in muscle activation ranging from 1 to 96 % of maximal voluntary contraction in leg muscles (Kramer, Venegas-Carro, et al., 2020). Therefore comparing AG to other forms of orthostatic strain (head-up tilt, lower-body negative pressure) would be difficult due their more passive nature. In the present study, however, cardiovascular responses were only compared within participant in different AG and ambient conditions; decreasing the impact of individual variability of muscle activation.

Finally, a consideration should be made for use of a SAHC vs a long arm human centrifuge (LAHC). In the present study, a SAHC was deemed more appropriate for two reasons. Firstly, the SAHC produces an acceleration gradient that acts in a head-to-foot direction, producing a leg-ward shift in fluid similar to that of lower-body negative pressure. Therefore the non-uniform acceleration of the SAHC provides a greater baroreflex stimulus for both high and low pressure baroreceptors located within the carotid sinus/aortic arch and the atria/pulmonary veins, respectively. As the aim of the study was to assess the interaction of baroreflex and thermoregulatory mechanisms in varying ambient conditions, this baroreflex activation was more appropriate. Secondly, while the primary aim of the study was to assess the mechanisms associated with cardiovascular control, a secondary aim was the assessment of haemodynamic responses to centrifugation via a SAHC due to its potential role as a spaceflight countermeasure.

4.6 Conclusion

To conclude, the present study demonstrates that human tolerance during passive centrifugation to 2GRF is significantly affected by the ambient conditions. Haemodynamic and lower limb blood flow responses, particularly in the higher temperature and Gz conditions, revealed a clear cardiovascular challenge which commonly led to pre-syncopal symptoms. Meanwhile, cool temperatures appear to produce tolerable conditions in which participants may benefit from longer passive or active SAHC sessions if conducted regularly.

Chapter 5

Implications of Sex Differences in Orthostatic Tolerance During Exposure to Acute Artificial Gravity

5.1 Foreword

Chapter 4 employed the use of three independent stressors (artificial gravity, temperature, partial pressure of oxygen) and assessed their impact on regional blood flow regulation and haemodynamics, in males only. It was therefore essential that the differences between sexes were also established, within the framework of the same study design and protocol as described in chapter four. During pilot testing for this study, it was discovered that technical limitations of the health monitoring equipment meant that unstable signals were produced during cool (18.4 ± 0.8 °C) sessions. Due to the frequent development of pre-syncopal symptoms during centrifugation, it was not possible to operate these sessions with safe practise so only two sessions were used with a hot T_A (29.1 ± 0.8 °C) and either normoxia or hypoxia. Thus, this chapter will discuss the possible differences in regional blood flow and haemodynamics between males and females in response to AG and differing partial pressures of oxygen whilst experiencing high T_A; and the implications for orthostatic intolerance.

The findings of study have been submitted to the European Journal of Applied Physiology under the following citation: Fisher, J.T., Ciuha, U., & Mekjavic, I.B. (Submitted) Implications of Sex Differences in Orthostatic Tolerance During Exposure to Acute Artificial Gravity. *European Journal of Applied Physiology.*

5.2 Introduction

As a consequence of the endeavours of Space Life Sciences, we have established a permanent presence in Earth's lower orbit with humans continuously inhabiting the International Space Station (ISS) in this century. Another milestone scheduled for this century is the interplanetary mission to Mars and potentially establishing a permanent presence on the Moon. This development has heightened the interest in the hazardous effects of prolonged exposure to weightlessness, as would be experienced by astronauts during such deep space missions. More importantly there is an urgent need to develop efficient countermeasures to minimise, or possibly eliminate the well-known adaptation of physiological systems to microgravity, including fluid changes, muscle atrophy and force reduction, hormonal changes, bone demineralization, cardiovascular deconditioning, and autonomic deconditioning, to name but a few (Coupe et al., 2009; Hughson, 2009; Navasiolava et al., 2020). One of the key indicators of cardiovascular deconditioning upon return to earth is the orthostatic tolerance (OT) test, which assesses an individual's ability to withstand the influence of gravity on their body and avoid syncope. Previous research has identified that differences in OT exist between females and males, both in terrestrial settings (Cheng et al., 2011; Fu et al., 2004) and upon astronaut's return-to-earth (Meck et al., 2001; Waters et al., 2002). On earth, pre-syncope during tilt-tests occurs in 69 % of females and 31 % of males (Diaz-Canestro et al., 2022), and the time to pre-syncope is reduced by ~23 %. Upon return from space it was found that 100% of females and 20% of males experienced pre-syncope during a 10-minute OT test after 5-16 days in space (Waters et al., 2002). Additionally, the duration of spaceflight appears to be correlated with a reduction in OT, with 25% of astronauts experiencing pre-syncope after short flights (8-16 days) and 83% after long flights (129-190 days); though no sex comparisons were reported (Meck et al., 2001). Possible reasons for these differences include anatomical and anthropometric factors, differences in sympathetic activity and vascular responses, hormonal autonomic control, and haemodynamic differences (Cheng et al., 2011; Fu et al., 2004; Platts et al., 2014). Therefore, without appropriate countermeasures the return to Earth will have serious health consequences for astronauts, but considerations should be made for the differences in response to these countermeasures between sexes.

One such countermeasure is exposing astronauts to periods of artificial gravity (AG) equivalent to at least that of Earth within space vehicles and future habitats. This can be achieved with a short-arm human centrifuge (SAHC). Several studies have assessed the acute cardiovascular responses to centrifugation on a SAHC, each presenting differing gravitational loading and exposure times; yet they each observe similar responses (Goswami, Bruner, et al., 2015; Habazettl et al., 2016; Laing et al., 2020; Verma et al., 2018; Watenpaugh et al., 2004). These studies, despite recruiting female participants, contain no analyses of sex differences. Watenpaugh et al. (2004) reported 4/7 females were disqualified due to low tolerance, and Goswami, Bruner, et al. (2015) reported unacceptable blood pressure and cerebral blood flow values leading to exclusion of 4/10 males and 6/10 females. To our knowledge, four studies have assessed sex differences in the cardiovascular response to passive centrifugation on a SAHC; however, one used an onboard cycle ergometer to power the centrifuge at low exercise intensities (Kourtidou-Papadeli et al., 2021), and another reported no other cardiovascular response besides protocol completion (Fong et al., 2007). During a graded Gz profile (1/2/1 Gz) using 4-minute stages, cardiovascular parameters were recorded in 12 males and 16 females (Masatli et al., 2018). Five females and zero males experienced an orthostatic event, and a significant increase in heart rate and blood pressure in both sexes; however, they concluded that the SAHC stimulated cardiovascular systems to a greater extend in females. Kourtidou-Papadeli et

al. (2021) did not utilise pure passive centrifugation, however they observed significantly higher cardiac output, cardiac power, and mean arterial pressure in males vs female participants; during 5-minute increments in Gz up to 2.0 Gz. One study assessing the effects of 90-minutes passive centrifugation below a predetermined presyncopal limit, in hypovolemic males and females, has been reported in reference to cardiovascular responses (Evans et al., 2018) and autonomic indices (Zhang et al., 2017). These studies found that single bouts of AG improved OT in both males (30%) and females (22%), and that females had overall higher heart rate, and lower stroke volume and arterial pressures than males. In addition, the single bout of AG improved male sympathetic response to orthostatic stress, but not in females. Finally, Fong et al. (2007) conducted a 60-minute AG test with 1 Gz at the heart level, in six males and 5 females. It was found that five males and one female were able to complete the session successfully, yet no reasoning was provided for these differences.

One additional consideration which need to be addressed in future space missions is the possibility of hypoxic exposure. As of September 2021, a total of 244 Extra Vehicular Activities (EVA) have been conducted over the past 20 years, indicating infrequency of ISS missions. As a consequence of the pressure gradient between the ISS and the space suits, the preparation for an EVA requires oxygen breathing combined with a slow decompression profile; thus avoiding decompression sickness. In contrast to ISS missions, EVAs will be conducted much more frequently during planetary explorations, so in order to minimise preparation time it is envisaged that future planetary habitats will be hypobaric with an enriched oxygen ambient; similar to that at an altitude in excess of 2000m (Bacal et al., 2008; Bodkin et al., 2006; Norcross et al., 2013). Even though it is well known that humans can acclimatize to the levels of hypoxia anticipated in future habitats (Milledge et al., 2007) ,the manner in which hypoxia might affect the physiological responses to artificial gravity countermeasures is not.

Therefore, the aim of the present study was to assess the sex differences in haemodynamic and blood flow responses to AG in the head-to-foot direction on a SAHC and reduced oxygen availability. This is likely to illicit interaction of homeostatic mechanisms towards either heat removal or orthostatic tolerance. It is hypothesised that 1) higher levels of gravitational loading will increase the magnitude of orthostatic intolerance, or reduce time to pre-syncope, in females compared to males, 2) reduced oxygen availability will further exacerbate orthostatic intolerance in both males and females, with a greater effect observed in females.

5.3 Materials and Methods

10 female and 10 male participants were recruited for the study, their mean ± SD anthropometrics are displayed in table 1. The protocol was approved by the National Committee for Medical Ethics at the Ministry of Health of the Republic of Slovenia (appendix D, approval no. 0120-180/2023/7); conforming to the guidelines of the Declaration of Helsinki.

5.3.1 Participant Information

A sample size calculation was conducted to assess the impact of environmental conditions of microvascular blood flow, utilising the results of a previous study (Ciuha, Tobita, et al., 2019). During heating, a difference of 4.9°C in proximal-distal temperature gradient ($\Delta Tsk_{p\text{-}d}$) was observed, producing an effect size (d) of 3.38 for the association between temperature and microvascular blood flow. Assuming an α of 0.05 and β of 0.99, eight

participants provide sufficient power to detect a statistical difference in this association, and assuming a N2/N1 ratio of 1, the required sample size for gender analysis between two independent groups is 8 participants per group (G*Power Version 3.1.9.2). 10 male and 10 female participants were recruited and completed the full session. None of the participants met the following exclusion criteria: smokers, physically inactive, diabetic, and/or a history of freezing or non-freezing cold injuries, cutaneous peripheral disease, or high or low blood pressure. Female participants were evaluated during the luteal phase of their menstrual cycle (day 14 – 28). Prior to the start of the study, participants were familiarized with the study protocol and procedures, and gave their written consent for participation. Participants were asked to refrain from caffeine, alcohol, smoking, and intense physical exercise in the 24-hours leading up the study.

Table 5.1: Participant anthropometric data.

	Females	Males
N	10	10
Age (years)	28.8 ± 8.5	27.9 ± 6.3
Height (cm)	170.0 ± 7.8	179.8 ± 6.1
Weight (kg)	63.5 ± 9.2	78.2 ± 10.3
Body mass index ($kg \cdot m^2$)	22.1 ± 3.5	24.2 ± 2.8
Body surface area (m^2)	1.7 ± 0.1	2.0 ± 0.2
Blood volume (L)	4.5 ± 0.4	5.3 ± 0.5
1GRF (%BW)	102.6 ± 3.4	99.9 ± 2.4
2GRF (%BW)	195.6 ± 6.4	187.0 ± 4.9

Body surface area (Mosteller, 1987) and blood volume (Nadler et al., 1962) were calculated using recorded body mass and body stature values.

5.3.2 Experimental Protocol

Female and male participants attended two testing session, where the temperature in the room was 29.1 ± 0.8 °C and ambient oxygen concentration was either normoxic (PO_2 = 133 mmHg) or hypoxic (PO_2 = 92 mmHg). Relative humidity (RH) remained constant at 49.5 ± 8.1 %. The participants experienced a protocol conducted on a SAHC located the Jožef Stefan Institute's Gravitational Physiology Laboratory (Planica, Slovenia).
The participants initially rested in thermoneutral conditions for a 10-minutes, before entering the SAHC chamber and being fully instrumented with all sensors; maximal cutaneous blood flow responses were also obtained (described in section 2.3). Figure 1 displays the protocol used in the present study, which involved collecting resting measurements in three postures across six conditions. During centrifugation, the definition of gravitational stimuli experienced by the participants was defined by ground reaction force (GRF) applied to a force platform the participants were 'stood' on. Table 1 displays mean ± SD %bodyweight associated with 1GRF and 2GRF. The six conditions are as follows; standing in normal gravity (NG), stationary supine on centrifuge (SUP1), 1GRF centrifugation (1GRF), stationary supine on centrifuge (SUP2), 2 GRF centrifugation (2GRF), stationary supine on centrifuge (SUP3). They were asked to lie or stand calmly with no movement in their right (instrumented) arm and leg.

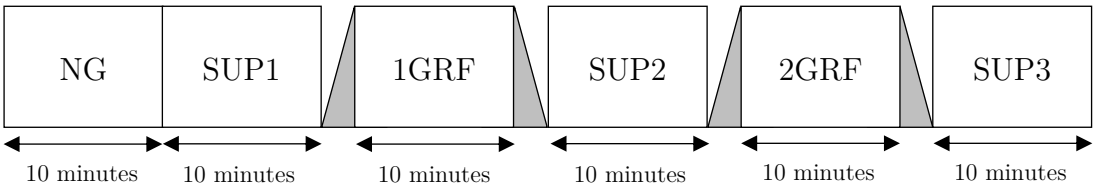

Figure 5.1: Session protocol displaying the order of conditions following time for acclimation and instrumentation (not shown in figure), identical in each of the four sessions. Grey areas represent the 90-second ramp up/down for centrifugation.

5.3.3 Data Collection Methods

Data collection methods are identical to those described in chapter 4.

5.3.3.1 Laser Doppler Flowmetry (LDF)

Laser Doppler Flowmetry (LDF) – measured at the forearm (ArmBF) and calf (LegBF), on the right side of the body, as a non-invasive measure of capillary, arteriole, and venule perfusion (Moors Instruments Laser doppler monitor, MoorVMS-LDF, Moor Instruments, UK). The device was calibrated using a fluid undergoing Brownian motion before each participant. Arbitrary units produced by the LDF (Laser doppler units: LDU) were converted into cutaneous vascular conductance (CVC) as a ratio of LDU to mean arterial pressure. They were then normalised against maximum blood flow for both the arm and leg in normothermic conditions in order to produce a relative measure of percentage maximum blood flow (%maxBF). Maximum blood flow values were obtained by locally heating the limb and producing a post-occlusion reactive hyperaemia (PORH) via occlusion then release the limb after 4 minutes.

5.3.3.2 Cardiovascular

Measurement of heart rate (HR, min^{-1}), stroke volume (SV, mL), cardiac output (CO, $L \cdot min^{-1}$), systemic vascular resistance (SVR, $mmHg \cdot min \cdot mL^{-1}$), rate pressure product (RPP, $mmHg \cdot min^{-1}$), systolic blood pressure (SBP, mmHg), diastolic blood pressure (DBP, mmHg), mean arterial pressure (MAP, mmHg), and oxygen saturation (SpO2, %) occurred continuously throughout the protocol (Finapres NOVA, Finapres Medical Systems B.V., Netherlands). Haemodynamic responses were calculated using the model flow algorithm (Wesseling et al., 1993) utilising the finger volume-clamp method and a 5-lead electrocardiogram (ECG). Reconstructed systolic and diastolic blood pressures were calculated via direct finger pressure measurements using waveform filtering and level correction (Westerhof et al., 2002), and normalised to the heart level via a height correction unit measuring the hydrostatic pressure difference. Additionally, participants held their arm in a sling with the measured finger at the heart level throughout all conditions.

5.3.3.3 Skin Temperature (T_{sk})

Continuous minute-by-minute skin temperatures was measured throughout the protocol using wireless iButton thermistors (type DS1921H, Maxim/Dallas Semiconductor Corp., USA) located at four sites (mid-belly of the bicep brachii, pectoralis major at mid-clavicular level, rectus femoris at femur midpoint, gastrocnemius on medial aspect) on the right side of the body. Weighed skin temperature (Tsk) was then determined using Ramanathan (1964) equation.

5.3.3.4 Gastrointestinal Temperature (T_{gi})

Deep body temperature was measured via ingested telemetric pills measuring gastrointestinal temperature. The pills were ingested 60-minutes before start of the exercise protocol, and participants avoided drinking water after ingestion.

5.3.3.5 Subjective Measures

Participants were requested to verbally provide their thermal sensation and thermal comfort every 5-min (ASHRAE, 2017). Temperature sensation is measured from -3 (cold) through to 3 (warm), and thermal comfort is measured from 0 (comfortable) through to 3 (very uncomfortable). Finally, for participant safety, motion sickness was recorded during centrifugation using a 6-point scale ranging from 0 (no symptoms) to 6 (vomiting).

5.3.4 Statistical Analyses

Following conversion of raw LDF data into %maxBF data (described above), these data were averaged to produce minute-by-minute values throughout the full protocol. Cardiovascular data, measured beat-by-beat, was also averaged to produce minute-by-minute average. Finally, T_{sk} and T_{gi} were averaged to produce minute-by minute values throughout the protocol. Three-way independent groups ANOVAs compared the effect of three dependent variables – level of artificial gravity (GRAVITY), participant sex (SEX), and oxygen concentration (OXYGEN) – on the independent variables (ArmBF, LegBF, HR, SVI, CI, SVR, RPP, SYS, DIA, MAP, T_{sk}, T_{gi}). Data was analysed using IBM SPSS statistics (Version 26, IL, USA) and Graphpad (Graphpad Prism 9, Version 9.1.2, USA); using an alpha value of $p < 0.05$.

5.4 Results

All female and male participants completed the 10-minute NG and 1GRF protocol phases successfully, in both conditions. Pre-syncopal symptoms were exhibited by several participants during the 2GRF centrifugation condition, resulting in premature termination on the condition. No participant experienced full syncope under any test condition. The time to test cessation at 2GRF was significantly lower in females than in males ($p = 0.004$, $F = 9.746$) irrespective of condition. Multiple comparison analyses identified that hypoxia alone produced a significant difference between sexes ($p = 0.008$). However, both males and females responded similarly to the different OXYGEN conditions, with regards to test duration. In normoxia, females lasted 7.2 ± 3.5 minutes and males lasted 9.1 ± 1.6 minutes; in hypoxia, females lasted 5.0 ± 3.3 minutes, and males lasted 8.5 ± 2.0 minutes (Fig. 5.2).

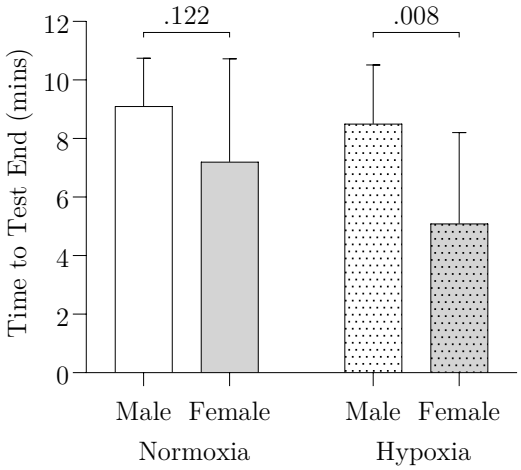

Figure 5.2: Time to test cessation at 2GRF in males and females, in normoxia and hypoxia. Significance of male vs. female difference detailed in each OXYGEN condition.

In addition, participants were thermally comfortable throughout NG (females: 0.0 ± 0.0, males: 0.6 ± 0.4), 1GRF (females: 0.0 ± 0.0, males: 0.4 ± 0.2) and 2GRF (females: 0.1 ± 0.0, males: 1.0 ± 0.2); with females more thermally comfortable throughout. Females also perceived a lower thermal sensation than males in NG (females: 0.8 ± 0.0, males: 1.3 ± 0.2), 1GRF (females: 0.2 ± 0.0, males: 0.5 ± 0.2), and 2GRF (females: 0.3 ± 0.0, males: 1.0 ± 0.4). Finally, ratings of perceived exertion also increased with increasing G-load; NG (females: 6.3 ± 0.4, males: 6.5 ± 0.2) compared to 1GRF (females: 6.7 ± 0.2, males: 7.3 ± 0.3) and 2GRF (females: 8.5 ± 0.1, males: 8.9 ± 0.4).

5.4.1 Skin Blood Flow (SBF)

Analyses of the arm M_{BF} only identified a significant effect of GRAV ($p = 0.036$, F = 3.665), and no effect of either SEX or OXYGEN ($p > 0.05$). Further analyses identified male M_{BF} in the arm was significantly reduced by the increase in GRAV (NG = 21.8 ± 8.5 %max, 1GRF = 20.4 ± 8.6 %max, 2GRF = 18.7 ± 10.7 %max; $p < 0.001$), whereas the female arm M_{BF} did not vary significantly (NG = 27.4 ± 15.8 %max, 1GRF = 21.4 ± 8.5 %max, 2GRF = 21.2 ± 11.6 %max); which may be due to higher individual variability in the female response.

Leg M_{BF} was also significantly affected by the increase in GRAV ($p < 0.001$, F = 27.43), displaying increases from NG up to 2GRF. Further analyses identified that the increase in GRAV significantly increased M_{BF} in both males (NG = 18.3 ± 6.6 %max, 1GRF = 23.8 ± 13.3 %max, 2GRF = 29.2 ± 13.3 %max; $p < 0.001$), and females (NG = 19.0 ± 7.8 %max, 1GRF = 22.0 ± 10.1 %max, 2GRF = 47.3 ± 22.6 %max; $p < 0.001$). In addition, the leg M_{BF} was also significantly altered by the interactions of GRAV + SEX ($p = 0.002$, F = 7.470), and GRAV + OXYGEN ($p = 0.001$, F = 3.777). Multiple comparisons analyses have identified the hypoxia in 2GRF condition as cause of this variation, as the leg M_{BF} in females was significantly higher (+30 %max, $p < 0.001$). Fig. 5.3 displays mean ± SD M_{BF} under differing ambient conditions and gravitational loads.

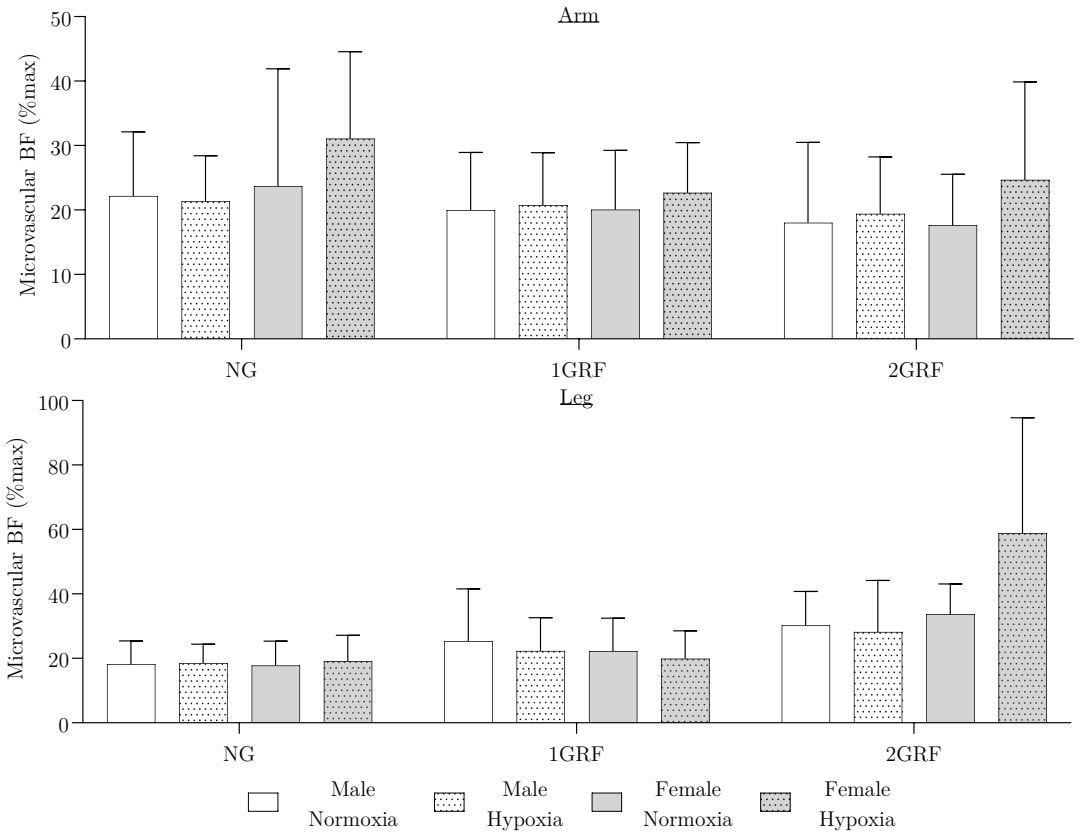

Figure 5.3: Microvascular blood flow under differing ambient conditions and gravitational loads.

5.4.2 Deep Body and Skin Temperatures

A significant effect of SEX was observed between females and males ($p < 0.001$); however no other interaction effect was observed. In the NG condition, the male T_{gi} was 37.1 ± 0.2 °C and female was 37.5 ± 0.3 °C. In 1GRF the male temperature was 37.2 ± 0.2 °C and female was 37.6 ± 0.2 °C. Finally, in 2GRF the male temperature was 37.2 ± 0.2 °C and female was 37.5 ± 0.3 °C. These values are provided as an average of both conditions in each sex, due to the lack of significant difference as a result of OXYGEN.

T_{sk} (Fig. 5.4) was significantly affected by SEX ($p < 0.001$, $F = 24.88$), as well as GRAV ($p < 0.001$, $F = 35.81$) and OXYGEN ($p < 0.001$, $F = 27.94$). Multiple comparisons analysis identified a significant difference from NG in both 1GRF and 2GRF conditions. In the NG condition, the male T_{sk} was 34.6 ± 0.2 °C and female was 34.8 ± 0.2 °C. In 1GRF the male T_{sk} was 34.0 ± 0.3 °C and female was 34.4 ± 0.3 °C. Finally, in 2GRF the male T_{sk} was 33.7 ± 0.3 °C and female was 34.2 ± 0.3 °C. As above, these values are provided as an average of both conditions in each sex, due to the lack of significant difference as a result of OXYGEN.

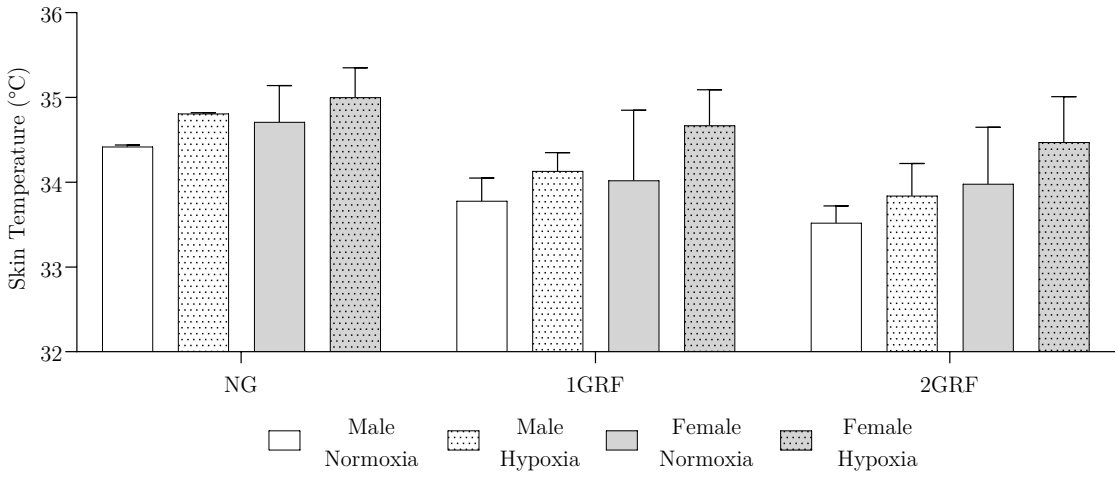

Figure 5.4: Skin temperatures under differing ambient conditions and gravitational loads.

5.4.3 Haemodynamic Responses

Mean ± SD of haemodynamic variables in both sexes, and all GRAV conditions are displayed in table 5.2. In response to SEX, HR was higher in females than in males (+ 15.0 $b \cdot min^{-1}$; $p < 0.001$, F = 27.55), SVI was higher in males than females (+6.7 mL; $p < 0.001$, F = 23.07), RPP was higher in females compared to males (+2478.8 $mmHg \cdot min^{-1}$; $p < 0.001$, F = 22.66), and SVR was higher in females compared to males (+0.2 $mmHg \cdot min \cdot mL^{-1}$; $p < 0.001$, F = 19.29).

The main effect of GRAV caused a significant effect in many variables, however multiple comparisons analyses identified that no differences existed between NG and 1GRF in any variables; significant differences were produced via the differences in NG vs. 2GRF and 1GRF vs. 2GRF. Table 2 details mean ± SD values at each GRAV condition, in both males and females. The increase in GRAV condition caused elevation in HR ($p < 0.001$, F = 25.78), RPP ($p < 0.001$, F = 23.80), SVR ($p = 0.027$, F = 3.752), SBP ($p = 0.009$, F = 4.865), DBP ($p < 0.001$, F = 15.19), and MAP ($p = 0.007$, F = 5.263). The increase in GRAV caused a decrease in SVI ($p = 0.001$, F = 7.178).

The main effect of OXYGEN caused an increase in HR ($p = 0.03$, F = 4.807) during the hypoxic trial, whilst a decrease in SRV ($p = 0.045$, F = 4.122) and SpO_2 ($p < 0.001$, F = 763.0) were observed.

Table 5.2: Mean ± SD haemodynamic values of all at each gravitational stimulus, in both males and females.

Haemodynamic Parameter	Sig.	GRAV condition	Male	Female
Heart Rate *($b \cdot min^{-1}$)*	a,b,c	NG	80.0 ± 10.9	95.6 ± 16.5
		1GRF	74.5 ± 10.8	90.3 ± 17.5
		2GRF	96.3 ± 20.2	118.9 ± 18.4
Stroke Volume *(mL)*	a,b	NG	74.5 ± 14.1	49.6 ± 16.8
		1GRF	73.8 ± 16.7	53.2 ± 19.6
		2GRF	60.4 ± 16.3	39.9 ± 14.2
Cardiac Index *($L \cdot min^{-1}$)*		NG	5.9 ± 1.1	4.6 ± 1.4
		1GRF	5.5 ± 1.2	4.6 ± 1.5
		2GRF	5.9 ± 1.9	4.5 ± 1.5
Rate Pressure Product *($mmHg \cdot min^{-1}$)*	a,b	NG	10713.9 ± 1883.0	12277.4 ± 3380.1
		1GRF	10654.2 ± 2017.1	12214.1 ± 2983.8
		2GRF	15379.0 ± 3412.3	16671.7 ± 3112.7
Systemic Vascular Resistance *($mmHg \cdot min \cdot mL^{-1}$)*	a,b,c	NG	1.0 ± 0.3	1.4 ± 0.4
		1GRF	1.2 ± 0.3	1.4 ± 0.5
		2GRF	1.3 ± 0.5	1.7 ± 0.7
Systolic Arterial Pressure *(mmHg)*	a	NG	130.0 ± 12.3	127.4 ± 13.3
		1GRF	140.9 ± 14.0	133.3 ± 13.5
		2GRF	151.2 ± 16.0	140.0 ± 20.3
Diastolic Arterial Pressure *(mmHg)*	a	NG	84.4 ± 12.0	90.2 ± 10.4
		1GRF	91.2 ± 12.1	92.4 ± 11.7
		2GRF	107.1 ± 14.9	106.2 ± 14.3
Mean Arterial Pressure *(mmHg)*	a	NG	101.9 ± 14.0	105.4 ± 17.6
		1GRF	110.8 ± 20.5	109.0 ± 16.2
		2GRF	124.8 ± 19.3	119.3 ± 20.3
SpO2 *(%)*	c	NG	90.9 ± 5.2	92.0 ± 6.0
		1GRF	90.9 ± 6.2	90.3 ± 7.2
		2GRF	91.8 ± 6.5	91.5 ± 6.1

a = significant effect of gravity, b = significant effect of sex, c = significant effect of oxygen, $p < 0.05$.

5.5 Discussion

Known differences exist in the response of sexes to an orthostatic stress; largely indicating a lowered tolerance in females compared to males. In the present study, female participants experienced pre-syncopal symptoms during hyper-gravity (2GRF) condition significantly earlier than males (hypothesis 1). In addition, exposure to a hypoxic stressor similar to those proposed for future space habitats (Bacal et al., 2008; Bodkin et al., 2006; Norcross et al., 2013) further influenced time to pre-syncope in both males and females, with a significantly reduced time to presyncope observed in females (hypothesis 2). These incidences of presyncope match those of previous research, however the present study

intends to elucidate reasons for these differences via assessments of haemodynamic and vascular responses to AG.

5.5.1 Orthostatic Tolerance

In those returning from space, the prevalence of OT is higher in females than in males. Following space shuttle missions lasting between 5 and 16 days, pre-syncope during postflight OT tests was reported in 70 - 100 % of females and 0 - 5 % of males (Blaber et al., 2004; Blaber et al., 2011; Waters et al., 2002). In a review of 165 astronauts during short duration spaceflight, 28 % of female astronauts and 7 % of male astronauts experienced pre-syncopal symptoms after 10 minutes in an upright posture (Harm et al., 2001). Fewer studies have assessed OT during AG exposure in males and females, however, those that have, calculated that 58% of females and 96% of males can successfully withstand 1 Gz centrifugation with varying session durations (Fong et al., 2007; Kourtidou-Papadeli et al., 2021; Masatli et al., 2018; Stenger et al., 2007). The present study, which utilised AG of up to 2 Gz, presyncope was reached by both males and females, yet females still experienced a reduction in the time to presyncope compared to males. This calls into question the suitability of a standardised centrifugation programme as a countermeasure for spaceflight-induced cardiovascular deconditioning; the use of an individualised approach seems to be more appropriate. By utilising centrifugation individualisation techniques for artificial gravity training, as described by (Goswami, Evans, et al., 2015), it may be possible to isolate sex differences in cardiovascular deconditioning, and response to training, during exposure to microgravity or one of its analogues (bedrest, dry immersion etc.,). Indeed, Stenger et al. (2007) revealed that 3-weeks of 1 – 2.5 Gz (foot level) centrifugation for 35 mins/day increased OT by 17.3 % in males and 9.3 % in females. It is unclear why the improvement was smaller in females, with the authors citing methodological issues in lower-body negative pressure (LBNP) rather than sex differences; yet it seems possible that in fact individualised training may have reduced the differences between sexes. To individualise AG training, (Goswami, Evans, et al., 2015) proposed using a 'pre-syncopal development test' which increased the AG load by 0.1 Gz every 3 minutes until pre-syncopal symptoms were reached; the training level was then defined as one level below pre-syncopal level. Using this method, Goswami, Evans, et al. (2015) noted increased OT after 45-minutes of training in 53% of females and 23% of males, and overall the tolerability of AG training improved; all participants completed the full 90-minute AG protocol.

5.5.2 Cardiovascular Responses

As observed previously (Goswami, Bruner, et al., 2015; Laing et al., 2020; Verma et al., 2018), significant differences in haemodynamic parameters were not observed between the NG and 1GRF conditions, in either sex. The increase in GRAV (i.e., NG/1GRF vs 2GRF), therefore, induced a strain on the human body that required a response from the cardiovascular system for maintenance of stable oxygenated blood delivery under severe environmental and gravitational stress. Despite increases in cardiovascular strain resulting from the 2GRF condition occurring in both sexes, there were also significant differences in haemodynamic variables between females and males. The increase in HR may be an autonomic response mechanism to the higher GRAV condition, to counteract the loss in circulating blood volume and lowered SV via blood pooling (Fu et al., 2004). It has been previously observed that females exhibit a greater sympathetic response under the same level of LBNP (Convertino, 1998; Frey et al., 1986; Montgomery et al., 1977), whereas males tend to exhibit a greater and more coordinated vascular response (Frey & Hoffler, 1988; Yang et al., 2012). Indeed, the difference in SVR between NG and 2GRF is larger in

males (0.2 mmHg · min · mL^{-1}). The changes in HR and SVI, and differences between sexes, may be a positive response to counteract the GRAV conditions; changes in RPP and SVI are more likely to be negative consequences of the overall decrease in circulating blood volume and consequent drop in blood pressure (Bundgaard-Nielsen et al., 2009; Van Lieshout et al., 2005). Another indicator of these detriments in haemodynamic function is observed in the microvascular blood flow. While no significant sex differences occurred, the interaction of GRAV + SEX was significant; largely due to the considerably higher M_{BF} in the leg in females (+ 30 %max). It is possible that these exaggerated leg M_{BF} values are either a general lack of vascular resistance to 2GRF in females or, more likely perhaps, it is the visualisation of blood pooling impacting venous/capillary flow (Antle et al., 2018). When considering the whole cardiovascular system contributing to the maintenance of orthostatic tolerance, the results of the present study indicate that a lack of vascular resistance in the lower limbs caused a cascade of haemodynamic responses which were ultimately unable to prevent pre-syncopal symptoms in female participants. In males, the known vascular resistance differences may have alleviated some of this haemodynamic strain, lengthening the time to pre-syncope.

5.5.3 Thermoregulation

Differences between sexes in deep body temperatures were considerable, albeit expected. During the chosen phase of the menstrual cycle (i.e., luteal), there is a known increase in resting deep body temperature of 0.3 – 0.7 °C (Baker et al., 2020); clearly observed in the present study. There were no other effects of GRAV or OXYGEN on the deep body temperature, however this may be due to the short duration of each phase of the protocol; in the absence of exercise, deep body temperature in 31.5 °C ambient temperature for 80-minutes has been shown to change by only ~0.1 °C (Huizenga et al., 2004). Skin temperature decreased from NG through 1GRF and 2GRF, which is likely due to the air flow produced via rotation of the SAHC; at higher speeds, the SAHC spins at ~40 kph, producing wind speeds as high as 4.2 m/s. The differences due to sex may be due to factors such as smaller body mass and surface area, lower metabolically active tissue, higher body fat, and greater insulation during vasoconstriction (Burse, 1979; Durnin & Womersley, 1974). The perception of temperature was also altered between sexes, with females perceiving a lower temperature throughout the study. As menstrual phase was controlled, and research tends to suggest that menstrual phase has little impact on thermal perception (Matsuda-Nakamura et al., 2015), it is likely that these differences are related to the sex differences as previously described. Despite the variations in deep body temperature, skin temperature, and thermal perception, it is also reported that increases in the autonomic thresholds for thermoregulation also occur from the early follicular vs the mid-luteal phases (Hessemer & Bruck, 1985). This may have resulted in a protective thermoregulatory /cardiovascular response to the combined high ambient temperatures and centrifugation whereby vasodilation required for thermoregulation in hot ambient temperatures occurs later. Centrifugation during earlier stages of the menstrual cycle may result in even earlier development of pre-syncopal symptoms due to greater levels of vasodilation, and thus must be considered in SAHC training prescription.

5.5.4 Limitations

We recognise two limitations within this study. Firstly, the use of high ambient temperature alone is not reflective of SAHC training conditions, as the high occurrence of pre-syncopal symptoms observed would be detrimental to short- and long-term training performance. The present study was conducted as part of a larger study assessing the

cardiovascular responses to differing temperatures, oxygen availability, and gravitational stress; discussing the mechanisms and responses to these stressors via the interaction of thermoregulatory and baroreflex interactions (Chapter 4). Due to technical limitations, stable measurement of haemodynamic signals in cold conditions in females were unobtainable; constituting an insurmountable safety risk as pre-syncope was not determinable. Nonetheless, the authors reaffirm the exacerbation of cardiovascular strain via heating, hypoxia, and hypergravity provides valuable data regarding the limits to sex differences in SAHC utilisation.

Secondly, female participants were recruited during the luteal phase of the menstrual cycle only. With the anticipated use of a SAHC as a full-time exercise countermeasure during future space missions, a consideration to the cardiovascular responses during a complete menstrual cycle must be considered. Previous research assessing the effects of the menstrual cycle on orthostasis provide a clear consensus; differing phases have no effect of orthostatic tolerance (Claydon et al., 2006; Meendering et al., 2005). However, when central hypovolemia is produced via LBNP, significant variations in cardiovascular response are displayed between follicular and luteal phases (Shankhwar et al., 2024); proposed to be related to changes in estrogen levels and sympathetic activity. Similar central hypovolemia occurs during centrifugation, therefore the role of the menstrual cycle during SAHC activity must be assessed.

5.6 Conclusions

Known sex differences in tolerance to a gravitational stimulus are also reflected in hypergravity, particularly when the ambient conditions exacerbate the cardiovascular burden. Female participants exhibited greater haemodynamic and microvascular strain, and encountered pre-syncopal symptoms earlier than male participants, during 2GRF centrifugation. For future research into the use of a SAHC as an exercise countermeasure, these differences must be considered and perhaps mitigated via individualised and tolerable gravitational loads.

Chapter 6

Discussion and Conclusions

The aim of this thesis was to investigate regional blood flow under a range of external stressors. Through a series of studies, a number of different stimuli were utilised to stress vascular control mechanisms in a way that would require interaction (and/or competition) for vasomotor control. The first study (Chapter 2) validated previous observations in the field of research, with a controlled HUT protocol during a simulated HW that specifically focussed on skin perfusion and thermoregulatory responses. This study demonstrated the existence of a regional blood flow response. The second study (Chapter 3) incorporated more appropriate SBF measurement techniques; including central and peripheral autonomic mechanism analyses. In the third study (Chapter 3), a more severe baroreflex stressor was introduced in the form of artificial gravity (AG) to examine the modulation of the baroreflex by the thermoregulatory system during exposure to differing ambient conditions. Finally, the fourth study (Chapter 5) measured regional blood flow variables in both females and males, and assessed their implications in relation to orthostatic tolerance. The findings of each of these studies are summarised below.

6.1 Study I (Chapter 2)

By simulating the effects of a three-day HW, and conducting a 60° HUT test on each of these days, the acute responses to high T_A and orthostatic stress was assessed. In addition, the prolonged and residual effects of the highly applicable HW conditions were also assessed. In response to the separate stressors of T_A and orthostasis, significant regional differences were observed. In particular, consistently higher cutaneous vasodilation was observed in arms than in the legs, particularly in the HW, suggesting a predominant thermoregulatory drive. The HUT also appeared to influence the legs predominantly, in both the non-HW and HW, which indicates a predominant tendency towards blood pressure regulation. The combined influence of both stressors also effected ΔT_{sk}F-F alone, however further analyses identified that this was largely due to the differences in the non-HW; indeed, during the HW conditions there is little influence of the HUT which therefore suggests thermoregulation is the predominant driver. Therefore, results obtained from study I appear to follow the same themes as observed in previous studies (Ciuha, Tobita, et al., 2019), whereby the arms are predominantly thermoregulatory, whilst the legs are predominantly baroreflex driven. The only addition to this previous research is that the arms also appeared to be modulated by the baroreflex during HUT, yet only in normothermic conditions. When dual stressors are established (i.e., high T_A and HUT) the arms return to predominant thermoregulatory control.

6.2 Study II (Chapter 3)

Study I was designed to assess regional SBF in the context of a larger study assessing cardiovascular responses to a simulated HW. Study II sought to build on previous research by utilising a highly controllable climatic chamber for accurate T_A, and gold-standard measurement techniques for SBF; laser-doppler flowmetry. By applying a significant thermal load up to ~40 °C, whilst in two postures, it was anticipated that the regional interaction of thermoregulation and the baroreflex would be discernible, and the transient nature of the thermal loading would enable observation of baroreflex modulation in a temporal view. In addition, central and peripheral autonomic mechanisms of SBF were also recorded in study II.

The results of the study indicated that SBF in the arms receives input (and subsequently interaction) from thermoregulatory and baroreflex mechanisms concurrently. In the forearm, a 95.1 % increase in CVC from cold to hot conditions in SUP was identified, yet during the STA this increase in vasodilation was clearly mediated by the baroreflex; only a 46.4 % in CVC was observed. In contrast, the leg appears to remain predominantly baroreflex-controlled, as calf CVC minimally responded to the significant increase in ambient temperature during the heating phase of the protocol, and thus was solely affected by the change in posture from supine to standing.

The threshold for vasomotor activity, particularly vasodilation, was assessed in study II by comparing T_{sk} to SBF. In agreement with the suggestion that the arm is predominantly thermoregulatory, the onset of vasomotor activity occurred at a T_{sk} 0.9 °C lower in the arm than the leg. In addition, the slope of the response was more than doubled in the arm vs. the leg; indicating the magnitude of SBF responses.

Autonomic analyses of HRV and WTA also initiated an understanding of the mechanisms related to the functional regional variations in SBF that were observed. As expected, centrally mediated sympathetic control was evident as a result of the increase in T_A and the posture change from SUP to STA; thereby suggesting thermoregulation and baroreflex activity is at least initially centrally mediated. By using WTA, it was also identified that the significantly higher myogenic and neurogenic activation occurred in the leg alone; indicating that baroreflex activation extends to the peripheral nervous system.

These results build on the work of study I, by revealing a significant modulation of the thermoregulatory response via centrally mediated baroreceptor unloading affecting the vasomotor threshold for vasodilation, as described in previous research (Johnson & Park, 1981; Roberts & Wenger, 1980).

6.3 Study III (Chapter 4)

Whilst study II was centred around a significant thermoregulatory stressor, the aim of study III was to induce a significant blood pressure stressor. In doing so, the detection of thermoregulation modulation of the baroreflex in differing ambient conditions may have been established. Despite the substantially higher gravitational stressor produced via AG on a SAHC, and significant thermal load (~30 °C), there appeared to be no modulation of the baroreflex response in the leg via thermoregulation. In fact, leg SBF was unaffected by the varying ambient conditions, and only significantly responded to the change in AG. This significant effect of AG centred on the difference between NG and 2GRF, in which AG was sufficient to overcome the baroreflex and allow pooling of blood in the lower limbs. As previously observed, arm SBF responded significantly to both the thermal and gravitational stressors. Despite the inclusion of hypoxia, the chemoreflex did not initiate any change in vascular activity.

6.4 Study IV (Chapter 5)

The final study of this thesis compared vascular responses to AG in high T_A and different partial pressures of oxygen between females and males. The results of this study indicate that there were no significant differences identified between sexes in any condition. However, there was a significant interaction of AG and sex, which was likely due to the considerably higher female leg SBF (+ 30 %max) than males in any another condition. It is possible that these exaggerated leg M_{BF} values are either a general lack of vascular resistance to 2GRF in females or, more likely perhaps, it is the visualisation of blood pooling impacting venous/capillary flow.

6.5 Conclusions

The findings of this thesis, which are the result of the above four studies are :

- SBF of the arm is predominantly controlled via thermoregulatory vascular mechanisms, particularly when no interaction of mechanisms is required (i.e., only heating is occurring). However, dual stressors (temperature and orthostatic) cause an interaction of more than one mechanism. It is likely that while the primary mechanism is still thermoregulation, a centrally mediated baroreflex unloading response to alter the vasomotor threshold for vasodilation occurs; to maintain suitable blood pressure. These responses appear to be modulated largely by central autonomic mechanisms altering sympathetic activity.
- SBF of the leg, despite substantial levels of heating, appears to be unaffected by thermoregulatory mechanisms and is almost entirely controlled by the baroreflex for maintenance of stable blood pressure. Mechanistic analyses of autonomic control highlight the use of both central and peripheral autonomic control.
- No significant difference in vascular mechanisms exist between females and males, in response to AG, high T_A, and variations in the partial pressure of oxygen. Though at significantly high levels of AG in the head-to-foot direction (i.e., 2 Gz +), vasoconstriction alone in females during passive AG may not be suitable to maintain blood pressure.

6.6 The Updated Core-shell Model

As discussed in the introduction (section 1.1.1), the well-established core-shell model of heat transfer in the human body (Aschoff & Wever, 1958) is suggestive of a uniform thermoregulatory response. The results of the present thesis may indicate that an alteration could be made to this model. The findings described in section 6.4 indicate that alterations to the temperature of the shell via external heating, particularly in the presence of orthostatic stress, produce a thermoregulatory controlled response in the arm that is not matched in the leg. It may be possible then that non-uniform alterations in the core-shell ratio occur throughout the body, likely due to the interaction of multiple mechanisms in the maintenance of overall homeostasis. Due to the limited changes in T_{core} throughout each study, however, it cannot be recommended that these regional variations will be maintained in occurrence of severe heat strain as a result of ambient heat accumulation and/or intense exercise.

6.7 Future Research

Whilst conducting this body of research, the opportunity for additional research has appeared in certain areas. Firstly, each of the studies conducted within the framework of this thesis relate to SBF alone. While regulation of SBF has a significant impact on many homeostatic mechanisms, it does not explain the regulation of the entire vascular system. Future research should look to continue with research into the regional regulation of whole-body blood flow by assessing the responses of areas such as the brain, muscle, and splanchnic regions; to name a few.

Secondly, it would be interesting to build on the work of previous research (Kakitsuba et al., 2007; Mekjavic et al., 1991) assessing the effects of T_{core} and T_{sk} on SBF; in particular the LCT and UCT of vasomotor activity. As observed in chapter 3 (Fisher et al., 2024), differing regions can be responsible for producing differing vasomotor thresholds for the onset of vasodilation and perhaps vasoconstriction. Additional research assessing the role of changes in T_{core} will also aid in the continued development of the core-shell model; with additional consideration made for the influence of regional variation in blood flow.

Appendix A

Predicting Deep Body Temperature (T_B) from Forehead Skin Temperature: T_B or Not T_B

A.1 Foreword

The detection of T_{core} via non-contact methods, such as infra-red thermography, are quick and commonly used in healthcare and industrial settings. However, they have been found to be inaccurate in detecting absolute T_{core} (Mekjavic & Tipton, 2021) using algorithms based on one T_{sk} site alone. It may be possible that by utilising the regional SBF information gathered from the present thesis, a more accurate and repeatable method of T_{core} prediction can be achieved. The study discussed in Appendix A develops this idea, and attempts to predict T_{core} via a number of sources such as the forehead, finger, and inner canthus of the eye $T_{sk,}$ as well as considering skin perfusion via T_{sk}F-F as an additional variable.

The findings of study were published in the Sensors under the following citation: Fisher, J. T., Ciuha, U., Tipton, M. J., Ioannou, L. G., & Mekjavic, I. B. (2022). Predicting deep body temperature (Tb) from forehead skin temperature: Tb or not Tb? *Sensors, 22*(3), 826.

A.2 Introduction

Two principal methods have been proposed to predict deep body temperature (T_b) from the measurement of heat loss from the skin surface. One method measures the conductive heat loss pathway (Gunga et al., 2008) and requires contact of the sensor with the skin surface, and the second is a non-contact method, monitoring radiative heat loss with infrared thermography. Common to both methods are their inaccuracy in estimating absolute T_b. Mekjavic and Tipton (2021) concluded the prediction of T_b from one skin region, namely the forehead, is inaccurate, resulting in false positives and negatives. They suggest that other facial sites, such as the inner canthus of the eye, may prove superior to forehead skin temperature (Tsk). They also recommend that T_{sk} gradients between proximal and distal sites, such as the forehead (proximal site) and fingertip (distal), may provide an improvement in the prediction of T_b. Namely, the proximal-distal skin temperature gradient (ΔT_{sk}P-D) reflects perfusion of distal sites, and may indicate whether elevated temperature is due to heat strain or fever, the former causing peripheral vasodilation, and the latter vasoconstriction.

Recently, the need to rapidly screen individuals using T_b prediction in industry has become more important for a number of reasons. Disregard for the control of greenhouse gases has resulted in global warming, with potentially devastating consequences for future generations. Among these consequences is the summer heatwave (HW), originally infrequent and occurring only during the peak summer months, now increasing in frequency, magnitude, and duration (Pogačar et al., 2018). In an industrial environment, HWs may affect the health and well-being of workers (Kjellstrom et al., 2009), and result in reduced labour productivity (Ciuha, Pogačar, et al., 2019; Flouris et al., 2018; Ioannou, Mantzios, Tsoutsoubi, Nintou, et al., 2021) as a result of occupational heat strain. It has been suggested that HWs may have a cumulative effect on workers, resulting in a residual effect several days after ambient temperature returns to normal (Ciuha, Pogačar, et al., 2019). To try and mitigate the debilitating effects of HWs in the working environment, many countermeasures are available to reduce metabolic heat production and enhance heat loss, if only in the short term. The countermeasures include availability of cold drinking water, cool and ventilated rooms during rest breaks, and cooling vests (Ioannou, Mantzios, Tsoutsoubi, Nintou, et al., 2021). However, the possibility of monitoring workers for impending signs of heat strain, such as monitoring Tb, has largely been ignored; a system of reactive rather than preventative monitoring is more common.

Additionally, the recent pandemic of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-COV-2), resulting in a global coronavirus disease starting in 2019 (COVID-19), caused a lockdown of industrial activity during peaks of the COVID-19 waves in 2020. The manufacturing industry maintained some operations, and has consequently taken the recommended precautions (i.e., masks, distancing, etc.) to safeguard the workforce. Some companies have implemented monitoring of workers' surface temperature using infrared thermography (IRT) to estimate T_b. Those identified by the scanners as having elevated body temperature, for whatever reason, are not allowed entry.

In view of increasing reliance on the prediction of T_b from T_{sk}, the present study evaluated whether contact measurements of T_{sk} can provide a suitable surrogate for direct measurement of T_b; for the purpose of screening workers for SARS-Cov-2 virus infection, and impending heat strain during summer HW. It was hypothesized that T_{sk} will produce a significant association with T_b, but measurement of more sites to generate a ΔT_{sk}P-D will produce a stronger association; as hypothesized by Mekjavic and Tipton (Mekjavic & Tipton, 2021).

A.3 Materials and Methods

This study was part of a program of research conducted within the framework of the European Commission Heat Shield project, investigating the effect of HW on the health, well-being, and labour productivity of workers in five key European industries (manufacturing, agriculture, construction, logistics, tourism). During four previous HWs, conditions within an industrial manufacturing plant employing 1500 workers (odelo d.o.o., Prebold, Slovenia) were monitored (Ciuha, Pogačar, et al., 2019). Due to the difficulty of continuous 24-hour physiological monitoring of workers during a HW, a study was conducted simulating the industrial process in controlled laboratory conditions (Ioannou, Mantzios, Tsoutsoubi, Panagiotaki, et al., 2021), using data from the HWs measured in central Slovenia. Consequently, measurements of T_{sk} and T_b were conducted hourly throughout a 9-day study, including both normothermic and simulated HW conditions, to assess the association with T_b using indirect measurements.

A.3.1 Experimental Design

The study was conducted at the PlanHab facility (European Space Agency ground-based research facility) at the Olympic Sport Centre Planica (Rateče, Slovenia). Participants were confined to the facility for 9-days, and had access to their rooms, a common area, laboratory, and dining area. They were provided with three meals and two snacks each day (breakfast, lunch, afternoon snack, dinner, evening snack) and could drink water ad libitum.

On arrival at the facility, the participants were acquainted with the entire facility and were familiarized with all the experimental procedures. They were instructed to refrain from venturing outside the designated areas of the facility, as the temperature and humidity was regulated only in the designated areas, using heaters controlled by temperature regulators. Ambient humidity within the laboratory remained constant ~45 %. The protocol was designed to mimic the routine daily activities in a manufacturing plant, as well as some of the activities at home, and is described in more detail in Ioannou, Mantzios, Tsoutsoubi, Panagiotaki, et al. (2021).

During the 9-day confinement, the temperatures within the living quarters and workplace (i.e., laboratory) were regulated as displayed in Table 1. The first 3 days (pre-HW) represented normal conditions. The simulated HW was initiated at midnight at the end of day 3, with temperatures increasing in all areas. At midnight on day 6, the night-time/daytime temperature profile was readjusted to the same profile as in the first 3 days (post-HW). Experiments took place in ambient conditions of 19.8 ± 1.8 Wet-Bulb Globe Temperature (from www.wunderground.com; accessed on 14 January 2022).

Table A1: Temperature during daily work and rest periods. Temperature is presented as a mean (SD) of each 3-day testing condition.

	Work (0840 – 1800hrs) Temperature (°C)	Rest/Sleep (1800 – 0840hrs) Temperature (°C)
Pre-HW	25.4 (0.3)	22.3 (0.5)
HW	35.5 (0.3)	26.3 (0.8)
Post-HW	25.5 (0.7)	23.1 (0.7)

HW: Heatwave. Pre-HW: Testing days 1-3. HW: Testing days 4-6. Post-HW: Testing days 7-9.

A.3.2 Participants

A sample size of seven participants was deemed to provide sufficient power to detect a statistical significance, assuming an α of 0.001 and β of 0.99 (G*Power Version 3.1.9.6, Germany) using an effect size of effect size (d) of 1.8834 (f = 0.9417), based on the results of a previous study (Ioannou, Mantzios, Tsoutsoubi, Panagiotaki, et al., 2021; Ioannou et al., 2017). Seven young, healthy males (mean ± SD; age = 21.1 ± 1.1 years; body stature = 180 ± 6.1 cm; body mass = 81.5 ± 15.6 kg; body mass index = 25.1 ± 4.4 kg.m^2) participated in the study, which had received prior approval (Approval no. 0120-402/2020/4: October 20th, 2020) by the Committee for Medical Ethics at the Ministry of Health (Republic of Slovenia). All were non-smokers, engaged in regular physical activity recreationally, and were free from known cardiovascular, respiratory, and autonomic disease. Prior to the commencement of the study, the participants were informed of the details of the experimental protocol and were familiarized with the procedures, before signing an informed consent. The participants were aware that they could terminate participation in the study at any time during the 10-day duration.

A.3.3 Measurements

Each morning the participants ingested a calibrated telemetric radio pill (Body Cap, Caen, France), and a thermistor was secured to their forehead ($T_{forehead}$) and distal phalanx pad of the middle finger (T_{finger}) (iButton, Type DS1921H, Maxim / Dallas Semiconductor Corp., USA). These devices provided continuous measurement of gastrointestinal temperature (T_{gi}) and T_{sk}, respectively, on each day. Validation of the calibrated telemetric radio pill against rectal thermistor during rest, water immersion, and steady state exercise revealed no significant differences; furthermore, the system produces effective validity and test-retest reliability (Bongers et al., 2017; Travers et al., 2016). Additionally, the validation of iButton thermistors against calibrated thermocouples revealed no significant difference during steady-state, though response time to changes in temperature were slower than thermocouples (van Marken Lichtenbelt et al., 2006).

A.3.4 Data Analyses

T_{gi} and T_{sk} were measured continuously, and an average of the last 10 minutes was taken in each hour for 23-hours, on every day. This averaging period was chosen to avoid potential artefacts by using a stable 10-minute period. Each day, telemetric pills were ingested at 0700hrs, immediately after waking up, and T_{sk} iButtons were attached to the skin in the evening at 2230 hrs. Temperature measurements were recorded during three distinct ambient conditions: 22 °C, 25 °C, and 35 °C. ΔT_{sk}P-D, an index of blood flow (Rubinstein & Sessler, 1990), was calculated between the forehead and fingertip ($\Delta T_{forehead-finger}$). When measured at the forearm-finger or calf-toe, a value $\geq$ 2 °C represents vasoconstriction and $\leq$ 0 °C represents vasodilation (House & Tipton, 2002; Keramidas et al., 2013). In the present study, in which the ΔT_{sk}P-D was assessed from T_{sk} at the forehead and fingertip, the thresholds for vasoconstriction and vasodilation may likely be dissimilar to those reported by previous studies using the forearm-fingertip skin temperature gradient as an index of perfusion. Holm et al. (2018) have previously investigated the use of the forehead-fingertip skin temperature gradient as an index of mortality in hospital patients.

Means, standard deviations and coefficient of variation (CoV) were calculated for T_{gi}, $T_{forehead}$, and T_{finger} (Table 2).

The data, following calculation of normality by a Shapiro-wilk test, were assessed using either a Pearson's Correlation Coefficient, or a Spearman's Rank Correlation Coefficient.

Additionally, a multiple linear regression using $T_{forehead}$, T_{finger} and $\Delta T_{forehead\text{-}finger}$ was conducted. All statistical tests were completed using an alpha value of $p < .05$ and conducted using IBM SPSS Statistics (Version 26, IL, USA).

In addition to the multiple linear regression, root mean square error (RMSE) was also calculated between measured T_{gi} and predicted T_{gi} as produced from a regression equation, using the following equation (Barnston, 1992):

$$RMSE = \left[\sum_{i=1}^{N} \frac{(Z_{fi} - Z_{Oi})^2}{N}\right]^{1/2}$$

Equation A1. Root Mean Square Error

Where,
Zfi = forecast values
Zoi = observed values
N = sample size

Table A2: Mean (± SD), and coefficient of variation (CoV) of Tsk and Tgi measurements at each ambient condition.

Ambient condition	Measurement	Mean ± SD	CoV (%)
22 °C	T_{gi}	36.7 ± 0.4	1.2
	$T_{forehead}$	34.2 ± 1.4	4.1
	T_{finger}	33.2 ± 0.5	1.5
25 °C	T_{gi}	37.0 ± 0.4	1.0
	$T_{forehead}$	33.9 ± 1.3	3.7
	T_{finger}	33.8 ± 0.5	1.4
35 °C	T_{gi}	37.3 ± 0.2	0.6
	$T_{forehead}$	35.9 ± 0.7	1.9
	T_{finger}	35.5 ± 0.6	1.7

T_{gi}: gastrointestinal temperature. $T_{forehead}$: forehead temperature. $T_{fingertip}$: fingertip temperature.

A.4 Results

All participants completed the 9-day confinement. There were no untoward effects of the 3-day HW. The physiological responses and labour productivity during the simulated normal weather and HW periods have been presented elsewhere (Ioannou, Mantzios, Tsoutsoubi, Panagiotaki, et al., 2021).

A.4.1 Relationship between Tsk and Tgi

To assess the true relationship between $T_{forehead}$ and T_{gi}, measurements from every day were compared simultaneously, encompassing all ambient conditions. The range of temperatures observed were greater for $T_{forehead}$ (32.2 - 36 °C) than for T_{gi} (36.1 - 37.7 °C), whereas the average temperature of all measurements was higher for T_{gi} (T_{gi} = 36.9 ± 0.4 °C; $T_{forehead}$ = 33.9 ± 1.4 °C), a significant difference ($p < 0.001$). A significant relationship was identified between the measurements of $T_{forehead}$ and T_{gi} ($r = 0.653$; $p < 0.001$).

A.4.2 Tsk and Tgi at Different Ambient Temperatures

The above correlation analysis of the relationship between $T_{forehead}$ and T_{gi} was repeated for the individual HW (35 °C) and non-HW (22 °C and 25 °C) ambient temperatures, as shown in Fig. 2. A significant relationship was observed for the 22 °C ($r = 0.591$; $p < 0.001$) and 25 °C ($r = 0.408$; $p < 0.001$) ambient conditions, whereas there was no significant relationship at 35 °C ($r = 0.263$; $p = 0.185$). Table 2 displays T_{sk} and T_{gi} values measured in each ambient condition.

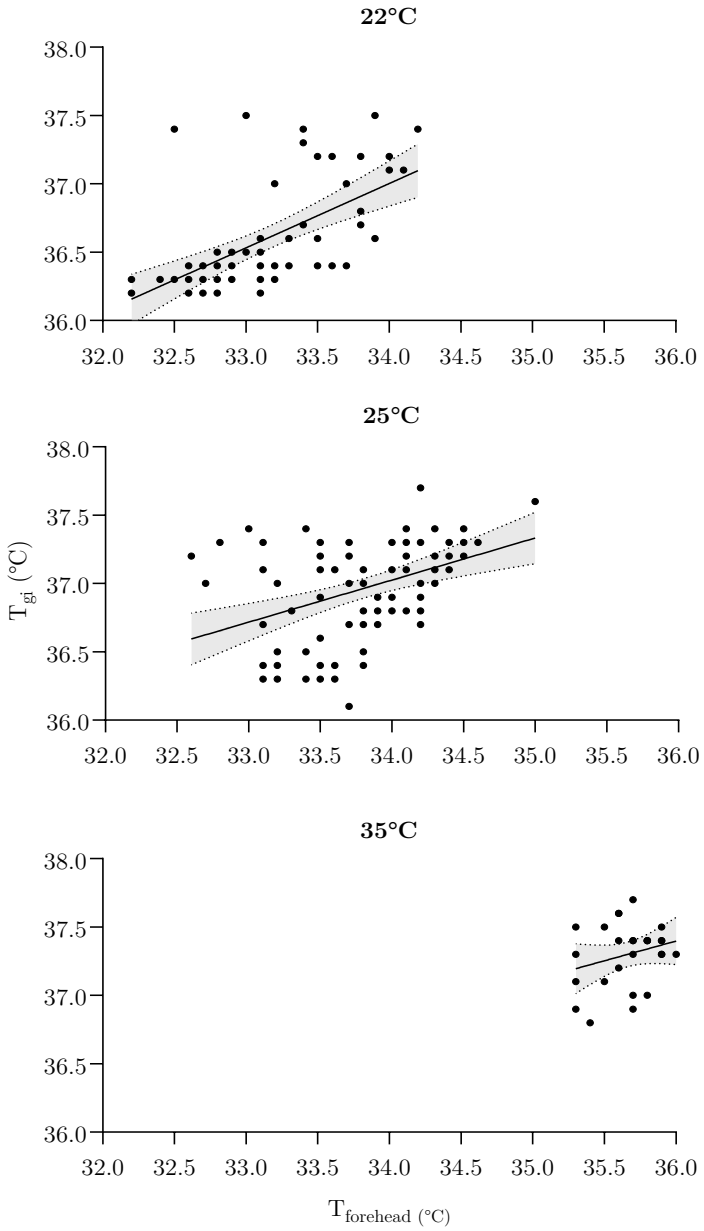

Figure A1: Gastrointestinal (T_{gi}) and forehead ($T_{forehead}$) temperature relationship. Measurements were obtained while participants were exposed to three ambient temperatures: 22 °C (upper panel), 25 °C (middle panel), and 35 °C (lower panel). Regression lines with associated 95% confidence bands for each temperature are also shown.

A.4.3 Proximal-distal Temperature Gradient Prediction

Mekjavic and Tipton (Mekjavic & Tipton, 2021) suggest that an index derived from measurements made at multiple sites might provide a more accurate temperature screening; primarily using areas where the skin is exposed (i.e., face and hands). When creating a T_{sk}P-D between the forehead and fingertip ($\Delta T_{forehead\text{-}finger}$), the correlation between this variable and T_{gi} was significant ($r = 0.637$; $p < 0.001$). Additionally, a multiple linear regression for prediction of T_{gi} using $T_{forehead}$, T_{finger}, and $\Delta T_{forehead\text{-}finger}$ produced a significant linear model using $T_{forehead}$ and $\Delta T_{forehead\text{-}finger}$ only ($r^2 = 0.588$; $F = 125.771$; $p < 0.001$):

$$Predicted\ T_{gi} = 29.349 + \left(0.225 \times T_{forehead}\right) + \left(0.154 \times\ \Delta T_{forehead-finger}\right)$$

Equation A2. Gastrointestinal Temperature Prediction

This linear regression model describes a suitable fit between the measured and predicted values of T_{gi}. RMSE analysis of this regression equation established an error of 0.26 °C between actual and predicted T_{gi}. Fig. 2 displays the correlation between the measured and predicted T_{gi}, which exhibits a plateau at higher measured T_{gi}. A second order polynomial trendline was chosen to best represent the associated fit of the correlation ($r^2 = 0.63$).

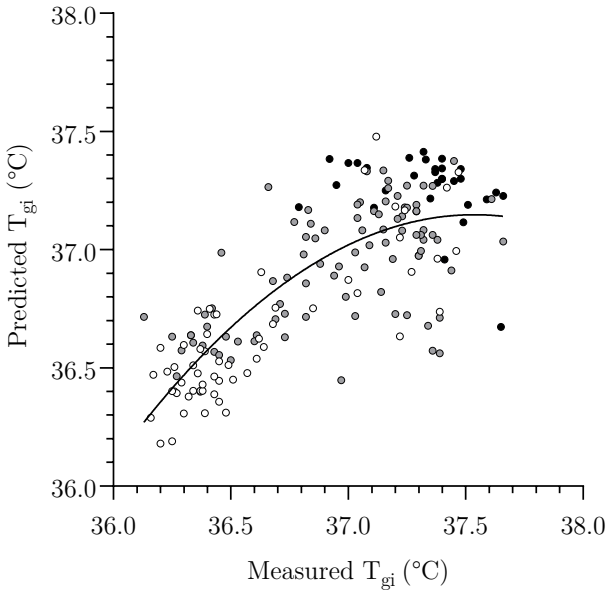

Figure A2: Gastrointestinal temperture (T_{gi}) and predicted T_{gi} relationship using equation 1. Measurements and predictions based on skin temperatures were obtained while participants were exposed to three ambient temperatures: 22 °C (white dots), 25 °C (grey dots), and 35 °C (black dots).

A.5 Discussion

Screening workers for elevated T_b has become of particular importance with the prevalence of two major global maladies, global warming, and the COVID-19 pandemic. Both of which cause dangerous elevations in T_b, and have potentially serious, if not fatal, consequences. Presently, workers in industry are being screened primarily for elevations in T_b arising from

a viral infection. However, in future, any such valid methodology has the potential to be used for monitoring workers for heat strain, particularly during episodes of summer HW. The assessment of the currently used approach for screening for elevated T_b was the aim of the present study. The principal finding was that neither single skin sites (i.e., hand, forehead), nor the proximal-distal skin temperature gradient in combination with Tforehead, were able to provide a physiologically accurate index of body core temperature (i.e., gastrointestinal temperature). The methodological approach of predicting T_b from $T_{forehead}$ is therefore not valid.

A.5.1 Prediction of T_b

The statistical analysis revealed significant correlations between the $T_{forehead}$ and T_{gi}, but the association with absolute T_b on this basis may vary by as much as 2 °C. Although it may seem like forehead is suitable through statistical significance, this correlation is of limited physiological relevance, as it may generate false positive/negative values. Of particular concern is the fact that the correlation becomes statistically non-significant during simulated HW conditions, thus in conditions when an accurate prediction in an industrial setting would be required the most. Whereas the present study used contact thermometry to measure skin temperature, the method of choice in industry is skin temperature measurements made with infrared thermography (IRT). Using this technology, the measurement of surface T_{sk} may be adequate, however, as demonstrated by the results of the present study, the subsequent derivation of T_{gi} from the measurement of T_{sk} at one site, the preferred site being the forehead, is not physiologically valid.

The recent proposal of Mekjavic and Tipton (Mekjavic & Tipton, 2021), which suggests additional sites to that of the forehead alone might provide a better outcome in the prediction of T_{gi}, was also evaluated by conducting a linear regression to calculate T_{gi} with the proximal-distal skin temperature gradient ($\Delta T_{forehead\text{-}finger}$), and skin temperatures. This regression proved statistically significant, resulting in smaller errors in the predictions of T_b. Furthermore, a polynomial curve fit to the relationship between measured and predicted T_{gi}, identified a plateau at higher levels of predicted T_{gi} (Fig. 3). This suggests that the association appears to be accurate at lower temperatures, however, begins to underestimate as T_{gi} gets higher. Residual analysis of independent variables in the regression equation identifies $T_{forehead}$ as a contributor to this plateau, due to increased variability and thus error in higher ambient temperatures. Additionally, whilst average T_{gi} in the HW conditions was 37.3 °C, $T_{forehead}$ only reached 35.5 °C which means it was incapable linearly matching rises in T_{gi} during higher ambient conditions. The combination of these two sources of error would likely cause the plateau in the relationship between measured and predicted T_{gi}, making it unsuitable to use $T_{forehead}$ as a prediction tool. It should also be emphasized that the industrial tasks simulated in the present study were that of checking the functioning of circuit boards; thus, a seated task. Any method for predicting heat strain in an industrial environment will need to be validated with tasks requiring elevated endogenous heat production, further increasing T_{gi} above $T_{forehead}$.

The ΔT_{sk}P-D between the forearm and fingertip has been demonstrated as an appropriate index of the perfusion of the fingers (House & Tipton, 2002; Keramidas et al., 2013; Rubinstein & Sessler, 1990). During exposure to a hot environment, as in the present study, a high distal (fingertip) T_{sk} would reflect vasodilation, thus activation of the thermoregulatory heat loss mechanism. We hypothesized that if $T_{forehead}$ was a valid surrogate of T_b, then combined with an index of peripheral perfusion, such as ΔT_{sk}P-D, this could provide an index of heat strain. However, unlike T_{gi}, $T_{forehead}$ varied with ambient temperature, such that the observed variations in T_{gi} of ± 1.5 °C, were accompanied by variations in $T_{forehead}$ of ± 3.8 °C, casting doubt on the validity of $T_{forehead}$ as a valid surrogate

measurement of T_b. Nevertheless, the $\Delta T_{forehead\text{-}finger}$ alone displayed a significant relationship with T_{gi}. Furthermore, a multiple regression combining ΔT_{sk}P-D with $T_{forehead}$ generated a regression equation, with an improved association with T_{gi}. The physiological validity of the derived regression model should be evaluated with a separate group of female and male subjects, of different ages, under conditions of elevated ambient temperatures, as would be experienced in industry, and during HWs.

A.5.2 Effect of Ambient Temperature

Mass screening of workers for elevated T_{gi} in an industrial setting may help to protect against heat stress or avoid the spread of viral disease. The ambient temperatures at which these measurements are taken may vary depending on the location of the measurement (indoor vs. outdoor), time of day (day shift vs. night shift), weather and season. The large variation in T_{sk}, with little change in T_{gi} is of concern with regard to the association of T_b with T_{sk}. In the present study, measurements taken in normal temperature (22 and 25 °C) ambient conditions provide a statistically significant relationship with T_{gi}, whereas measurements conducted during simulated HW (35 °C) conditions provided no statistically significant relationship. In the present study, increases in T_b were the result of high ambient temperatures. In contrast, a febrile temperature is the result of elevated endogenous heat production combined with decreased heat loss (vasoconstriction). Any method proclaiming to be able to predict T_b of active and/or febrile individuals regardless of the ambient temperature, should be appropriately validated. Manufacturers of currently available scanners based on IRT technology do not provide the algorithms used to predict T_b based on $T_{forehead}$, nor do they provide any information regarding the validation of such algorithms. Due to the proven global importance of screening individuals for elevated T_b, it should only be a matter of time before this is regulated.

A.5.3 Accuracy of IRT to Contact Thermography

The aim of the present study was to assess the association of T_{sk} with T_b using contact thermography, and not to validate IRT as a method for predicting T_b. However, IRT is the most commonly used method of measuring skin temperature in applied settings such as workplaces and hospitals, and its validity and accuracy should be considered in future T_{sk} predictions. The validity of IRT as a measurement of T_{sk} has been heavily debated, particularly with reference to its overestimation and comparison to a 'gold standard' of T_{sk} measurement. Maley, et al. (Maley et al., 2020) propose that during hand rewarming, following cold water immersion, IRT overestimates Tsk measured by contact thermometry by 1.8 °C. However, this was countered by Havenith and Lloyd (Havenith & Lloyd, 2020) who suggest that methodological issues such as camera accuracy and calibration commonly occur, and that contact thermometry cannot be considered a 'gold standard'.

Any system for mass screening of workers based on the prediction of body temperature from forehead T_{sk} derived with IRT would need to utilize an infrared camera of high accuracy as differences occur commonly. Ng, et al. (Ng et al., 2005) reported significant differences among the three infrared scanners used to measure $T_{forehead}$. The differences among these scanners were as high as ± 2.0 °C. Such discrepancies among infrared cameras are also reflected in their ability to accurately measure T_{sk} when compared to contact thermography. Although a strong correlation between contact thermometry and non-contact IRT thermography has been reported (Maley et al., 2020; van den Heuvel et al., 2003), the authors reported that T_{sk} measured with IRT was 2.3 °C lower than that measured with a thermistor (van den Heuvel et al., 2003). The above comparisons were made during a sleep study (van den Heuvel et al., 2003) and at rest (Maley et al., 2020).

During dynamic movement and exercise, as would be anticipated in an industrial setting, the agreement between contact and IRT measurements of Tsk is poor (de Andrade Fernandes et al., 2014; James et al., 2014). Irrespective of the validity achieved by IRT, the type of device specifications stipulated by the 'Journal Temperature Toolbox' (Foster et al., 2021), in application, are unsuitable for many work-places.

A.5.4 Prediction of Deep Body Temperature

Infrared scanners providing a predicted value of T_b based on a measurement of T_{sk} at a single site, do so using proprietary algorithms, which are not available for scrutiny. This is unsatisfactory and unacceptable, considering the impact elevated body temperature, whether due to viral infection or summer HW, has had not only on industry, but all aspects of our lives globally. The present study illustrates the errors in association of T_b with T_{sk} that occur under controlled laboratory conditions, in which the measurements were conducted by trained individuals. It also emphasizes the need to discern between statistical and physiological significance. For example, the correlation between $T_{forehead}$ and T_{gi} (Fig. 1) may be statistically significant, indicating that an increase in one variable is observed as an increase in the other, this relation does not, however, provide an accurate assessment of T_b. Alternatively, using a regression equation of multiple measurement sites provided a significant prediction of T_{gi}, the physiological significance of which is made clear using RMSE. This analysis of the regression equation proposes that the error between actual and predicted T_{gi} is as low as 0.3 °C; enabling more accurate extrapolation of T_{gi} from T_{sk} to occur. For measurements of T_b the difference in values at one site could be the difference between a healthy temperature and heat strain, or fever. It is most likely that future strategies of predicting T_b from exposed T_{sk} may need to incorporate several sites rather than one, as suggested by Mekjavic and Tipton (Mekjavic & Tipton, 2021).

A.5.5 Limitations

As detailed above, differences lie in the mechanisms relating to changes in T_b, leading to differential heating and perfusion responses during either ambient heating or fever. The present study produced an equation for prediction of T_b using several sites, when participants were experiencing ambient heating at rest. Additional testing should consider the T_{sk} and T_b responses to the unique aspects of fever and exercise as methods of heating the human body. In addition, the participants in the present study, young healthy males, did not appear to experience undue heat strain based on their T_{gi}. Though these participants were exposed to the conditions of previously recorded HW (Ciuha, Pogačar, et al., 2019), suggesting other non-thermal factors such as morphology, gender, acclimation, etc., should be considered in prediction algorithm produced. Due to the relatively small and homogenous sample, the results of the present study should only be used as an example of the type of error associated with T_{sk} prediction. Finally, while the study design reflected certain applied conditions such as working schedules and tasks, the external validity should be cautioned and additional research with larger sample sizes in applied conditions advised.

A.6 Conclusions

Measurement of contact T_{sk} at the forehead appears to be a suitable site from which T_{gi} can be extrapolated at lower ambient temperatures. However, while statistically significant, this relationship cannot be considered physiologically appropriate due to an error of ~1 °C. The measurement of multiple sites, including a proximal-distal temperature gradient, may provide a more suitable prediction of T_b with a lower error (0.3 °C), however again this is

not appropriate due to a plateauing of the prediction efficacy at higher temperatures, likely due to lower and more variable T_{sk} measurements. The methodological approach of predicting T_b from T_{sk} is therefore not physiologically valid in young males, particularly in higher ambient temperatures. In future, indirect T_{sk} measurements should consider the effect of ambient temperature, the use of multiple sites including a perfusion index, and the source of raised T_b in their algorithms.

Appendix B

Ethics Document Study 1

dr. Leonidas G. Ioannou
Institut Jožef Stefan
Jamova 39
1000 Ljubljana

Številka: 0120-402/2020/4
Datum: 27. 11. 2020

Zadeva: Ocena etičnosti predložene raziskave
Zveza: Vaša vloga z dne 2. 9. 2020

Komisija Republike Slovenije za medicinsko etiko (KME RS) je dne 2. 9. 2020 prejela vlogo za oceno etičnosti za raziskavo z naslovom »Učinki vročinskega vala na zdravje in produktivnost
delavcev«.

Gre za temeljni podoktorski projekt Z7-9412, katerega vodja je dr. Urša Ciuha v njem bo sodeloval dr. Leonidas G. Ioannou. Raziskava se bo izvajala v okviru ARRS raziskovalnega programa P2-0076 in Obzorje 2020 projekta Heat Shield (št. 284438). Za varnost v raziskavi bo poskrbela prof. dr. Polona Jaki Mekjavic, dr. med. Raziskva bo potekala v Olimpijskem športnem centru Planica (Rateče, Slovenija), v njej bo sodelovalo 10 zdravih moških preiskovancev. Preiskovanci bodo čez dan opravili več različnih kognitivnih, fizičnih, fizioloških in delovnih testov. Sprememba telesne sestave in hidracijskega stanja bo ocenjena na podlagi antropometričnih meritev ter vzorca krvi, ki bodo opravljeni pred in po študiji. Psihološki parametri bodo ocenjeni večkrat na dan z uporabo subjektivnih lestvic.

KME RS je na seji 20. oktobra 20201 obravnavala prejeto vlogo in ugotovila, da je vloga popolna ter ocenila, da je predlagana raziskava etično sprejemljiva. S tem vam za njeno izvedbo izdaja svoje soglasje.

Pri nadaljnjih dopisih v zvezi z raziskavo se obvezno sklicujte na številko tega dopisa.

S spoštovanjem,

dr. Božidar Voljč, dr. med.,
predsednik KME RS

Appendix C

Ethics Document Study 2

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Jason.fisher@ijs.si

Številka: 0120-301/2022/13
Datum: 11. 5. 2023

Zadeva: Ocena etičnosti predlagane raziskave
Zveza: Vaša vloga z dne 15. 7. 2022 , 13. 10. 2022 in 13. 3. 2023

Na Komisiji Republike Slovenije za medicinsko etiko (v nadaljnjem besedilu: KME RS) smo dne 15. 7. 2022 prejeli vlogo raziskave z naslovom »Regionalni pretok krvi kot odziv na spreminjajoče se okoljske pogoje in telesno držo«.

Raziskava bo potekala v okviru Inštituta Jožef Stefan, Avtomatika, biokibernetika in robotika.
V raziskavi bodo sodelovali naslednji raziskovalci:
Mag. Jason Fisher MSc. – glavni raziskovalec
Prof. dr. Igor B Mekjavić - mentor
Prof. dr. Polona Jaki Mekjavić
Dr. Urša Ciuha

KME RS je na seji 16. avgusta 2022 obravnavala prejeto vlogo in ugotovila, da je bilo potrebno vlogo dopolniti:

1. Prosimo vas, da Komisiji posredujete vlogo v slovenskem jeziku;
2. V vlogi so navedeni zgolj akademski nazivi sodelujočih raziskovalcev. Prosimo vas, da navedete tudi strokovne nazive;
3. Natančno opredelite odgovornega zdravnika, ki bo skrbel za varnost oseb, ki bodo sodelovale v raziskavi. Sprememba zunanje temperature od 15^0 C do 40^0 C in obratno, vsako minuto za 1^0 C ter sprememba koncentracije kisika, so po mnenju Komisije dejavniki, ki lahko sprožijo pri sodelujočih v raziskavi življenjsko ogrožujoče stanje. Hkrati vas želimo seznaniti še z dodatnimi dilemami na katere Komisija pričakuje odgovore: kako bo spremljana hipoksija v krvi preiskovancev, podatek o pričakovanih spremembah srčne frekvence ni jasen, kašne so še dopustne spremembe srčne frekvence brez škode za preiskovanca. Nadalje manjkajo medicinski podatki o podhlajevanju telesa. Navedeno je, da bo notranja temperatura spremljana s kapsulo, toda, z medicinskega vidika podhlajevanje telesa ni nedolžna zadeva. Kapsula za merjenje

notranje temperature, (najverjetneje po istem principu kot UZ v kapsuli za pregled tankega črevesa), ne bo imela enotne funkcije, ker bo odvisna od peristaltike posameznika, z različno hitrostjo pasaže skozi telo. Zaradi vsega navedenega je mnenje komisije, da bi moral biti pri preizkusih prisoten zdravnik specialist interne medicine – specialist intenzivne interne medicine. Prav tako smo mnenja, da bi morala biti nenehno navzoča ekipa / team usposobljenega medicinskega kadra in oprema za reanimacijo;

4. Mnenje komisije je, da je skupina sodelujočih, to je študentov še posebej ranljiva skupina. Glede na pričakovano plačilo za sodelovanje v raziskavi, je treba pred vključitvijo teh oseb v raziskavo, poleg pregleda anamneze, opraviti tudi ustrezen zdravniški pregled. Naše mnenje je, da lahko predvideno plačilo pritegne k sodelovanju osebe, ki bi zaradi plačila zamolčale morebitne pomembne zdravstvene težave;
5. Besedilo v obrazcu o zavestni in svobodni privolitvi osebe v raziskavo je nerazumljivo za posameznike, ki nimajo medicinske izobrazbe. Prosimo, da obrazložitev pripravite tako, da bo razumljiva vsakemu sodelujočemu, ne glede na njegovo izobrazbo in znanje;
6. Komisija meni, da mora biti raziskava, ki jo želite izvesti izpeljana kakovostno in predvsem varno. Verjetno obstajajo mednarodni standardi za izvedbo tovrstnih raziskav. Prosimo vas, da navedete kateri so ti standardi in v kolikšni meri ste jim pri zasnovi te raziskave sledili;
7. Ponovno poudarjamo, da je mnenje komisije, da bi se take raziskave morale izvajati v specializiranih laboratorijih ob prisotnosti osebja, ki na podlagi svojega medicinskega znanja in izkušenj varno in kakovostno oskrbi udeleženca, pri katerem se pojavi negativni učinek oziroma nevarna / življenjsko ogrožajoča sprememba vitalnih znakov.

KME RS je dne 13. 10. 2022 prejela dopolnitev vloge ter jo skrbno pregledala in obravnavala na seji 15. novembra 2022. V prejetih dopolnitvah ste upoštevali večino naših priporočil, za kar se vam zahvaljujemo, vendar je bilo potrebno vlogo ponovno dopolniti in sicer:

1. V zvezi s tem vam sporočamo, da je stališče KME RS, da vam omenjene raziskave **ne odobri**, dokler na jasen in dokumentiran način ne predložite ekipe (imena, priimki, strokovni nazivi, usposobljenost in dokazila o licencah za uporabo reanimacijske opreme in izvajanje ukrepov v primeru življenjske ogroženosti), ki bo nenehno bedela in spremljala stanje udeležencev v poskusu, ki ga predvideva vaša raziskava. Izrecno poudarjamo, da mora biti to ekipa, ki nima zgolj teoretičnih znanj glede postopkov oživljanja, ampak mora biti to izkazano tudi v praksi. To pomeni, da so člani ekipe, ki v postopkih oživljanja sodelujejo tedensko / dnevno. Pričakujemo, da bo vodja te ekipe zdravnik specialist z eno od naštetih specializacij: anesteziologija, reanimatologija in perioperativna intenzivna medicina, interna medicina, intenzivna medicina ali urgentna medicina.
2. Glede na to, da v poteku raziskave obstaja tudi možnost uporabe reanimacijske opreme, KME RS meni, da bi bilo treba udeležence, ob podpisu zavestnega pristanka v raziskavo, jasno seznanit z možnostjo zapletov, ki lahko zahtevajo reanimacijske postopke.
3. Prav tako je mnenje KME RS, da tako nevarnih eksperimentov ne bi smeli izvajati na populaciji študentov. Menimo, da v naši visokošolski in pravni kulturi velja, da sme študirajoči računati, da bo pri učiteljih svoje univerze (ne le neposredno nadrejenih) predvsem varen, da od njih zanj ne bodo izhajale nevarnosti za življenje in zdravje ali skušnjave za ogrožanje tako pomembnih dobrin. Učitelj se mora načeloma zadržati in vzdržati, spravljati študirajoče svoje univerze v nevarnost, tudi v zvezi z očitno resno tveganimi znanstveni raziskavami na študentih, ki niso v njihovo neposredno zdravstveno korist.

Dne 13. 3. 2023 ste vlogo dopolnili. KME RS je na seji 18. aprila 2023 ponovno obravnavala vašo vlogo in ugotovila, da je vloga sedaj popolna in raziskava etično sprejemljiva. S tem vam za njeno izvedbo daje svoje soglasje.

Pri nadaljnjih dopisih v zvezi z raziskavo se obvezno sklicujte na številko tega dopisa.

S spoštovanjem,

dr. Božidar Voljč, dr. med.
predsednik KME RS

Appendix D

Ethics Document Studies 3 & 4

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Številka: 0120-180/2023/7
Datum: 27. 7. 2023

Zadeva: Ocena etičnosti predlagane raziskave
Zveza: vaša vloga z dne 14. 4. 2023

Na Komisiji Republike Slovenije za medicinsko etiko (v nadaljnjem besedilu: KME RS) smo dne 14. 4. 2023 prejeli vlogo za oceno etičnosti raziskave z naslovom »Vzajemno delovanje termoregulacije in regulacije krvnega tlaka na regionalno periferno perfuzijo med izpostavljenostjo umetni gravitaciji«.

Namen raziskave je ovrednotiti vpliv spreminjajoče se okoljske temperature na vazomotorični odziv spodnjih in zgornjih okončin. Raziskavo bodo delali pri preiskovancih v ležečem in stoječem položaju in želijo preveriti hipotezo, da vazomotorični odziv v zgornjih okončinah ureja predvsem termoregulacijski sistem, vazomotorični odziv v spodnjih okončinah pa je podrejen regulaciji krvnega tlaka. V raziskavi nameravajo preveriti še dva dejavnika, ki sta pomembna pri regulaciji krvnega pretoka in sicer hipoksija in umetna težnost. S centrifugiranjem bodo vzpostavili umetno zemeljsko težnost, pri čemer bo občutek povečane težnosti podoben nošenju nahrbtnika s težo 30 % telesne teže preiskovanca. Evropska vesoljska agencija je preverila in potrdila varno delovanje centrifuge, mednarodna ekipa zdravnikov pa je prav tako potrdila varnost uporabe centrifuge za preiskovance.

V predlogu je navedeno, da se bo centrifugiranje izvajalo v hipoksičnem okolju, ni pa podatka o tem, za kakšno hipoksijo gre oz. kolikšen bo tlak kisika. Nadalje med izključitvenimi dejavniki nista navedeni koronarna- srčna in možgansko-žilna bolezen, ki se lahko ob hipoksičnih pogojih poslabša, temveč je kot izključitveni dejavnik navedena le periferna arterijske bolezen. Ni tudi opisan nadzor nad morebitnimi zapleti, ki bi se lahko zgodili med centrifugiranjem v hipoksičnih pogojih, zato je vlogo potrebno dopolniti.

KME RS je na seji 16. maja 2023 obravnavala prejeto vlogo in ugotovila, da je bilo vlogo potrebno dopolniti.

Dne 15. 6. 2023 ste vlogo dopolnili s podatkom, da bo delni tlak kisika znašal 133 mm Hg,

prisimulaciji nadmorske višine 4000 m pa bo delni tlak kisika 90 mm Hg. Vlagatelji tudi navajajo, da bodo izključili bolnike s koronarno srčno in možgansko žilno boleznijo. Poleg

tega bo izvedbo celotnega eksperimenta nadzorovalo zdravstveno osebje, ki bo nadziralo življenjske funkcije.
KME RS je na seji 18. julija 2023 obravnavala prejeto dopolnitev vloge in ugotovila, da je vloga popolna in etično sprejemljiva. S tem vam za njeno izvedbo izdaja svoje soglasje.

Dopolnitev vloge (ne pa celotne vloge) pošljite na gp.mz@gov.si.

Pri nadaljnjih dopisih v zvezi z raziskavo se obvezno sklicujte na številko tega dopisa.

S spoštovanjem,

dr. Božidar Voljč, dr. med.
predsednik KME RS

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Publications Related to the Thesis

This thesis is based on the following papers:

Journal Articles

Fisher, J. T., Ciuha, U., Ioannou, L. G., Simpson, L. L., Possnig, C., Lawley, J., & Mekjavic, I. B. (2022). Cardiovascular responses to orthostasis during a simulated 3-day heatwave. *Scientific Reports, 12*(1), 19998.

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Biography

Jason Fisher is a PhD candidate at the Jožef Stefan International Postgraduate School of Ljubljana and a member of the Environmental and Gravitational Physiology Laboratory at the Jožef Stefan Institute. His research focuses on cardiovascular responses to an array of environmental stressors including temperature, hypoxia, and artificial gravity; with a particular focus on the regulation of blood flow. His supervisor for his doctoral dissertation is Professor Igor B. Mekjavic and his co-supervisor is Dr. Urša Ciuha.

The candidate completed his degree in BSc (Hons) Sport and Exercise Science at the University of Portsmouth in 2019. The title of his undergraduate thesis was: "Correct movement patterns in a squat: The relationship between segment lengths and joint angles during a back squat exercise" and mentored by Dr. Joseph O'Halloran.

In 2020, the candidate completed his MSc in Sports Performance at the University of Portsmouth. His masters' thesis was titled "Float first": Categorising physiological and anthropometric factors effecting floating in the general population during water immersion" under the supervision of Dr. Heather Massey and Professor Mike Tipton.

From October 2019 to March 2020, the candidate continued his work in the University of Portsmouth's Extreme Environments Laboratory as a research assistant on a project tilted "The Pathophysiology of Non-Freezing Cold Injuries" under the mentorship of Dr. Jennifer Wright, Dr. Clare Eglin, and Professor Mike Tipton.