UNIVERSITY OF BIRMINGHAM

THE EFFECT OF HEAT THERMOTHERAPY ON CARDIOVASCULAR FUNCTION AND CARDIOMETABOLIC HEALTH

by

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ABSTRACT

Heat thermotherapy (HT) is a promising strategy to improve cardiovascular (CV) function. HT modalities such as sauna bathing and hot water immersion (HWI) have been shown to induce positive acute CV responses and long-term CV adaptations, including artery structural remodelling, lower resting blood pressure (BP), and improved endothelial function; all associated with a lower risk of CV disease. Furthermore, HT could be used alongside high-intensity interval exercise (HIIEx) to augment positive CV adaptations associated with HIIEx alone. Unfortunately, substantial heterogeneity within the HT literature prevents evidence-based HT prescription for CV health. Additionally, common HT strategies can be prolonged and uncomfortable for participants. Therefore, this thesis investigated the efficacy of HT to improve CV function (meta-analysis, **Chapter 2**) and assessed different HT protocols and their acute cardiovascular response in young, healthy adults (**Chapters 4 and 5**).

The meta-analysis (**Chapter 2**) revealed HT improves CV function, as shown by the positive CV response after a single bout of HT (reduced diastolic blood pressure (DBP), mean arterial pressure (MAP) and increased flow-mediated dilation)) and the longer-term CV adaptations (reduced resting DBP and MAP) following multiple bouts of HT. Health status nor age did not affect these changes, demonstrating that HT can improve CV function in various population demographics. **Chapter 4** investigated whether a short, hot, neck-level, 30-minute bathing session (HWI30) can elicit a similar acute CV response as the 60-minute, shoulder/waist level immersion bathing session (HWI60). Additionally, it was hypothesised that there would be no meaningful CV response (i.e. lower BP) in the thermoneutral water immersion condition

(TWI). There was a condition-by-time interaction effect for DBP, MAP, HR and core body temperature (T_c). Despite the higher T_c achieved during and post-bathing for HWI60 than HWI30, the heart rate (HR) elevation and reduction in DBP and MAP were similar for HWI30 and HWI60. Meanwhile, there was a reduction in DBP and MAP during bathing for TWI; however, DBP and MAP values returned to pre-bathing values immediately post-bathing. This demonstrated that HWI30 was a more time-efficient approach that elicited the same BP response as HWI60.

Chapter 5 investigated the acute CV response of HIIEx followed by HWI30 (ExHWI) versus HIIEx followed by TWI (ExTWI). There was a condition-by-time interaction effect for DBP, MAP, HR and T_c. ExHWI led to a greater hyperthermic and CV response than ExTWI, as shown by the greater elevated T_c and HR during bathing and immediately post-intervention. DBP and MAP were similarly reduced during bathing for ExHWI and ExTWI while only ExHWI saw a reduction in DBP and MAP immediately post-intervention. Therefore, ExHWI led to a greater hyperthermic response and lower BP response (immediately post-intervention) than ExTWI. ExHWI, HWI30, and HWI60 caused a robust CV response, including a hypotensive effect during bathing and immediately post-intervention in young, healthy adults. However, this effect was short-lasting, as blood pressure was restored by one-hour post-intervention and did not change 24 hours post-baseline. The thesis has revealed that despite the popularity of researchers using HWI60 within the literature, shorter HT protocols such as HWI30 can result in a similar hypotensive response during and immediately after bathing in young, healthy adults. Yet whether HWI30 or ExHWI can lead to long-term CV adaptations in young, healthy adults is yet to be determined.

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LIST OF ABBREVIATIONS

Abbreviation	Definition
ANS	Autonomic nervous system
BMI	Body mass index
BP	Blood pressure
cAMP	Cyclic adenosine monophosphate
CRP	C-reactive protein
CV	Cardiovascular
CVD	Cardiovascular disease
CVP	Central venous pressure
DBP	Diastolic blood pressure
ExHWI	High-intensity exercise followed by hot water immersion
ExTWI	High-intensity exercise followed by thermoneutral water immersion
FMD	Flow-mediated dilation
HIIEx	High-intensity interval exercise
HIIT	High-intensity interval training
HR	Heart rate
HSP	Heat shock protein
HT	Heat thermotherapy
HWI	Hot water immersion
IL-6	Interleukin-6
LMEM	Liner mixed-effect model
MAP	Mean arterial pressure

MDD	Major depressive disorder
MICE	Moderate-intensity interval exercise
MICT	Moderate-intensity exercise
NO	Nitric oxide
PV	Plasma volume
RH	Relative humidity
ROS	Reactive oxygen species
SBP	Systolic blood pressure
SR	Shear rate
SNS	Sympathetic nervous system
T_c	Core body temperature
T_{rec}	Rectal temperature
TWI	Thermoneutral water immersion
$V0_{2peak}$	Peak oxygen consumption
$V0_{2max}$	Maximal oxygen consumption
WI	Water immersion
W_{peak}	Peak power output

1. GENERAL INTRODUCTION

1.1 Cardiovascular function and disease

1.1.1 Incidence and prevalence of cardiovascular disease

Cardiovascular disease (CVD) is an umbrella term for all diseases of the heart and circulation, including coronary heart disease, stroke, myocardial infarction and congestive heart failure (Cheema et al., 2022). CVD is commonplace within the UK, with over 7.6 million people diagnosed with the condition ((ONS), 2023). In fact, over 50% of the UK population will have a heart or circulatory condition within their lifetime ((ONS), 2023). Alongside the health-related implications of CVD, the estimated £10 billion per annum health care costs to the UK economy are substantial (Waterall, 2019). Therefore, strategies to attenuate CVD in the UK are highly advantageous to the UK population and its economy.

1.1.2. Key components of the cardiovascular system

The cardiovascular system (CV) is a complex organ system composed of the heart, vasculature, and blood. These components transport and exchange respiratory gases, nutrients, waste products and hormones, maintain thermal balance and regulate blood pressure (BP). The medulla oblongata is located within the brainstem. It communicates with higher brain centres, such as the hypothalamus (for temperature regulation (Saper and Lowell, 2014, Lipton, 1973)), and sensory signals from the spinal cord to regulate respiratory and CV control (e.g., heart rate (HR) and BP; (Lipton, 1973)). It governs the CV responses by controlling autonomic nervous system activity ((Ten Donkelaar et al., 2020); ANS). The ANS

consists of sympathetic and parasympathetic activity, which can regulate the CV response by manipulating factors such as vascular tone and heart rate (Shanks and Ramchandra, 2021).

Specifically, elevated sympathetic activity increases HR, whilst elevated parasympathetic activity decreases HR (Kaplan and Talajic, 1991). Additionally, the withdrawal of parasympathetic activity increases HR, whilst the withdrawal of sympathetic activity decreases HR. The ANS controls HR by modifying the repolarisation and depolarisation characteristics of the heart's pacemaker cells (Freeman et al., 2006). When the sympathetic nervous system is activated, it releases norepinephrine, which binds to β1-adrenergic receptors on the heart's pacemaker cells (sinoatrial node). This initiates a series of events that lead to increased depolarization of the heart, resulting in an elevated heart rate (Kaplan and Talajic, 1991). In contrast, elevated parasympathetic activity increases the release of acetylcholine by the vagus nerve. Acetylcholine binds to M_2 muscarinic receptors within the sinoatrial node. These receptors oppose β1-adrenergic receptors and cause hyperpolarization of the sinoatrial node cells, thus slowing down the frequency of action potentials generated by sinoatrial cells and lowering HR (Kaplan and Talajic, 1991).

Stroke volume is elevated by increasing preload (the volume of blood returned to the heart during diastole), reducing afterload (the force opposing the ejection of blood from the heart), and increasing contractility (the myocyte force of contraction, which is independent of preload and afterload (Crandall and Wilson, 2015)). Cardiac output can be increased by elevating HR, stroke volume, or both. While resting, cardiac output is ~6 L/min; manipulating HR and stroke volume can increase this significantly (Rowell, 1974), although cardiac output

is affected by gender, age and aerobic training (Lavie et al., 2015). For example, During maximal exercise, an untrained individual's cardiac output is ~20L/min, whereas an elite aerobic athlete's can reach ~40 L/min (Rivera-Brown and Frontera, 2012, Arena et al., 2008). Alongside fitness, males on average have a 25% higher cardiac output than females, with ageing causing a progressive decline in both men and women (Rivera-Brown and Frontera, 2012).

1.1.3 Blood flow regulation

Blood vessels (arteries, veins, and capillaries) contribute to the transportation and exchange of respiratory gases and nutrients. As the CV system is a closed-loop system, two factors affect the rate and amount of blood flow: the pressure differential (e.g., the BP from the aorta vs. the right atrium) and the resistance to flow (Badeer and Hicks, 1992). According to the unified theory of power laws for flow resistance (Chen, 1991), the relationship between pressure differential and resistance to flow is as follows:

$$Flow = \frac{Pressure\ differential}{Resistance\ to\ flow}$$

The pressure differential between the aorta and the right ventricle (e.g., 70-100 mm Hg vs. 0-4 mm Hg (Bellhouse, 1969)) results in forward flow from the aorta through the circulatory system and back to the right ventricle. Within the CV system (i.e., arteries, arterioles, veins, venules and capillaries), the arterioles (due to a large amount of smooth muscle) cause the greatest resistance to blood flow and subsequent drop in BP within the circulatory system (Zelis, 1983); whereas the veins within the lower periphery, such as the legs, have the

greatest impact on blood flow to the heart (venous return) via increased/decreased venous tone (Rothe, 1986).

According to Poiseuille's law (Pfitzner, 1976), blood viscosity and a vessel's cross-sectional area impacts resistance to flow through the vasculature of the circulatory system. As shown in the following equation (Pfitzner, 1976):

$$R = \frac{8\eta\ell}{\pi r^4}$$

Where R, resistance; η, blood viscosity; ℓ, length of vessel; r, radius of vessel

Blood viscosity usually remains constant (although it can increase from prolonged exposure to hot environments due to dehydration (Ahmadizad et al., 2011)). Therefore, a vessel's cross-sectional area is the primary determinant of blood flow resistance. Indeed, throughout the arterial arm of the CV system, blood flow is controlled by manipulating the vascular smooth muscle, wherein constricting or dilating the arterioles increases/decreases the blood flow passing through a specific vessel (Zelis, 1983). Vascular smooth muscle is usually in some state of contraction, also known as vascular tone (Williams, 1998). Basal vascular tone is achieved by constant sympathetic activation via the release of norepinephrine, which binds to α-adrenergic receptors and causes smooth muscle contraction (Amiya et al., 2014). Vascular tone can be elevated, typically via sympathetic activation, leading to increased peripheral resistance, vasoconstriction and reduced blood flow (Williams, 1998).

Vascular tone is modulated via CV control centres in the brain, local stimuli (e.g. heating of a specific limb (Williams, 1998)), changes in vessel transmural pressure (Blair et al., 1959),

metabolic substances (e.g. carbon dioxide (Shi et al., 2020)) and hormones (e.g. angiotensin II, adrenaline and cortisol (MacGregor et al., 1981)). Neurohormonal control of vascular tone occurs through the medullar CV control centres in the brain (which control the ANS and stimulate the production of hormones, including catecholamines) by responding to sensory feedback from chemoreceptors and baroreceptors (detailed further in section 1.1.4) and the renin-angiotensin-aldosterone system (Amiya et al., 2014). This pathway is important to maintain healthy BP control and blood flow (MacGregor et al., 1981). However, consistently elevated levels of renin are associated with an increased risk of hypertension and kidney disease (Carey, 2015).

Whilst central factors such as the CV control centre are important for vascular tone, local factors such as transmural pressure and metabolic substances are still important. For example, metabolic substances such as adenosine, carbon dioxide, and hydrogen ions (Shi et al., 2020) are released as by-products during elevated metabolic activity, i.e. strenuous exercise, which results in smooth vascular muscle relaxation and causes the vessel to dilate, thereby increasing blood flow (Shi et al., 2020). Meanwhile, for local control of the vascular smooth muscle via myogenic autoregulation (response to transmural pressure), the equation is as follows:

$$Wall\ tension\ = (Transmural\ pressure\ \times\ Vessel\ radius)/Wall\ thickness$$

Transmural pressure (BP, which affects the whole blood vessel), shear stress (the dragging force of the blood against the intima layer where the endothelium is located), the elasticity of the vascular wall, and endothelial function regulates changes in vessel diameter, ultimately affecting blood velocity and therefore flow within the vessel (Green and Smith,

2018). For example, increased transmural pressure results in vascular smooth muscle contraction and vasoconstriction (Blair et al., 1959), which prevents an increase in blood flow further downstream (i.e., to the capillaries). This relationship is the same if transmural pressure decreases as the smooth muscle relaxes, dilates the vessel, and prevents a reduction in blood flow. Therefore, changes in transmural pressure and metabolic substances have an important role to play in manipulating vascular tone.

Alongside the utility of vascular smooth muscle to regulate blood flow, the compliance (elasticity) of the vessel helps regulate blood flow and BP and ensure a steady, rather than pulsatile, blood flow in the conduit arteries and beyond (Belz, 1995). For example, the aorta stretches during systole when blood is ejected from the left ventricle, but during diastole the aorta recoils, acting as a "secondary pump", pushing blood into the conduit arteries (Nichols et al., 2022). The purpose of this is twofold. Firstly, this means that there is a steady supply of blood flow to the coronary arteries. Secondly, it dampens BP and changes blood flow from pulsatile to steady, preventing any BP-related damage (Nichols et al., 2022). Unfortunately, due to ageing, arteries begin to stiffen due to elastin degradation within the artery (Pierce et al., 2022). As a result, the elastin is replaced by collagen which makes the artery less compliant (Lacolley et al., 2017). Thus, a linear relationship exists between ageing, aortic stiffness and increased pulse pressure (Said et al., 2018).

1.1.4 Blood pressure regulation

Arterial baroreceptors in the carotid sinuses and the aortic arch regulate short-term changes in BP, responding to BP increases or decreases within two heartbeats (Thrasher, 2005). When

BP increases, the baroreceptors send a signal to the medulla oblongata, which sends parasympathetic nerve impulses to the heart and periphery and suppresses sympathetic nerve impulses. This reduces HR and increases vasodilation of the conduit arteries, subsequently reducing venous return, preload and cardiac output, thus lowering BP (Wehrwein and Joyner, 2013). On the other hand, a drop in BP increases sympathetic nerve impulses, increasing HR, vascular tone and venous return, and subsequently restoring BP (Wehrwein and Joyner, 2013). Effective baroreceptor function is important for postural positioning; for example, when moving from a supine to a standing position, without effective baroreceptor functioning, blood would pool in the lower extremities, resulting in syncope (Mosqueda-Garcia et al., 1997). Alongside the baroreceptor response, peripheral chemoreceptors also regulate BP (Nattie and Li, 2012). Chemoreceptors are sensitive to the concentrations of hydrogen ions in the blood. Increased hydrogen ions increase chemoreceptor activation, which increases pulmonary ventilation. This increased pulmonary ventilation (due to increased hydrogen ion concentration) can inhibit the medullary vagal centre, thus decreasing parasympathetic nerve impulses to the heart. This, in turn, increases HR and BP (Nattie and Li, 2012). Peripheral chemoreceptors also increase sympathetic nerve impulses, causing an increase in vascular tone, HR and BP (Guyenet et al., 2009). Therefore, the baroreceptor and chemoreceptor responses are important in controlling BP.

1.1.5 Endothelial function

A monolayer of endothelial cells within a blood vessel's intima helps maintain vascular function (Krüger-Genge et al., 2019). These endothelium cells regulate systemic vascular resistance by increasing or decreasing vascular tone via the release of vasoactive agents,

including nitric oxide (NO) for vasodilation and endothelin-1 for vasoconstriction (Deanfield et al., 2007). A healthy endothelium is essential as it also provides anti-inflammatory and antithrombotic functions, helping to prevent plaque buildup (Eelen et al., 2015). Endothelial function is the ability of the endothelium within conduit arteries to respond to an increase or decrease in transmural pressure (Green and Smith, 2018). It is mediated by mechanotransduction, which begins a cascade of events resulting in the production of vasodilator/vasoconstricting substances (Green and Smith, 2018). Endothelial function is central to attenuating vessel damage from increased BP as it helps to regulate blood flow to downstream organs and tissue by increasing or decreasing the vascular conductance of large resistance arteries (Lind et al., 2011).

Shear stress is the frictional force of blood flow against the intima of the vessel wall (Papaioannou and Stefanadis, 2005). Typically, blood viscosity is assumed to be consistent across participants and over time (Gnasso et al., 1996). Therefore, shear rate (SR) is commonly used in the literature and is assessed non-invasively via ultrasound. The equation for SR is as follows:

$$Shear\ Rate\ = 4\ \times\ (mean\ blood\ velocity/\ diameter)$$

Increased antegrade (forwards) SR and decreased retrograde (backwards) SR have been established as the pathway to improved vascular function (Green et al., 2017). To illustrate this, Carter et al. (2013a) performed simultaneous bilateral SR and artery diameter measurements in the radial and brachial arteries while manipulating SR in one arm (via supra-systolic cuff inflation on the upper arm) during leg cycling. They found that no

manipulation of antegrade SR in the non-cuffed arm increased radial and brachial artery diameter following leg cycling. In contrast, inhibiting SR in the cuffed arm caused no changes in radial or brachial artery diameter (Carter et al., 2013b)). Similar findings have been observed in local arm heating (Carter et al., 2013b) and lower-limb heating (Carter et al., 2014). In a study by Carter et al. (2014), young healthy adults completed eight weeks of bathing (24 sessions for 30 minutes at 40 °C (waist-level immersion)(Carter et al., 2014). During bathing, a BP cuff was inflated on one forearm to prevent an increase in SR. Post-intervention, brachial artery FMD increased in the uncuffed arm (5.2±1.9 to 7.7±2.6%), but there was no FMD increase in the cuffed arm. Therefore, the authors postulated that preventing an increase in shear stress prevented the increase in brachial FMD post-intervention. Similar effects have been seen with exercise. For example, brachial artery wall thickness reductions were not observed after eight weeks of handgrip exercise training in the cuffed arm compared to the uncuffed arm (Thijssen et al., 2011). Therefore, it appears that increased antegrade SR is a necessary mechanism to cause functional (improved endothelial function as shown by increased FMD) and structural changes (such as arterial remodelling) in the vasculature.

Increased antegrade SR results in a cascade of molecular mechanisms within the endothelial cells which eventually leads to increased blood vessel vasodilation (Carter et al., 2013b). Increased antegrade SR is detected by mechanosensation within the endothelial cells, which is converted into a cascade of intracellular signalling pathways (Desjardins and Balligand, 2006). Firstly, there is an influx of calcium ions into the endothelial cells, which leads to the activation of endothelial nitric oxide synthase (eNOS) via activating AMP-activated protein

kinase. eNOS catalyses the conversion of L-arginine to L-citrulline, and NO is created during the process. NO diffuses into the smooth muscle, which upregulates cyclic guanosine monophosphate production and leads to muscle cell relaxation (Desjardins and Balligand, 2006). Increased antegrade SR is associated with improvements in acute endothelial function, as demonstrated by an increase in FMD. Tinken et al. (2009) showed that antegrade SR was increased by 30 minutes of handgrip exercise, cycling exercise, or forearm heating in young adults, as demonstrated using an FMD assessment before and 30 minutes post-interventions. Furthermore, this increase in antegrade SR was associated with increased brachial artery FMD within all three conditions. Interestingly, despite the different mechanisms involved in increasing antegrade SR (for example, with handgrip exercises, FMD increases are metabolically driven whilst forearm heating is thermoregulatory driven), FMD increases were similar across conditions. Thus, the authors proposed that antegrade SR increases were the ultimate mechanism for acute FMD changes, possibly leading to long-term artery structural adaptations.

1.2 Cardiovascular disease pathology

CVD-related deaths are often caused by a condition known as atherosclerosis, which involves the thickening and hardening of the arterial walls (Rafieian-Kopaei et al., 2014). Atherosclerosis begins with the accumulation of lipids in the artery walls during childhood and can progress to form atherosclerotic plaques (McGill et al., 2000). These plaques can lead to vascular smooth muscle hypertrophy and, in conjunction with the buildup of foam cells and fatty lipids, can obstruct blood flow (Frąk et al., 2022). A reduction in oxygenated blood flow results in tissue ischemia (Rafieian-Kopaei et al., 2014). Ultimately, the excessive

buildup of extracellular lipids can weaken and rupture the arterial wall, releasing a thrombus that can block smaller upstream vessels (Frąk et al., 2022). Atherosclerosis in critical areas such as the coronary arteries significantly increases the risk of myocardial infarction and heart failure (Cheung et al., 2000). Crucially, atherosclerosis typically begins with endothelial dysfunction and is more likely to occur in a pro-inflammatory environment (Frąk et al., 2022).

1.2.1 Endothelial dysfunction

Endothelial dysfunction is the inability of the endothelium within conduit arteries to respond to an increase/decrease in transmural pressure and the reduced production of vasodilator/vasoconstricting substances (Endemann and Schiffrin, 2004). Endothelial dysfunction is characterised by a proinflammatory and prothrombotic state, with reduced vasodilation and the beginning of artery thrombus formation (Gallo et al., 2021). Several reviews have discussed the causes of endothelial dysfunction (Poredos et al., 2021, Cai and Harrison, 2000), which demonstrate that the likely causes are a pro-inflammatory environment, such as increased reactive oxygen species damaging endothelial cells, increased pro-inflammatory cytokines, and increased leukocyte adhesion to the artery walls. By initiating the vascular remodelling of resistance arteries (Masi et al., 2019), endothelial dysfunction is a precursor to hypertension and ultimately CVD (Ross, 1999).

1.2.2. Hypertension

In adults, a healthy BP range is defined as a systolic (SBP) of 100-120 mm Hg and a diastolic (DBP) BP of 60-80 mm Hg (Flack and Adekola, 2020). Hypertension is defined as consistent

systolic BP (SBP) >140 mm Hg and diastolic BP (DBP) >90 mm Hg (stage one (Stergiou et al., 2021)). The severity of the hypertension experienced leads to a higher risk for CVD-related incidences; for example, stage two hypertension (SBP > 160 mm Hg; DBP > 100 mm Hg) has a higher likeliness of CVD-related incidents than stage one (Stergiou et al., 2021). Hypertension is caused by sustained elevated systemic vascular resistance, cardiac output, or both (Delacroix et al., 2014). In hypertensive individuals, cardiac output is typically elevated via increased blood volume, which increases ventricular preload and thus stroke volume. This increased blood volume is sustained by renal sodium and water retention, typically seen by individuals with a high dietary salt intake (Delacroix et al., 2014). Meanwhile, increased systemic vascular resistance is associated with overactive sympathetic activity (Joyner et al., 2008), chronic low-grade inflammation (Kressel et al., 2009) and atherosclerosis (Chobanian and Alexander, 1996). Modifiable lifestyle factors such as diet, hypertension, smoking, and physical inactivity are attributable to 79% of CVD-related deaths in the UK (Cheema et al., 2022), and of these, hypertension is the leading contributory risk factor for CVD-related deaths in the UK (Cheema et al., 2022). Thus, it is not surprising that hypertension is of significant concern in the UK, with over 33% of adults predicted to have high BP and up to 50% not receiving any treatment ((ONS), 2023). Therefore, by preventing/treating hypertension, the risk of CVD-related mortality will be lower.

1.2.3 Mood and cardiovascular disease risk

Alongside traditional risk factors, disturbed sleep (Wang et al., 2021), chronically elevated stress (Lagraauw et al., 2015), and depression (Krittanawong et al., 2023) can increase CVD risk. Indeed, CVD and depression are interrelated, whereby individuals with CVD are

predisposed to depression and the severity of depression correlates with the likelihood of developing CVD and vice-versa (Frasure-Smith and Lespérance, 2005, Carney and Freedland, 2017, Whooley and Wong, 2013). As described above, consistently elevated vasoconstriction and inflammation can lead to endothelial dysfunction and hypertension, thereby increasing the risk of CVD (Gallo et al., 2021). Alongside depression, chronically elevated levels of stress are associated with developing CVD (Batty et al., 2014, Rosengren et al., 2004). Stress increases sympathetic nervous system (SNS) activation, thus increasing the inflammatory cell input and establishing chronic low-level inflammation within the vasculature (Brotman et al., 2007). Therefore, both depression and stress increase pro-inflammatory responses and are linked to a higher risk of endothelial dysfunction, ultimately leading to the development of CVD. Depression, sleep quality and chronic stress are typically interlinked with individuals having a combination of these factors (Wallace et al., 2017). Interventions that can address these independent or combined risk factors would reduce the likelihood of developing CVD.

1.2.4 Young adults and cardiovascular disease

Healthy young adults (<35 years) have a lower CVD risk than their older counterparts (Dhingra and Vasan, 2012). This is because ageing is associated with systemic chronic low-level inflammation (Sanada et al., 2018), arterial remodelling (due to elastin being lost and replaced by collagen (Laurent and Boutouyrie, 2020)) and plaque buildup leading to advanced atherosclerosis (Wang and Bennett, 2012). However, lifestyle choices may increase the risk of CVD development at a younger age. Firstly, obesity is prevalent in the UK, with over 33% of inhabitants having a body mass index above 30 kg/m^2 (Abarca-Gómez et al., 2017). Secondly, young adults (aged 16-24 years ≈ 14%) in the UK do not meet the physical

activity guidelines (Services and Safety, 2011), which increases CVD risk (Cleven et al., 2020). Thirdly, young people in the UK are associated with high perceived levels of stress (Act, 2011) and regularly experience anxiety/depressive symptoms (Griffin, 2010). As discussed previously, endothelial function is affected by the pro-inflammatory environment that physical inactivity, depression, and stress contribute to. Therefore, strategies to combat CVD risk are imperative for young adults in the UK.

1.3 Common strategies to reduce the risk of developing CVD

Addressing modifiable lifestyle factors such as hypertension is an effective strategy to reduce the risk of CVD-related incidents. BP reductions have clinically meaningful CV health implications for both normotensive and hypertensive individuals (Cook et al., 1995). For example, a 2 mm Hg reduction in diastolic BP (DBP) results in a 6% reduction in CVD risk for hypertensive individuals (Cook et al., 1995). Furthermore, a meta-analysis (Bundy et al., 2017) showed that individuals with a SBP within the range of 120-124 mm Hg had a lower incidence of CVD than those with an SBP ≥134 mm Hg. A typical approach to prevent and treat CVD is physical activity and exercise(e.g. meeting the recommended 150 min/week of moderate-intensity or 75 min/week of vigorous-intensity aerobic exercise (GOV.UK, 2019)). Pharmaceutical interventions may also be used, yet these are costly and have a low adherence rate. A study by Sung et al. (2009) demonstrated that over 50% of patients stop taking antihypertensive medication after 12 months (Sung et al., 2009). Similarly, despite its efficacy in reducing CVD risk (Fiuza-Luces et al., 2018), exercise has low adherence rates due to time constraints, cost of participation (e.g.. gym memberships) and confidence to engage inan exercise programme (Standage and Ryan, 2012). Therefore, there is a need for

alternative interventions that are cost-effective, practical, and have the potential for higher adherence rates compared to traditional intervention strategies.

1.4 Thermoregulation

1.4.1 Biophysical factors

Maintaining core body temperature (T_c) is essential for human survival and metabolic function. Human thermoregulation is influenced by internal (body) and external (environmental) factors affecting heat retention or loss (Cramer and Jay, 2016). These factors include metabolic energy expenditure, convection, conduction, radiation, and evaporation from the skin's surface (Gagge and Nishi, 1977). The biophysics of human heat balance can be summarised using the following equation (Parsons, 2019).

$$S = M - W_k \pm K \pm R \pm C - E_{res} - E_{sk} \, [W]$$

Where S; body heat storage, M; metabolic energy expenditure, W_k; external work, K; conduction, R; radiation, C; convection, E_{res}; evaporative heat loss from the respiratory tract, E_{sk}; evaporative heat loss from the skin, W; Watts. Heat balance is defined as S=0, whilst S>0 indicates increased body heat storage.

Metabolic heat production is divided into metabolic heat expenditure (80%) and mechanical work (20%). As exercise intensity increases, metabolic heat expenditure also increases markedly. For example, the body produces 100 W of metabolic heat while standing, but when running at 16 km/h it can increase to 1500 W (Barnes and Kilding, 2015). In addition to exercise intensity, external factors also play a significant role in body heat storage.

Radiation and convection are the primary sources of external heat gain in warm environments. Radiation is the heat transferred from electromagnetic waves between the environment and the human body (Parsons, 2019) and is particularly prevalent in outdoor environments (i.e., from direct sunlight) but can also occur indoors (i.e., in infrared saunas). The absorbency of radiant heat depends on the exposure, the surface area exposed, and the absorbency of the clothes worn (Cramer and Jay, 2016). Convection is the transfer of heat exchange from fluids or gases to the human body, such as warm air or hot water. The absorbency of convective heat depends on the surface area exposed and the thermal convection rate of the gas/fluid (Cramer et al., 2022). For example, convection in still air depends upon the skin-air temperature gradient and air density (Nishi and Gagge, 1977). Conduction is the transfer of heat between solid surfaces in contact with each other. Typically, conduction in an external environment is considered negligible unless the skin is in contact with a solid surface for a long period, such as when the skin is exposed to ice vests (Ross et al., 2013) or warming blankets (Giuffre et al., 1991).

1.4.2 Thermoregulatory responses to heat stress

Heat stress can either be passive (T_c increases while resting in a hot environment) or active (T_c increases while exercising in a hot environment). Thermoreceptors in the skin (cutaneous thermoreceptors) and within the preoptic-anterior hypothalamus detect changes in skin and T_c , respectively (Schlader et al., 2018). This sensory afferent feedback is sent to the preoptic/anterior hypothalamus, which initiates efferent heat loss responses to return T_c to homeostasis and allostasis (Nakamura, 2011). For example, during heat stress, the hypothalamus responds by sending signals to effectors like skeletal muscles, skin blood

vessels, and sweat glands to restore T_c to baseline values. (Kanosue et al., 2010). Thus, peripheral and brain thermoreceptors allow humans to sense and determine the response to external environmental temperatures (e.g. moving away from an environment or initiating physiological heat loss mechanisms (Bulcao et al., 2000)).

1.4.2.1 Physiological heat loss mechanisms

Increases in T_c ($\Delta \geq 0.3°C$) can activate physiological heat loss mechanisms such as sweating (Brengelmann and Savage, 1997, Gisolfi and Wenger, 1984). Ultimately T_c is the primary driver for autonomic thermoeffector activation; however, as both skin and T_c inputs are integrated in the CNS, skin temperature can modify autonomic thermoeffector activation (Kanosue et al., 2010). For example, elevated skin temperature can lower the T_c threshold for when sweating is initiated. Elevated skin temperature leads to redistribution of blood flow from the internal organs to the cutaneous vasculature, which represents the hallmark of the CV response induced by heat stress (Rowell, 1974). As skin temperature rises, cutaneous blood flow will increase until a plateau (at ~7-8 L/min) once skin temperature has reached ~42-43°C (Rowell, 1974). Ultimately, the redistribution of blood flow means that over 50% of the total cardiac output is distributed to the cutaneous vasculature when experiencing heat stress (see Figure 1.1; (Rowell, 1974)).

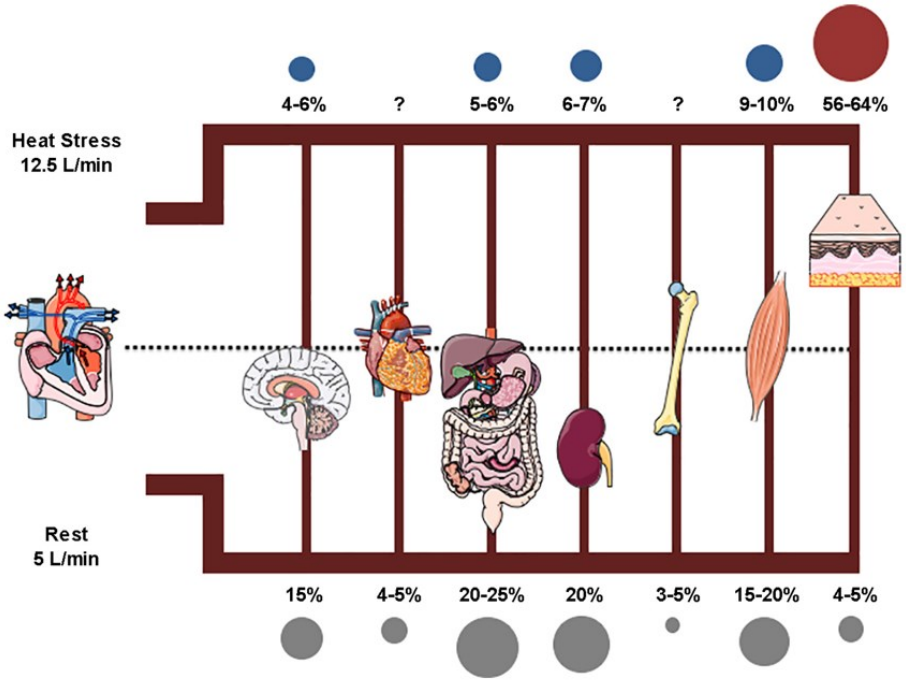

Figure 1.1 The change in blood flow from resting to during whole-body passive heat stress. The figure is from Cheng *et al*. (2019) with permission from the editor. Grey circles indicate the percentage of cardiac output at rest, and red circles indicate the percentage increase in cardiac output. In contrast, blue circles show decreased cardiac output due to whole-body passive heat stress.

Changes in skin blood flow can occur via local control (skin temperature changes) and reflex control (SNS activity) (Schlader and Vargas, 2019). Reflex control is mediated by sympathetic vasoconstrictor and active vasodilatory systems. During heat stress, sympathetic vasoconstrictor neural activity is reduced and sympathetic vasodilatory activity is increased (Johnson et al., 2011). As T_c is elevated, increased sympathetic activity causes the stimulation of sympathetic cholinergic nerves, which release acetylcholine (Kellogg Jr et al., 1995). Acetylcholine causes NO and prostaglandin production and release from endothelial cells, with these vasodilatory substances diffusing into the smooth muscle (Kellogg Jr et al., 1995). This results in the relaxation of vascular smooth muscle and increased vasodilation of the arteries (Greaney et al., 2016). Ultimately, cholinergic nerves account for approximately 80-95% of cutaneous blood flow increases during passive heat stress (Greaney et al., 2016), primarily mediated by elevated T_c. For local control, elevating the skin temperature in a

specific limb increases cutaneous blood flow in that area, which plateaus when skin temperature reaches 42-43°C (Minson et al., 2001). This process is mediated by an axon reflex that reduces the activity of cutaneous vasoconstrictor nerves (Schlader et al., 2018), which is then followed by a rise in NO to facilitate a sustained elevation in cutaneous blood flow (Minson et al., 2001).

Evaporation of sweat from the skin surface is the most effective means of reducing body heat storage, with each litre of sweat evaporated from the skin's surface transferring up to 2400 kJ of heat energy to the environment (Cramer and Jay, 2016). Evaporation is a two-step process. Firstly, sweat is secreted onto the skin's surface and is turned from a liquid into a vapour. Secondly, the vapour is diffused across the boundary layer of the skin into the external environment (Cramer et al., 2022). Air velocity, skin wetness, and the exposed surface area influence sweat evaporative heat loss. For example, increased air velocity enhances evaporative and convectional cooling of the skin (Adams et al., 1992, Clifford et al., 1959), which may occur in outdoor environments (or indoors if a fan is used). Despite its effectiveness in reducing body heat storage in dry conditions, evaporative heat loss via sweating is less effective in humid conditions (Gagnon and Crandall, 2018). Therefore, in hot, humid conditions, humans are at a higher risk of a sharp rise in body heat storage and potentially hyperthermia.

Respiratory heat exchange is another physiological mechanism for reducing body heat storage. During breathing, the respiratory tract inspires, warms, and humidifies the air before releasing (expiring) the warm, humid air into the external environment. Respiratory heat loss

is most effective in cold, dry environmental conditions; however, as air has poor thermal conductivity, the respiratory system has a minimal impact on whole-body heat loss (Cramer and Jay, 2016). Respiratory heat loss will also be impaired if the person breathes in warm, humid air, such as in tropical environments or steam rooms.

1.4.2.3 Heart rate

Increased cutaneous blood flow due to heat stress results in a large decrease in vascular resistance (Rowell, 1977), which lowers venous return and, subsequently, central venous pressure (CVP). If CVP is not restored, BP will fall (Crandall et al., 2008). To prevent a reduction in BP, cardiac output increases via increased HR alongside renal and splanchnic blood flow restriction (Rowell, 1974). The magnitude of the increase in cardiac output depends on the duration, heat severity, and heating modality utilised (Crandall and Wilson, 2015). Generally, the greater the heat stress, the greater the cardiac output needed to match the increased demand for cutaneous blood flow.

The increased ANS activity and T_c mediate HR elevations during heat stress (Jose et al., 1970, Gorman and Proppe, 1984). Increases in sympathetic activity and suppression of parasympathetic activity elevate HR by accelerating the pacemaker signal to adjacent myocytes (enhancing conductivity) (Crandall and Wilson, 2015, Kontaxis et al., 2019). This ANS activity accounts for 60% of the rise in HR during heat stress (Gorman and Proppe, 1984). The remaining rise in HR is likely caused by increased metabolic activity due to the Q_{10} coefficient (Commission, 2001). According to this principle, cellular components have increased kinetic energy at higher temperatures, which speeds up biochemical processes and

physiological functions such as HR conductivity and contractility (Jose et al., 1970). Indeed, it has been shown that following a pharmacological autonomic blockade (preventing ANS-mediated HR responses), a 7 $b \cdot min^{-1}$ increase in HR still occurred per 1°C increase in T_c, accounting for 40% of the increase in HR during heat stress (Jose et al., 1970). Therefore, HR during heat stress is affected by increased metabolic and sympathetic activity and the need to increase cardiac output due to the redistribution of blood flow to the cutaneous vasculature.

1.4.2.4 Stroke volume

Passive heating seems to minimally effect stroke volume (Crandall and Wilson, 2015), but it does increase cardiac sympathetic outflow, increasing the contractility of atrial and ventricular myocytes (Shibasaki et al., 2015). In a controlled environment, an inotropic increase in HR is accompanied by increased stroke volume, suggesting that increased myocardium contractility should improve stroke volume (Ilebekk and Kiil, 1979). However, passive heating also evokes physiological responses that reduce stroke volume. Specifically, heat stress-related reductions in CVP and blood volume leads to a decrease in cardiac preload and, subsequently, stroke volume (Crandall et al., 2008). Nonetheless, diastolic function improves due to reduced afterload and increased inotropy (Nelson et al., 2010) which causes a leftward and upward shift on the Frank-Starling curve (Wilson et al., 2009). Therefore, diastolic function does improve during passive heat stress; however, changes in cardiac output are still extensively mediated by heart rate.

1.4.2.5 Blood pressure

Heat stress leads to increased cardiac output to the cutaneous vasculature for cooling (Rowell et al., 1969). Eventually, this can lead to an imbalance between cardiac output and venous return, reducing CVP and, ultimately, DBP (Crandall et al., 2008). For example, under normal body temperature conditions, previous research has shown that a reduction in venous return (achieved through lower body negative pressure) leads to a decrease in CVP and, therefore, a reduction in DBP (Johnson et al., 2014). However, venous return is reduced during heat stress without the need to apply a lower body negative pressure intervention. For example, in a study by Crandall et al. (2008), ten young adults underwent a passive heating protocol in a water-perfused suit (46-48°C water) until T_c increased ≥ 1°C. Cutaneous blood flow increased fivefold at this T_c, whilst CVP decreased from 5.5±0.7 to 0.2±0.6 mm Hg. This resulted in a mean arterial pressure (MAP) reduction of ~-6mm Hg (and presumably a reduction in DBP, although this was not measured directly) Crandall et al. (2008). Skin temperature was also clamped at ~38°C, resulting in a consistently elevated cardiac output to the cutaneous vasculature. Reducing the cardiac output to the cutaneous vasculature can restore BP to pre-heat stress values. Indeed, changes in skin temperature can significantly impact systemic vascular resistance as hyperthermic-related drops in BP are restored with skin cooling (Lucas et al., 2010). Therefore, reducing cardiac output to the cutaneous vasculature can address the imbalance between cardiac output and venous return during heat stress, thus restoring BP values.

1.4.2.6 Behavioural thermoregulatory response to heat stress

Behavioural thermoregulation is a key non-physiological mechanism in controlling T_c (Schlader et al., 2011) and can be considered the "first line of defence" in preventing a sharp rise in T_c (Taylor et al., 2008). Changes in thermal sensation (Mower, 1976) and thermal comfort (Cabanac, 1971) lead to conscious behavioural adjustments, such as removing layers of clothing, seeking cooler environments, and having a greater desire for cold beverages (Taylor et al., 2008). As T_c changes are too slow to be a primary signal (Weiss and Laties, 1961), skin temperature is likely the main input for thermoregulatory behaviour changes (Schlader et al., 2009). The main advantage of skin temperature as the primary behavioural input is that a change in behaviour, such as removing a layer of clothing, can occur before the need to activate energy-consuming autonomic thermoregulatory responses (Romanovs.ky, 2007). Therefore, thermoregulatory behavioural responses are key to preventing increases in T_c.

1.5 Heat thermotherapy

Heat stress can be detrimental to sports performance (de Korte et al., 2021) and health (e.g., during heat waves (Franklin et al., 2023)); however, when the correct protocol is used, regular bouts of heat stress can lead to positive long-term adaptations to CV function (Cheng and MacDonald, 2019). For example, increased cutaneous blood flow during heat stress can lead to long-term structural artery adaptations that can lower CVD risk (Brunt et al., 2016a, Larson et al., 2020). Heat Thermotherapy (HT) is the application of a passive (non-exercising) heating stimulus that increases T_c and results in a beneficial health outcome (Cheng and

MacDonald, 2019). Recent literature has demonstrated the positive cardiometabolic health and CV function benefits of HT, including reductions in BP (Roxburgh et al., 2023), inflammatory markers (Ely et al., 2019a), and improved endothelial function (Cheng et al., 2021), all of which are associated with a reduction in CVD-related mortality. CV function and cardiometabolic health improvements have been observed in various populations, ranging from young and healthy cohorts (Brunt et al., 2016a) to chronic heart failure patients (Kihara et al., 2002). Additionally, adherence rates to HT are higher than those observed in exercise interventions (Akerman et al., 2019, Naumann et al., 2020). Therefore, preliminary evidence suggests that HT may be a practical and easily accessible method to help prevent the progression of non-communicable diseases in various population groups.

1.5.1 Heat thermotherapy characteristics

Within the literature, HT protocols vary considerably (Price et al., 2020a; see Chapter 2); however, hot water immersion (HWI) and sauna bathing are the two most common methods used to date (as summarised in Table 1.1 below). Whilst studies using sauna exposure have used a wide range of durations, the average time ranges from 16 to 45 minutes when air temperature is >63 °C. For HWI, the most common water temperature is >39.9 °C, with sessions lasting between 10-90 minutes. Other HT methods reported in the literature (though not included in Table 1.1) are water-perfused suits and partial limb HWI.

Table 1.1 Heat thermotherapy protocol characteristics. Table from Price et al. (2020a)

Descriptive Factor (Initial Thermotherapy conditions)	**Number of Papers**	**Mean Duration (minutes)**	**Duration Range (minutes)**	**Mean Core Body Temperature Change (°C)**	**Core Body Temperature Change Range (°C)**
<u>Hot Water Immersion</u>					
Temperate water temperature (≤38 °C)	3	27	20-30	Not Reported	Not Reported
Warm water temperature (>38; ≤39.9 °C)	4	53	10-120	1.6	1.5-1.6
Hot water temperature (>39.9 °C)	14	44	10-90	1.5	0.6-1.8
<u>Heated Air</u>					
Moderately high air temperature, low humidity (≤63 °C; ≤30% RH)	4	39	15-110	0.6	0.6-0.6
Moderately high air temperature, high humidity (≤63 °C; >30% RH)	1	240	-	0.8	-
High air temperature, low humidity (>63 °C; ≤30% RH)	5	32	25-45	1.7	0.8-2.7
High air temperature, high humidity (>63 °C; >30% RH)	1	16	-	Not Reported	Not Reported

Note. For HWI papers: core temperature was not reported in 3/3 of the temperate water (38 °C) papers, 2/4 warm water (>38; 39.9 °C) papers and 5/14 of the hot water (>39.9 °C) papers. For heated air papers, core temperature was not reported in 3/4 of the moderately high temperature, low humidity (63 °C; 30% RH) papers, 1/5 high temperature, low humidity papers (>63 °C; 30% RH) and 1/1 of the high temperature, high humidity (>63 °C; >30% RH) papers. Overall, 15/39 heated air and HWI papers included in the table did not report core temperature change.

1.5.2 Blood pressure responses to heat thermotherapy

HT acutely reduces BP during and after heating, although the extent of this response varies within the HT literature. Decreased BP has been observed 30-60 minutes after HT in both older (MAP ~-8 mm Hg) (Romero et al., 2017a) and young participants (MAP, ~-7 mm Hg); (Francisco et al., 2021)). These effects have been observed with different HT modalities, including saunas (Gravel et al., 2020), water-perfused suits (Hemingway et al., 2022), and HWI (Campbell et al., 2022). It has previously been shown in hypertensive individuals that BP (SBP ~-7mm Hg) remains lower 24 hours after a single 40-minute duration HWI session (Roxburgh et al., 2023). However, there is no evidence to date that this also applies to normotensive individuals (Didier et al., 2022, Campbell et al., 2022). Therefore, HT appears to lower BP immediately after a single session, while also lowering resting BP after multiple sessions (Roxburgh et al., 2023).

1.5.3 Peripheral vascular response to heat thermotherapy

During acute heat stress, the SR in conduit arteries changes from an oscillatory pattern, with both antegrade and retrograde shear, to a purely antegrade profile with high average shear, similar to SR values measured during low-intensity aerobic exercise (Francisco et al., 2021). Endothelial cells detect this increased antegrade SR and subsequently release vasodilatory substances such as NO, which diffuse into the smooth muscle in the artery wall. This results in smooth muscle relaxation, and thus vasodilation (Green et al., 2017). Antegrade SR is sharply elevated during heat stress in young individuals (Larson et al., 2021) and their older counterparts (Thomas et al., 2017). Therefore, the increase in antegrade SR during HT is likely

a universal response. Following HT, an elevated SR is acutely observed, with previous studies reporting a post-HT SR elevation in both younger and older participants (Engelland et al., 2019, Thomas et al., 2017). How long such changes in SR persist following HT appears to vary across studies. Francisco et al. (2021) demonstrated that SR changes continue in the brachial artery for approximately 40 minutes and in the femoral artery for approximately 20 minutes following chest-level HWI. Thus, different arteries appear to have different acute temporal SR responses following HT.

Acute HT can temporarily improve post-HT FMD. For instance, after a 45-minute lower-leg HWI session, an increase in brachial FMD was observed post-immersion (Cheng et al., 2021). The mechanisms for this increase in FMD are likely the rise in antegrade SR (Cheng et al., 2021), and the increased availability of vasoactive substances, including NO (Hoekstra et al., 2018). The rise in FMD following an acute bout of HT is inconsistent across the literature. For example, 60 minutes of HWI did not improve brachial artery FMD in young, healthy adults (Brunt et al., 2016b) or older adults with type two diabetes mellitus (Behzadi et al., 2022). In contrast, Behzadi et al. (2022) reported a significant increase in antegrade SR, typically associated with an increase in FMD. Therefore, it is unclear whether HT increases FMD acutely and whether such increases are moderated by factors such as age, CVD risk factors or a HT session's thermal load.

Within exercise physiology literature, there is an understanding that after a bout of moderate-intensity or high-intensity exercise, there is a bi-phasic FMD response (Dawson et al., 2013, Bailey et al., 2017, Yoo et al., 2017). The theory is that immediately after exercise,

there is an FMD decrease “nadir” followed by “supra normalisation” FMD increase approximately one hour after exercise, which normalises 24-48 hours after exercise (Green and Smith, 2018). This bi-phasic response is affected by the exercise modality (higher intensity exercise leading to a stronger nadir (Johnson et al., 2012)), oxidative stress levels (increased oxidative stress reduces NO availability, leading to a stronger nadir (Ohara et al., 1993)) and increased baseline diameter (e.g. an increased baseline diameter may prevent a further increase in FMD as the endothelium is already stimulated, leading to a stronger nadir This is known as a “diminished dilator reserve” (Gori et al., 2010)). The nadir is followed by an increased production of vasodilatory substances (Cosio-Lima et al., 2006) and an antioxidant response (Tyldum et al., 2009b), which causes the supra-normalisation FMD response that is typically seen 60 minutes post-intervention (Dawson et al., 2013, Bailey et al., 2017, Yoo et al., 2017). It is unknown whether there is a biphasic post-HT FMD response as no previous study to date has reported measuring FMD twice within 90 minutes after an acute HT bout Additionally, unlike exercise (Tjønna et al., 2011), it is presently unknown whether the elevated FMD response post-HT is still prevalent 24 hours post-intervention.

1.5.4 Temperature thresholds for heat thermotherapy

Within the HT literature, the HT intervention target to reach T_c of 38.5-39°C appears to originate from the Brunt et al. (2016b) study, which investigated the effects of an eight-week HWI intervention in young, healthy adults. The authors from this study based the rationale for their T_c target on a longitudinal study (Laukkanen et al., 2015), where after a 20-year follow-up the greatest reduction in CVD risk was associated with Finnish sauna sessions over 19 minutes long in 80-100°C. Note, T_c was not recorded in this longitudinal study. Therefore,

the rationale for the 38.5-39°C temperature threshold was to mimic the peak rectal temperature (T_{rec}) achieved by heat-adapted individuals within a 30-minute sauna session (air temperature 80-100°C); (Leppäluoto et al., 1986). Despite the stated rationale to achieve a peak (T_{rec}) between 38.5-39°C, it is unclear why Brunt and colleagues extended their HWI protocol to 90 minutes, given that the desired (T_{rec}) was reached by 30 minutes in this study (Brunt et al., 2016a). Following this study, Brunt and colleagues reduced the protocol duration to one hour so that participants spent ~30 minutes above 38.5°C rather than 60 minutes.

Due to the improvements in CV function (i.e., reduced arterial stiffness, increased FMD, and reduced resting MAP) with the Brunt et al. (2016b) HWI intervention and following several successful follow-up studies from the same group (Brunt et al., 2016b, Ely et al., 2019b), other researchers (James et al., 2023, Behzadi et al., 2022) have utilised the T_c threshold of 38.5°C for their own HT interventions. However, it has been demonstrated that HT interventions targeting a lower T_c (~38°C) also improve CV function. For example, 12 weeks of HWI (post-bathing T_c: 37.9 ± 0.3°C) reduced resting SBP in peripheral artery disease patients (Akerman et al., 2019). Therefore, it is unclear what the optimal T_c threshold is for eliciting the optimum CV function adaptations.

1.5.5 Sauna bathing

Sauna bathing typically involves sitting or lying supine in a heated room (60-100°C), with some studies having rest periods in the middle of sessions for 5-10 minutes before re-

entering the sauna (Gravel et al., 2019, Laukkanen et al., 2018). Sauna bathing is typically in a low-humidity environment (~10% relative humidity), so the sweating response more effectively attenuates a T_c rise compared to humid environments (Cramer and Jay, 2016). During the first 10-30 minutes of sauna bathing at 80°C, T_{rec} can increase up to 1°C (Hannuksela and Ellahham, 2001). Regular sauna bathing is associated with improved CV function. For example, Laukkanen et al. (2018) conducted a 20-year follow-up study with over 2000 Finnish adults aged 42 to 61 years old. After 20 years, those who completed 4-7 sessions a week for >19 minutes per session had a lower risk of CVD and all-cause mortality than those who did not have a sauna bath. Although this is a longitudinal study and thus does not demonstrate a causative effect, the results show the long-term health benefits of general sauna bathing and heat therapy.

1.5.6 Hot water immersion

HWI typically involves partial limb immersion or whole-body immersion (from the waist upwards) in warm-to-hot water. HWI can be advantageous over saunas in elevating T_c temperature because the thermal convection of water is ~24 times that of air (Parsons, 2019). Thus, HWI protocols typically use water temperatures around 37 - 42°C to increase T_c (Price et al., 2020a). The level of HWI affects the rate of rise of T_c. For example, T_c increases at ~0.7 °C/h (Engelland et al., 2019) with partial limb immersion (waist immersion) compared to ~1.5 °C/h (neck immersion; (Francisco et al., 2021)).

When immersed in water, T_c elevation can be faster than sauna bathing; for example, 20-30 minutes of neck level HWI can increase T_{rec} by 1.2°C (Brunt et al., 2016b) compared to the

0.2–1.0°C rise seen with sauna bathing (Hannuksela and Ellahham, 2001). Notably, most HWI protocols using the 60-minute immersion approach change the immersion level from the neck to the waist after 20-30 minutes of immersion (Behzadi et al., 2022, Francisco et al., 2021). This attenuates the rate at which T_c rises during the second half of such 60-minute protocols. The faster T_c rise in HWI vs. sauna bathing is due to the ineffectiveness of the sweating response for immersed skin surface areas (Cramer and Jay, 2016), plus the clamping of skin temperature for immersed skin surface areas (which diminishes heat loss via convection from the surface of the skin (Cramer and Jay, 2016)).

Compared to sauna bathing, HWI has the added effect of hydrostatic pressure. During bathing, hydrostatic pressure increases venous return and improves arterial compliance (Arborelius et al., 1972). Therefore, HWI could have additional CV function benefits to sauna bathing. HWI is associated with improved CV function. (Brunt et al., 2016a) conducted an 8-week HWI intervention in young sedentary individuals consisting of 36 sessions (4-5 per week) with 90 minutes of HWI (40.5°C) per session. At the end of the 8-week intervention, participants had lower BP (drop of ~4mm Hg for DBP and MAP) and improved arterial stiffness and endothelial function compared to baseline. Additionally, improved CV function and anti-inflammatory responses have been shown in participants with polycystic ovary syndrome (Ely et al., 2019b), whereby muscle sympathetic muscle activity and BP were lower. Furthermore, endothelial function (as measured using FMD) was greater in participants who completed an 8–10-week HWI intervention (60 min in 40.5°C, 4-5 times per week) as compared to a control group which undertook no intervention (Ely et al., 2019). In summary, the combined effects of hydrostatic pressure and heat stress following hot water

immersion can lead to positive CV function adaptations, such as reduced resting BP and improved endothelial function.

1.5.7 Heat thermotherapy mechanisms for long-term cardiovascular adaptation

Long-term physiological adaptations resulting in reductions in BP, improved endothelial function, and reductions in arterial stiffness are associated with a reduced risk of CVD incidence (Rafieian-Kopaei et al., 2014, Ross, 1999). Fortunately, proposed long-term CV adaptations following HT are increases in angiogenesis (Brunt et al., 2019), improved endothelial function (Brunt et al., 2016a) and positive vascular structural changes in response to repeated antegrade SR (Brunt et al., 2016a). These HT improvements in endothelial function are proposed to promote an anti-atherogenic state (Eelen et al., 2015), decrease platelet aggregation, reduce inflammatory reactive oxygen species (ROS), and increase NO production (Brunt et al., 2019). All of these factors are associated with a lower CVD risk.

In theory, improvements in CV function following HT are driven by increasing cutaneous blood flow (Rowell et al., 1969) and antegrade SR through the conduit arteries (Cheng and MacDonald, 2019) alongside temporary reductions in BP due to a decrease in CVP and venous return to the heart (Crandall et al., 2008). Repeating this process, by completing 5-12 HT sessions, appears to result in functional CV improvements such as increased FMD (Imamura et al., 2001) or reduced fasting glucose values (Hoekstra et al., 2018). Longer-term CV adaptations such as reduced arterial stiffness and BP are likely due to structural changes such as artery remodelling (shown by an increased artery baseline diameter) and angiogenesis, alongside a reduction in sympathetic activity (in those where sympathetic

activity was elevated at baseline (Ely et al., 2019b)). These adaptations have been seen in studies that have repeated HT 4-5 times a week over 8-12 weeks in young, sedentary individuals (Brunt et al., 2016a) and those with CVD risk factors (Ely et al., 2019b).

Despite the positive CV adaptations seen across the three aforementioned studies, there are some variance in reported findings. Whilst all three studies observed a decrease in resting SBP, only two studies observed an increase in brachial FMD (Brunt et al., 2016a, Ely et al., 2019b). This could be due to several factors. Firstly, there are differences in participant demographics, specifically, older adults with peripheral arterial disease vs. young and healthy vs. young with polycystic ovary syndrome. With ageing and advanced peripheral arterial disease, less compliant arteries may limit HT-related changes in endothelial function (Königstein et al., 2021, Lind, 2007). Indeed, previous studies have shown no change in endothelial function (as measured using FMD) following 12-week HT interventions in older peripheral arterial disease participants (Akerman et al., 2019). In contrast, after eight weeks of HT, endothelial function improvements have been demonstrated in a younger cohort (with CVD risk factors such as polycystic ovary syndrome) (Ely et al., 2019). In summary, long-term CV function adaptations have been demonstrated with HT, in particular in younger cohorts (Ely et al., 2019b, Brunt et al., 2016a). Despite the promising long-term CV function adaptations following some HT studies, it is not certain if these changes are consistent and what the optimal HT protocol is to derive long-term CV function adaptations across different participant health statuses and ages.

1.5.8 Cardiometabolic health and heat thermotherapy

Obesity and a chronic inflammatory environment can lead to poor cardiometabolic health, increasing the risk of CVD (Ely et al., 2018). Obesity enlarges adipose tissue, potentially causing adipose tissue ischemia (Trayhurn et al., 2008). This triggers interactions between the adipose tissue and inflammatory cells, leading to increased release of inflammatory cytokines such as interleukin-6 (IL-6), tumour necrosis factor and a reduced release of adiponectin (Díez and Iglesias, 2003, Trayhurn et al., 2008). Low levels of adiponectin, along with heightened inflammatory cytokines, impede insulin action and reduce insulin sensitivity (Facchini et al., 2001). This sequence of events results in hyperglycaemia, hyperlipidaemia, meta-inflammation, and insulin resistance (Ely et al., 2018).

In response to insulin resistance, the body increases its insulin production, raising BP and hypertension risk by increasing sympathetic outflow (Anderson et al., 1992, Malpas, 2010). Systemic insulin resistance is associated with impaired endothelium-dependent dilation and microvascular function (Steinberg et al., 1996). Hyperinsulinemia reduces vasodilatory response due to limited bioavailable NO and increased oxidative stress (Scherrer and Sartori, 2000). The meta-inflammatory state of obesity also leads to impaired vascular remodelling, causing increased arterial stiffness (Safar et al., 2006) and intima-media thickness (Dalmas et al., 2013). Consequently, poor cardiometabolic health fosters an inflammatory environment and contributes to impaired CV function, as evidenced by endothelial dysfunction, vascular remodelling, increased risk of atherosclerosis, and elevated BP.

Long-term adaptation to HT can improve poor cardiometabolic health, reducing the risk of CVD development (Ely et al., 2018). The mechanisms through which HT achieves this are as follows. Firstly, HT increases heat shock protein (HSP) proliferation (Faulkner et al., 2017), which is associated with improved NO production (Hoekstra et al., 2018), improved insulin sensitivity (Morera et al., 2012), and decreased inflammation (Hoekstra et al., 2020). HT can also reduce sympathetic outflow, thus reversing some insulin-mediated hypertension (Ely et al., 2019). Ely et al. (2019) conducted an 8–10-week HWI intervention (30 sessions of 60-minute HWI at 40.5°C) with polycystic ovary syndrome patients. After the intervention, those in the HWI group exhibited a decrease in muscle sympathetic nerve activity (associated with sympathetic activity), reductions in fasting glucose and insulin, and C-reactive protein alongside reductions in BP and carotid wall thickness. These improvements in both CV function and cardiometabolic health indecreased known to experience chronic low-grade inflammation and an elevated risk of CVD, demonstrate the potential of HT to be an effective tool to reduce CVD-related mortality.

1.5.9 Heat thermotherapy and mood

There is a relatively small body of HT literature that has examined the effect of HT on mood state (Naumann et al., 2016, Naumann et al., 2020, Janssen et al., 2016). This literature is promising however, with one study showing a suppression in depressive symptoms that lasted for six weeks after one HT session (Naumann et al. (2018). Specifically, in this eight-week study (Naumann et al., 2020), participants with major depressive disorder (MDD) were randomly assigned to receive either a HWI or an exercise program. The HWI group

completed biweekly HWI sessions consisting of 20 minutes in 40 °C water, immersed up to the neck, followed by 20 minutes resting covered in blankets. In contrast, the exercise group completed biweekly supervised sessions, consisting of 45-50 minutes of warming up, stretching, jogging and resistance exercises. Notably, the HWI group showed a reduction in depressive symptoms after two weeks, and these improvements were maintained throughout the remaining six weeks of the intervention. Conversely, there was only a significant reduction in depressive symptoms for the exercise group after six weeks of their intervention. Thus, it is possible that HT could elicit a faster positive change in depressive symptoms than typical pharmaceutical strategies, which can take over eight weeks to work (Thompson, 2002). Despite several studies investigating the benefits of HT for MDD patients (Naumann et al., 2016, Naumann et al., 2020, Janssen et al., 2016), there are currently no studies that have assessed positive and negative mood state after HT in non-MDD individuals.

1.6 High-intensity interval exercise

Whilst the potential for HT to improve CV function is the focus of this thesis, other strategies can lead to long-term CV function adaptations. For example, exercise interventions have been demonstrated to lower resting BP and improve FMD (Ramos et al., 2015). Recently, researchers have started to investigate whether there is efficacy in combining HT and exercise strategies together to improve CV function (Steward et al., 2024, Cullen et al., 2020, Lee et al., 2021).

During exercise, there is an increased demand for blood flow to active skeletal muscle beds. To meet this demand, cardiac output increases, mediated by increased HR and SV. Additionally during exercise, there is a linear increase in SBP (mainly due to increased cardiac output, sympathetic activation, and changes in vascular resistance (Mohammed et al., 2020, Joyner et al., 2010)), while DBP remains relatively stable (Kelley et al., 2001). Following exercise, a reduction in SBP and DBP has been observed (Romero et al., 2017b, Price et al., 2020b). This post-hypotensive effect can reduce SBP for several hours and may lead to long-term CV adaptations, including lower resting SBP and improved endothelial function (Romero-Vera et al., 2024, Ramos et al., 2015). Post-exercise hypotension is more pronounced in higher intensity exercise protocols vs. moderate intensity protocols (Ramos et al., 2015). Therefore, it may be more advantageous to focus on HIIEx protocols than moderate intensity exercise-based protocols.

Post-exercise hypotension following HIIEx is caused by the resetting of arterial baroreceptors alongside a sustained vasodilation (Romero et al., 2017b). Post-HIIEx, the arterial baroreflex shifts downwards and to the left, so a drop in blood pressure causes a dampened baroreceptor and sympathetic activity response (Romero et al., 2017b). Meanwhile, increased peripheral vasodilation, primarily driven by local release of histamine and receptor activation (Romero et al., 2017b), occurs in previously active skeletal muscle beds (Halliwill et al., 1996) and to a smaller extent nonactive skeletal muscle beds (Hara and Floras, 1995). This post-exercise peripheral vasodilation is minimally influence by cutaneous vascular beds under thermoneutral conditions (Wilkins et al., 2004). Therefore, a combination of HIIEx and HT would be expected to induce greater peripheral vasodilation response.

High-intensity interval exercise (HIIEx) involves alternating periods of high-intensity aerobic exercise with periods of lower-intensity active recovery (Taylor et al., 2019). HIIEx creates a greater physiological stimulus and adaptation than moderate-intensity continuous exercise (MICE), leading to greater cardiorespiratory fitness, vascular function, and metabolic benefits that can prevent cardiometabolic diseases (Ramos et al., 2015). HIIEx can be categorised based on the intensity (60 – 120% peak wattage) and duration of the exercise bouts, with high-volume protocols accumulating ≥15 minutes of high-intensity intervals and all others being defined as low-volume protocols (Taylor et al., 2019). To categorise by intensity, the first lactate threshold (when blood lactate begins to accumulate above baseline) is the typical boundary for whether the HIIEx is considered moderate or heavy (Coates et al., 2023). The difference between heavy and severe HIIEx is the critical power threshold (the highest sustainable aerobic intensity before an elevated increase in non-oxidative metabolism) (Poole et al., 2016). Severe HIIEx, or sprint interval exercise, involves shorter bursts lasting 10-30 seconds, whilst moderate HIIEx intervals can last up to over four minutes (Taylor et al., 2019). Research has shown that low-volume, high-intensity interval training protocols (>6 weeks) produce comparable improvements in cardiorespiratory fitness to high-volume, high-intensity interval training and are superior to moderate-intensity continuous exercise interventions (Ramos et al., 2015).

Acutely, post-exercise brachial FMD increases have also been seen after a single bout of HIIEx. Weston et al. (2022) investigated the impact of HIIEx and the acute endothelial response in young, healthy adults. One hour and three hours after HIIEx (condition one: 5x3-minute intervals at 75% maximal oxygen consumption ($\dot{V}o_{2max}$) and condition two: 5x3-

minute intervals at 90% $\dot{V}o_{2max}$)), both conditions saw an increase in brachial artery FMD (~2%) with no change in the MICE condition or the resting control. This improvement in endothelial function approximately one hour after HIIEx is likely due to the increased conduit artery antegrade SR, the post-exercise hypotensive state, and the increase in anti-inflammatory markers, subsequently increasing NO availability (Dawson et al., 2013). High-intensity interval training (multiple HIIEx bouts) has also improved endothelial function in children and adults (Early et al., 2017). Brachial artery FMD was assessed following a high-intensity interval training programme lasting 10 – 78 sessions and compared to a resting control group, which included subgroup analysis comparing the effect of age and baseline diameter (Early et al., 2017). The meta-analysis of 66 publications demonstrated that high-intensity interval training interventions (2-5 sessions per week for 4-24 weeks) increased FMD by 9.29% compared to moderate intensity continuous exercise (3.63%), while age and baseline diameter did not have an effect. In summary, HIIEx after a single or multiple bouts increases FMD to a greater extent than moderate-intensity continuous exercise.

1.7 High-intensity interval exercise & passive heating

Few studies have investigated the combined or individual effect of exercise and HT on CV function. Previously, in healthy individuals it has been shown that after an acute bout of cycling (15 minutes at 75% HR max) followed by a sauna session (15 minutes at 73±2°C), MAP and pulse pressure are lowered, and these changes were sustained at 30 minutes post-intervention (Lee et al., 2020). In another study, middle-aged participants (49±9 years old) with at least one CVD risk factor were randomly assigned to three conditions: (1) 60 minutes of resistance (10-minute full body warm-up followed by 20 minutes of lifting upper and

lower body weights) and aerobic exercise (30 minutes cycling at 65-80% HR maximum) only, (2) 60 minutes of resistance and aerobic exercise (65-80% HR maximum) training followed by 15 minutes of sauna, or (3) a resting control (Lee et al., 2022). These sessions were repeated thrice weekly for eight weeks and BP, cardiorespiratory fitness and arterial stiffness were assessed after the intervention. After the intervention, the exercise-only group saw an increased cardiorespiratory fitness and had a lower fat mass than the control group (compared to baseline), but no change in resting BP was observed. In comparison, the exercise and sauna group had a lower fat mass than the control group, a lower SBP (~-8 mm Hg) and a greater increase in cardiorespiratory fitness than the exercise-only group and the control group. Therefore, the combination of exercise and HT resulted in a beneficial BP outcome that did not occur with exercise only, plus an enhanced cardiorespiratory fitness response. To our current knowledge, no study has assessed CV responses to the combination of HIIEx and HT.

1.8 Summary of individual chapters

The primary objective of this thesis was to assess the efficacy of HT (specifically HWI) in eliciting a positive CV response that could eventually lead to long-term positive CV adaptations in young, healthy individuals. The secondary objective was to understand how HWI can be manipulated (duration and immersion level) to elicit a positive CV response, whether this can be achieved more pragmatically (less time and thermal discomfort), and how these HWI interventions affect mood responses.

To achieve this, a systematic review and meta-analysis (**Chapter 2**) was conducted to evaluate the efficacy of HT in enhancing CV function and cardiometabolic health and to identify any existing gaps in the literature. Specifically, **Chapter 2** aimed to investigate the potential CV and cardiometabolic health benefits of HT comprehensively and empirically, comparing how these benefits respond to acute versus multiple HT bouts. **Chapter 3** outlines measurement techniques used in this thesis, highlighting strengths and limitations.

The experimental chapters examined the effects of different HWI protocols on acute CV responses (**Chapter 4**) and the impact of HIIEx and HWI on acute CV responses (**Chapter 5**). Specifically, **Chapter 4** investigated whether a short, hot, neck-level, 30-minute HWI session can elicit the same, meaningful CV and cardiometabolic health benefits as a 60-minute, shoulder/waist level immersion HWI session. The secondary aim was to investigate how HWI affects mood, stress, anxiety, and sleep quality over 24 hours post-HWI. It was hypothesised that whilst both HWI strategies would improve CV, mood and sleep outcomes to a greater extent than the thermoneutral water immersion (TWI) condition, they will not significantly differ from each other. For **Chapter 5**, the study examined the acute CV response to a combined HIIEx and HWI protocol (ExHWI) compared to a combined HIIEx and TWI (ExTWI) protocol. The secondary aim was to investigate how ExHWI and ExTWI affect mood, stress, anxiety, and sleep quality over 24 hours post-HWI. It was hypothesised that ExHWI (HWI & HIIEx) would result in lower BP, a greater increase in FMD and plasma blood volume expansion than ExTWI (TWI & HIIEx). The overall findings of the thesis will be summarised, and recommendations for future research will be provided in **Chapter 6**.

2. HEAT THERMOTHERAPY TO IMPROVE CARDIOVASCULAR FUNCTION: A SYSTEMATIC REVIEW AND META-ANALYSIS

Acknowledgements: This chapter is under review in the Journal of Experimental Physiology.

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Contributions: B.P. led this chapter's conception, interpretation, drafting and revision.

S.L., A.P., & R.L. contributed to this chapter's conception, interpretation, drafting and revision.

B.P. led this chapter's data extraction process.

B.P., R.G. & R.L. contributed to this chapter's data extraction process.

A.P. supported the data analysis of this chapter.

2.1 INTRODUCTION

Heat thermotherapy (HT) is the application of a passive (non-exercising) heating stimulus that increases core body temperature (T_c) and results in numerous beneficial health outcomes (Brunt et al., 2016a, Ely et al., 2019a, Naumann et al., 2020). Specifically, HT has been shown to reduce BP, inflammatory markers, fasting glucose, glycated haemoglobin and improved endothelial function, all associated with a reduction in CVD related mortality (Fiuza-Luces et al., 2018, Ras et al., 2013). These HT-related CV and cardiometabolic improvements have been observed in young, sedentary, healthy cohorts (Brunt et al., 2016a) and chronic heart failure patients (Kihara et al., 2002), but it is unclear whether the magnitude of HT-related improvement differs between populations.

Numerous narrative reviews have discussed how HT improves CV and metabolic health (Cheng and MacDonald, 2019, Brunt and Minson, 2021, Ely et al., 2018, Hoekstra et al., 2020). These reviews indicate the interest in, and the potential use of, HT as a therapeutic tool. However, these narrative reviews did not systematically review the literature or provide empirical evidence to support purported HT mechanisms. A previous meta-analysis by Pizzey et al. (2021) examined resting BP and FMD responses to HT (specifically, >10 HT sessions) in healthy and clinical populations. A total of 12 papers were included in this meta-analysis, which showed that repeated use of HT reduced BP and improved FMD. However, this meta-analysis did not include studies examining acute BP and FMD responses. Subsequently, it remains unclear how acute HT responses translate to long-term adaptations. Identifying acute responses to HT is essential in guiding future research and clinical application (i.e., optimising HT to enhance health).

Two previous systematic reviews have examined glycaemic HT responses (Sebok et al., 2021, Maley et al., 2019). Maley et al. (2019) found that glycaemic control was not affected in non-diabetics but was acutely impaired in diabetics following HT. Meanwhile, Sebok et al. (2021) found that fasting glucose was unaffected in diabetic participants following HT. Inflammation has a strong association with glycaemic control and overall cardiometabolic health (Ely et al., 2018) and, thus, it would be advantageous to understand how it is affected by HT. However, key cardiometabolic responses (e.g., HSPs) and inflammatory markers such as CRP have not been systematically examined. Therefore, this systematic review and meta-analysis aimed to investigate the potential for HT to improve CV and cardiometabolic parameters by comparing how these parameters respond to acute and multiple HT bouts.

2.2 METHODS

2.2.1 Overview

This review followed the Preferred Reporting System for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009). The protocol for this review is published on the PROSPERO register (https://www.crd.york.ac.uk/prospero) under the registration number **CRD42018103246.** The register details the complete sample search query, inclusion and exclusion criteria, data extraction, and analysis. Full details of the search terms used, alongside an example search for the Medline database, can be found via the PROSPERO register.

2.2.2 Information sources and search strategy

Bibliographic databases, MEDLINE, EMBASE and Web of Science, were primarily searched for relevant publications. An additional manual search (via Google Scholar) was then conducted to retrieve all relevant publications. Databases and manual searches included publications from the earliest start date to the 1st of June 2023. Search results were extracted to EndNote (Clarivate Analytics, Philadelphia, Pennsylvania, USA) and duplicates were removed before continuing the screening process.

2.2.3 Study inclusion/exclusion process

The inclusion and exclusion process used the patient, intervention, comparison, and outcome (PICO) framework (Schardt et al., 2007). This PICO was designed to include studies

that examined adult human populations (≥ 18 years) with or without diagnosed health conditions, used a passive heat stress intervention (i.e., non-exercising heat stimuli, such as hot water immersion or sauna bathing) to increase limb or body temperature, used a randomised, causational study design, and measured CV or cardiometabolic responses. Passive heating studies were included if they heated a single limb to full body immersion, lasting from 10 minutes onwards. Studies were excluded if the heat stimulus occurred in addition to exercise, pharmaceutical interventions, or any other concurrent intervention, or there was no control comparison. The CV parameters included for this meta-analysis were blood pressure, arterial stiffness, FMD, and total shear rate (SR). The cardiometabolic parameters were interleukin-6 (IL-6), glucose (fasted, mixed meal and after a glucose tolerance test, which were grouped together due to the limited number of reported values), heat shock proteins (HSP; which were grouped together due to the limit number of reported values), and C-reactive protein (CRP). Cardiometabolic data for IL-6 came from either serum or plasma, and for HSPs data came from muscle, plasma, serum and adipose tissues. To account for the heterogeneity within the cardiometabolic protocols (i.e. serum vs. plasma), the data were presented as a standardised mean difference in the meta-analysis.

Titles and abstracts of citations identified in the search were independently screened by reviewers (B.P. & R.G.), according to the inclusion/exclusion criteria above. To ensure the publication selection procedure was applied consistently, a random sample of 20% citations was screened by both reviewers (B.P. & R.G.), and results were compared. Any disagreements regarding a study's eligibility were discussed with a third reviewer (R.L.).

2.2.4 Data extraction

Full texts deemed eligible for inclusion underwent data extraction by both reviewers (BP & RG) using a data extraction form. Data were checked for errors by an additional reviewer (AA) before analysis. The mean difference and standard deviation/standard error of the mean were extracted from all eligible studies. Where possible, results were expressed as absolute values (mean ± SD), with authors contacted via email to retrieve any missing data. If studies reported results as a mean difference with 95% confidence intervals, the Cochrane Handbook method (7.7.7.2) for calculating the standard difference from 95% CI's was applied (Li et al., 2019). In studies reporting non-parametric results (i.e., median and interquartile ranges), the mean and standard deviation were estimated from the sample size, median and interquartile range (Wan et al., 2014). If unavoidable, unavailable data was calculated using an online software package (WEBPLOT DIGITIZER; https://apps.automeris.io/wpd/).

2.2.5 Risk of bias

A Risk of Bias Assessment (Cochrane RoB 2) was conducted using COCHRANE Guidelines (Sterne et al., 2019). This assessment determined the risk of type one or type two error by assessing five domains, specifically: potential bias arising from the: (1) randomisation process, (2) deviations from intended interventions, (3) missing outcome data, (4) measurement of the outcome, and (5) selection in the reported result. One reviewer completed the risk of bias (BP) with a second reviewer (RL) consulted to resolve any discrepancies.

2.2.6 Data synthesis and analysis

The current study aimed to distinguish between acute (e.g., minutes/hours after an HWI bout) and chronic (e.g., days following an HWI intervention) CV and cardiometabolic responses to HT. The acute response was recorded as soon as possible after the first HT bout whilst chronic responses were recorded within 24 hours – 1 week after the last HT bout. Therefore, extracted data were divided into these two discrete categories (i.e., single bout (1 heating bout), and multiple bouts (>1 heating bouts)), to reduce the possibility of a unit of analysis error and appropriately represent the physiological process within the data.

For single-bout and multiple-bouts categories, mean differences between intervention and control groups were calculated, and overall effect estimates (raw effect or Hedge's G) were calculated using generic inverse variance models and random effect models. Hedge's G values of 0.15, 0.40 and 0.70 indicate small, medium and large effect sizes, respectively (Lovakov and Agadullina, 2021). Estimated significance (p-value; $\alpha>0.05$) and heterogeneity (I^2) were examined, with $I^2>50\%$ and $I^2>75\%$ indicative of substantial and considerable heterogeneity, respectively (Higgins et al., 2003). Where heterogeneity was substantial ($I^2 \geq 50\%$), subgroup analysis was performed to investigate variables (i.e., heating modality, duration of HT bout, and participant demographics). For example, the heating modality (e.g. sauna vs. bathing) alongside the duration (e.g. 10 minutes vs. 60 minutes) of the HT session can dramatically affect an individual's acute physiological response and physiological adaptation over several HT sessions. Meanwhile, a participant's health status can change the impact of an HT session; for example, the reduction in BP may be greater for hypertensive

individuals compared to normotensive individuals. Additionally a meta-regression was completed for each outcome variable when applicable.

Small study effects, including potential publication bias, were assessed with Egger's test, the trim and fill method, and p-curve analysis. The impact of influential points on the pooled summary effect size was estimated with an influence analysis using multiple indicators (DFFITS, Cook's distance, covariance ratio). Finally, to avoid unit of analysis error, singular control groups from trials with more than one intervention arm were split (Deeks et al., 2019). All statistical analyses were completed using packages (Tidyverse (Wickham et al., 2019), Meta (Balduzzi), Metafor (Viechtbauer, 2010) and Dmetar (Harrer et al., 2019)) written for R (Core Team, 2014) and implemented in RStudio (Allaire, 2012).

2.3 RESULTS

2.3.1 Systematic search

Figure 2.1 shows the search, screening, and selection process for eligible publications. In total, 44 peer-reviewed publications were included, and their data were extracted and included in the meta-analysis.

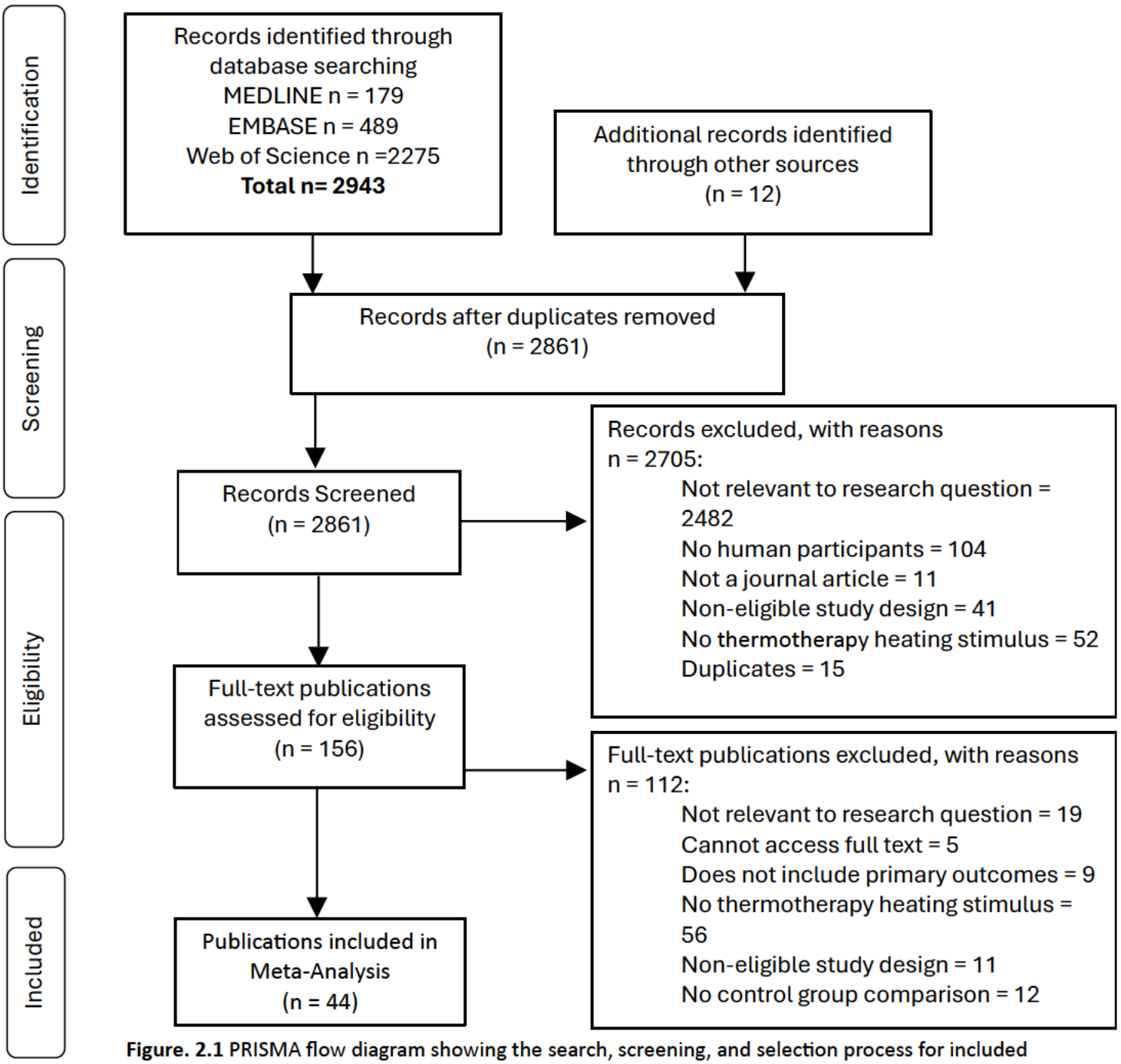

Figure. 2.1 PRISMA flow diagram showing the search, screening, and selection process for included publications in the meta-analysis.

2.3.2 Publication characteristics

Of the 44 included publications, 10 were conducted in Europe (UK, Hungary and Austria), 7 in Asia (Japan and China), 24 in the Americas (USA, Canada and Brazil) and 3 in Australasia (Australia and New Zealand). Across all included publications there was a total of 817 participants. Participants were classified as healthy (no CV or cardiometabolic risk factors; n = 338), or unhealthy (>1 CV or cardiometabolic health risk factor; n = 479). The following demographic information was also extracted: participant age [young (18-35 years, n = 313), middle-aged (36-59 years, n = 73), or elderly (>60 years, n = 431)] and sex (male, n = 495; female, = 322). Within the 44 included publications, some participants were reported to have taken medication (including oral contraceptives, ACE inhibitors, statins, antiplatelets, beta-blockers, corticosteroids, and antidepressants) and this information was also extracted.

The included publications used three different passive heating modalities (HWI, sauna, and water perfusion suit). There were 26 HWI, 11 sauna, and 7 water perfusion suit publications. Sauna bathers were exposed to the highest environmental temperatures, followed by the water-perfused suit and HWI modalities (69 ± 14°C vs. 48 ± 2°C vs. 42 ± 4°C, respectively).

2.3.3 Heat thermotherapy effects on cardiovascular and cardiometabolic outcomes

The meta-analysis included 30 HT single-bout and 14 HT multiple-bouts publications. For single-bout publications, the median HT duration was 60 (IQR: 38.26) minutes. In multiple-bouts publications the number of HT bouts ranged from 2 to 60 and the median total HT

duration was 505 (1290) minutes. All pooled effect sizes and subgroup data are presented in Table 2.2.

MAP was significantly lowered following single, and multiple HT bouts (Figure 2.3), largely facilitated by significantly reduced DBP (Table 2.1), especially after a single bout of HT (figure 2.2). SBP did not change following a single HT bout. When significant subgroups were identified (BP measures), effects appeared more pronounced when the control group was an exercise condition, and when exposed to HT via a sauna (compared with HWI). There were no other significant modulating effects for BP apart from single-bout DBP, (heating modality), with the sauna causing a greater DBP reduction than HWI (see Forest plot; figure 2.5).

Table 2.1. Summary subgroup and meta-regression data for cardiovascular and cardiometabolic responses to a heat thermotherapy intervention

Outcome Measure	Publications (*n*)	Effect Estimate	*P* Value	Outliers Removed	Outliers Effect Estimate	*P* Value	Heating Modality Subgroup (*p* value)	Control Condition Subgroup (*p* value)	Health Status Subgroup (*p* value)	Meta Regression Correlation	Meta-Regression *P* Value
Single Bout Response											
DBP (mm Hg)	25	-5 [-9 to -2]	**<.01**	6	-2 [-4 to 0]	**.017**	0	0	N/A	0.70	.295
SBP (mm Hg)	25	-3 [-9 to 4]	.396	10	-2 [-7 to 3]	.377	.226	0	N/A	0.59	.992
MAP (mm Hg)	19	-8 [-12 to -5]	**<.01**	2	-6 [-9 to -4]	**<.01**	.590	0	N/A	0.55	.836
FMD	12	0.40 [0.06 to 0.74]	**.026**	1	0.31 [0.06 to 0.56]	**.019**	.014	N/A	N/A	0.28	.590
HSP	11	-0.14 [-0.44 to 0.20]	.373	0	-0.14 [-0.44 to 0.20]	.373	NA	.517	N/A	0.37	.341
IL_6	8	-0.07 [-0.69 to 0.55]	.803	0	-0.07 [-0.69 to 0.55]	.803	.016	.022	N/A	0.51	.267
Shear Rate	14	4.63 [1.96 to 7.30]	**<.01**	4	3.88 [2.59 to 5.16]	**<.01**	N/A	.650	N/A	0.93	.876
Multiple Bouts Response											
SBP (mm Hg)	6	-5 [-10 to 1]	.075	0	-5 [-10 to 1]	.075	N/A	.746	.081	0.75	.992
DBP (mm Hg)	6	-5 [-7 to -2]	**<.01**	0	-5 [-7 to -2]	**<.01**	N/A	.431	.443	0.70	.335
MAP (mm Hg)	4	-4 [-7 to -1]	**.031**	0	-4 [-7 to -1]	**.031**	N/A	<.01	.129	0.70	.360
Glucose	7	<0.01 [-0.59 to 0.60]	.985	1	0.08 [-0.07 to 0.23]	.214	N/A	N/A	N/A	0.47	.079
CRP	4	-0.61 [-1.87 to 0.65]	.219	0	-0.61 [-1.87 to 0.65]	.219	N/A	N/A	N/A	0.70	.331
FMD	7	1.37 [-0.64 to 3.39]	.147	1	0.61 [-0.17 to 1.38]	.100	N/A	.087	.284	0.71	.789
HSP	5	0.29 [-0.49 to 1.07]	.358	0	0.29 [-0.49 to 1.07]	.358	N/A	N/A	N/A	0.50	.281
IL_6	2	-0.53 [-0.97 to -0.08]	**.042**	0	-0.53 [-0.97 to -0.08]	**.042**	N/A	N/A	N/A	0.99	N/A
Combination of Single and Multiple Bouts Responses											
Arterial Stiffness	8	-0.36 [-0.78 to 0.06]	.083	0	-0.36 [-0.78 to 0.06]	.083	N/A	.221	N/A	0.70	.506

Note: Multiple bouts refer to publications examining responses to >1 heat thermotherapy bouts; Combined refers to single-bout and multiple-bout study designs (i.e., for Arterial Stiffness outcomes). Multiple-bouts response for glucose is a combination of fasting and postprandial glucose measurements. The Heat Shock Protein response combines different intracellular and extracellular heat shock proteins. Meta-regression data are reported after any identified outliers were removed. Abbreviations: CRP = C-reactive protein; SBP = Systolic blood pressure; DBP = Diastolic blood pressure; MAP = Mean Arterial pressure; FMD = Flow Mediated Dilation; IL-6 = Interleukin 6; HSP = Heat Shock Protein. Glucose represents fasting glucose and postprandial glucose responses. Blood pressure values are expressed as mm Hg,

whilst all other variables are expressed as an effect size (Hedge's G). Subgroup heating mobility determined if there was a difference between hot water immersion, sauna and water perfused suit; the control condition subgroup determined if there was a difference between a resting control vs. an exercise control group; and the health status subgroup determined if there was a difference between participants with no cardiovascular risk factors vs. participants with more than one cardiovascular disease risk factor.

FMD and peripheral artery shear rate (Figure 2.4) were significantly improved following a single-bout of HT but this did not persist for the multiple-bouts conditions. IL-6 decreased following multiple bouts of HT although the number of publications was limited (*n*=2). There was no significant difference in arterial stiffness, glucose, HSP, or CRP, potentially due to the small number of publications included for those variables. There was no significant modulating effect of cumulative minutes (log-transformed) on the treatment effect for any CV or cardiometabolic outcome measure.

The heterogeneity scores for most CV and cardiometabolic outcomes were substantial despite removing outliers (I^2>75%). Only HT single-bout FMD and HT multiple bouts MAP achieved low to moderate levels of heterogeneity (I^2<50%). Despite conducting Baujat Diagnostics (Baujat et al., 2002), leave one out analysis and DFFITS analysis (Cohen, 2013) to identify and remove outliers, this did not change the heterogeneity. Most of the funnel plots for the outcome measures displayed symmetry, and none of the Eggers' tests were significant (<0.05) which demonstrates that the meta-analysis was unlikely influenced by publication and small study bias. The p-curve analysis (Simonsohn et al., 2014) demonstrated a skew to the left for all meta-analysis variables (most publications that were significant were <0.02 rather than 0.05). Therefore, the meta-analysis variables are unlikely to have publications with selective statistical reporting, e.g., where authors increase the number of participants until a p-value of 0.05 is achieved (Simonsohn et al., 2014) or only selectively report significant p-values.

Overall, included publications were deemed to have some concerns (n=38) or a high risk of bias (n=6). The main area of concern was related to the randomisation process, of which 41/44 publications were rated as high-risk (n=6) or some concerns (n=35). The lowest area of concern was the risk of bias due to deviations from the intended interventions, in which all 44 publications were deemed to be of low risk of bias.

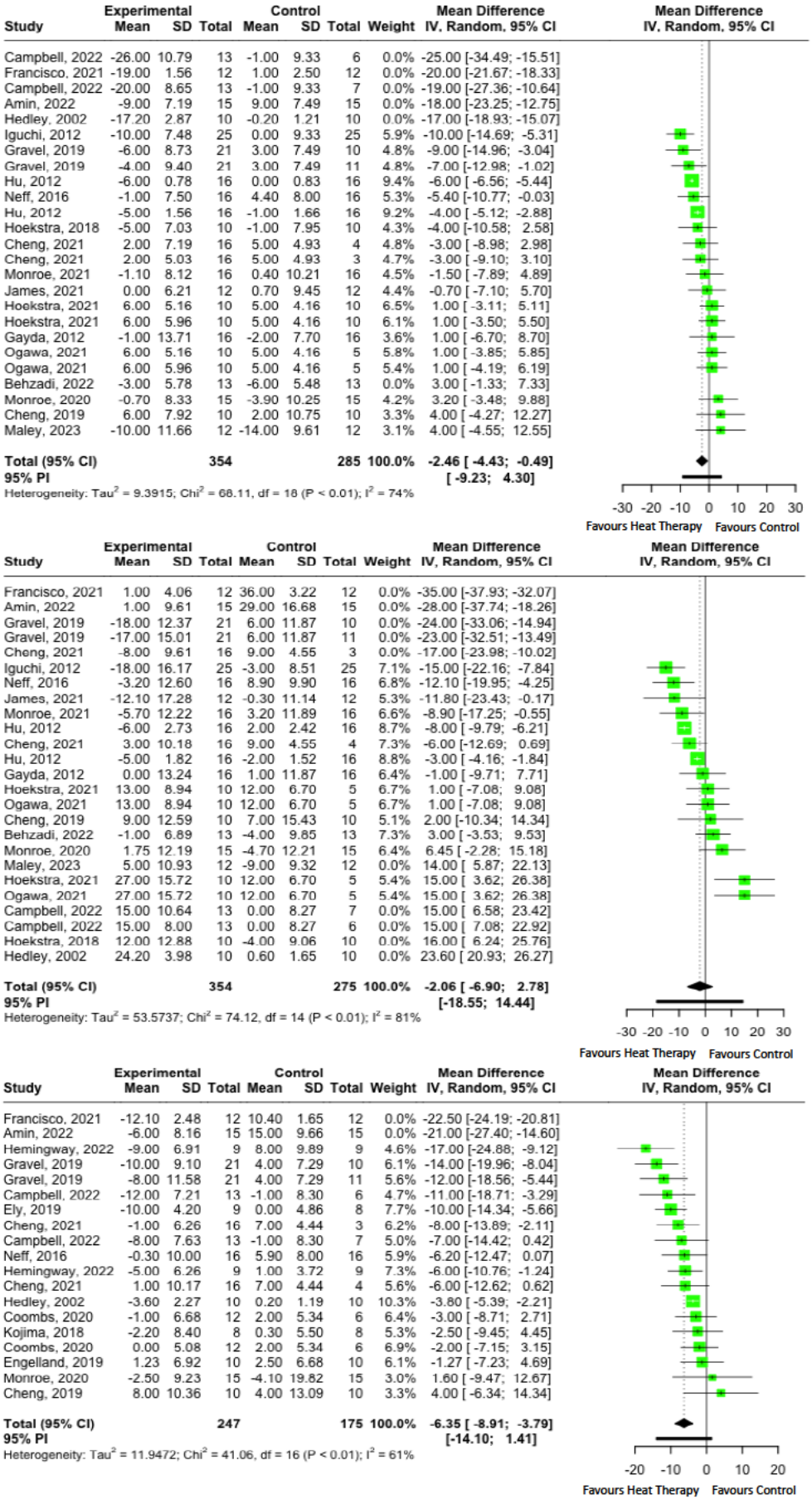

Figure 2.2 The pooled effect estimate, Blood pressure response to a heat thermotherapy (HT) single-bout when publication outliers are removed. A) Diastolic Blood pressure response to an HT single bout; B) Systolic blood pressure response to an HT single-bout; C) Mean arterial pressure response to an HT single-bout.

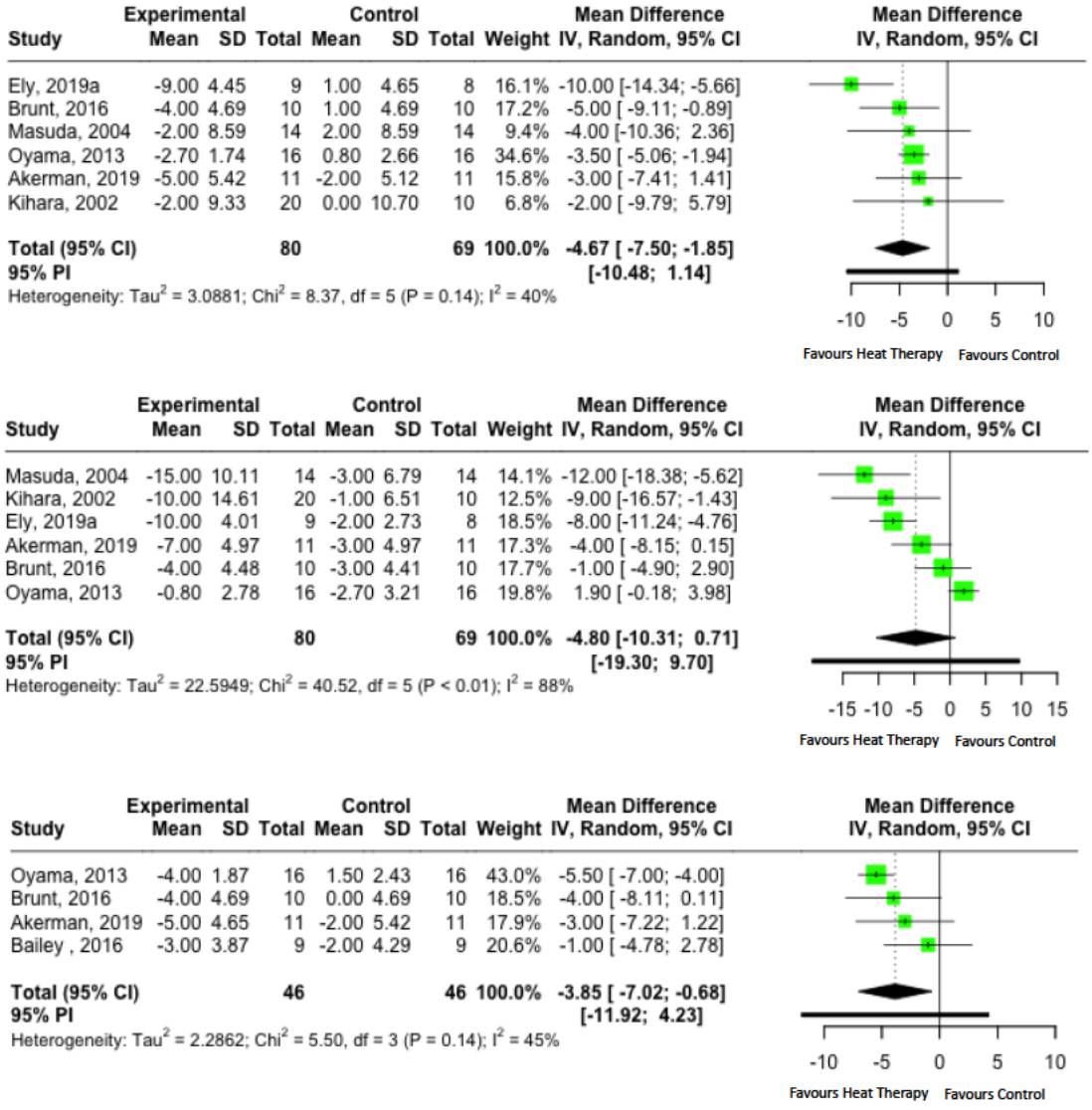

Figure 2.3. The pooled effect estimate blood pressure response to heat thermotherapy (HT) multiple-bouts when publication outliers are removed. A) Diastolic blood pressure response to HT multiple-bouts; B) Systolic blood pressure response to HT multiple-bouts; C) Mean arterial pressure response to HT multiple-bouts.

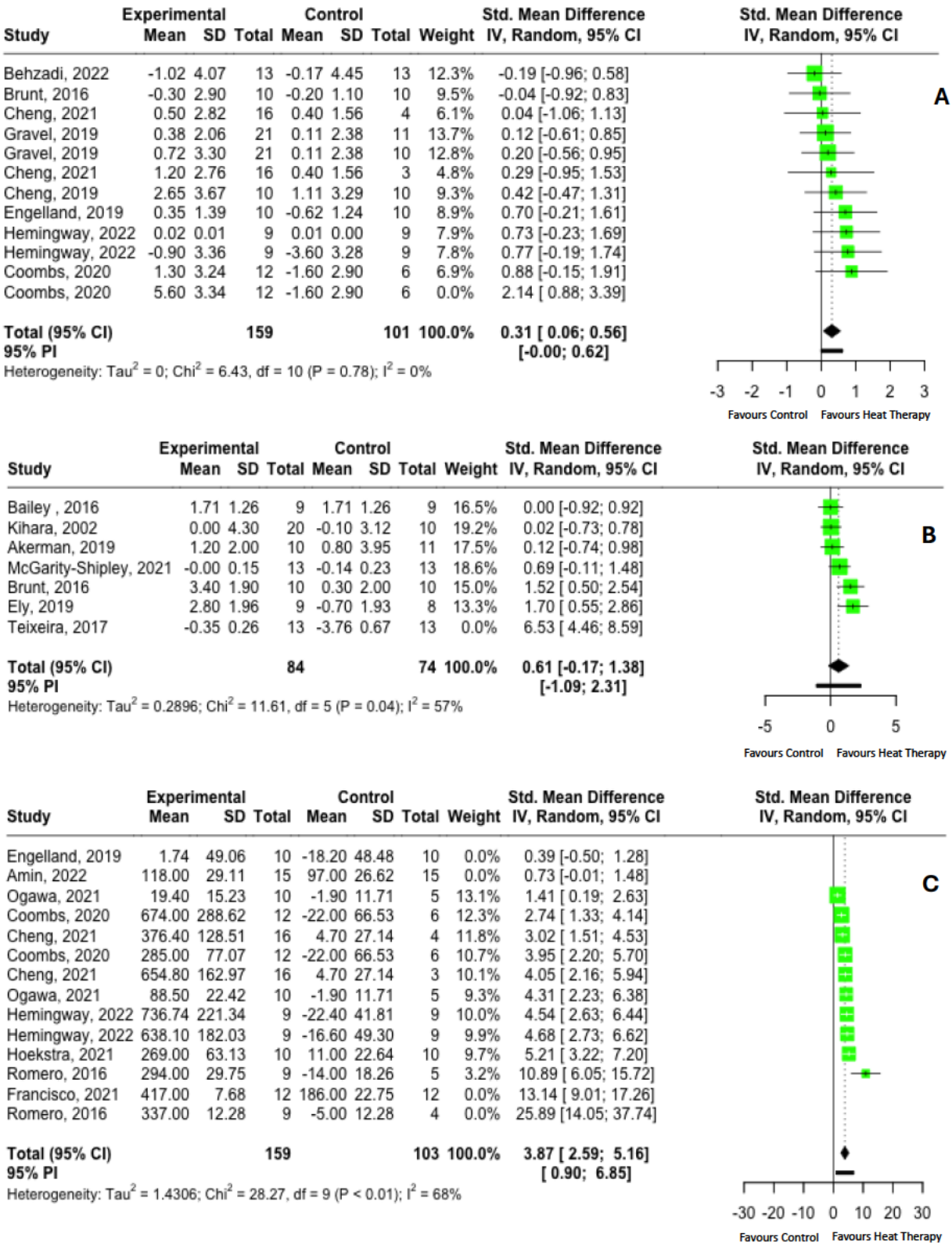

Figure 2.4. The pooled effect estimate, flow-mediated dilation (FMD) to heat thermotherapy (HT) single and multiple-bouts as well as the pooled effect estimate shear rate response to HT single-bout when publication outliers are removed. A) FMD response to HT single-bout. B) FMD response to HT multiple-bouts. C) Total shear rate response to a HT single-bout.

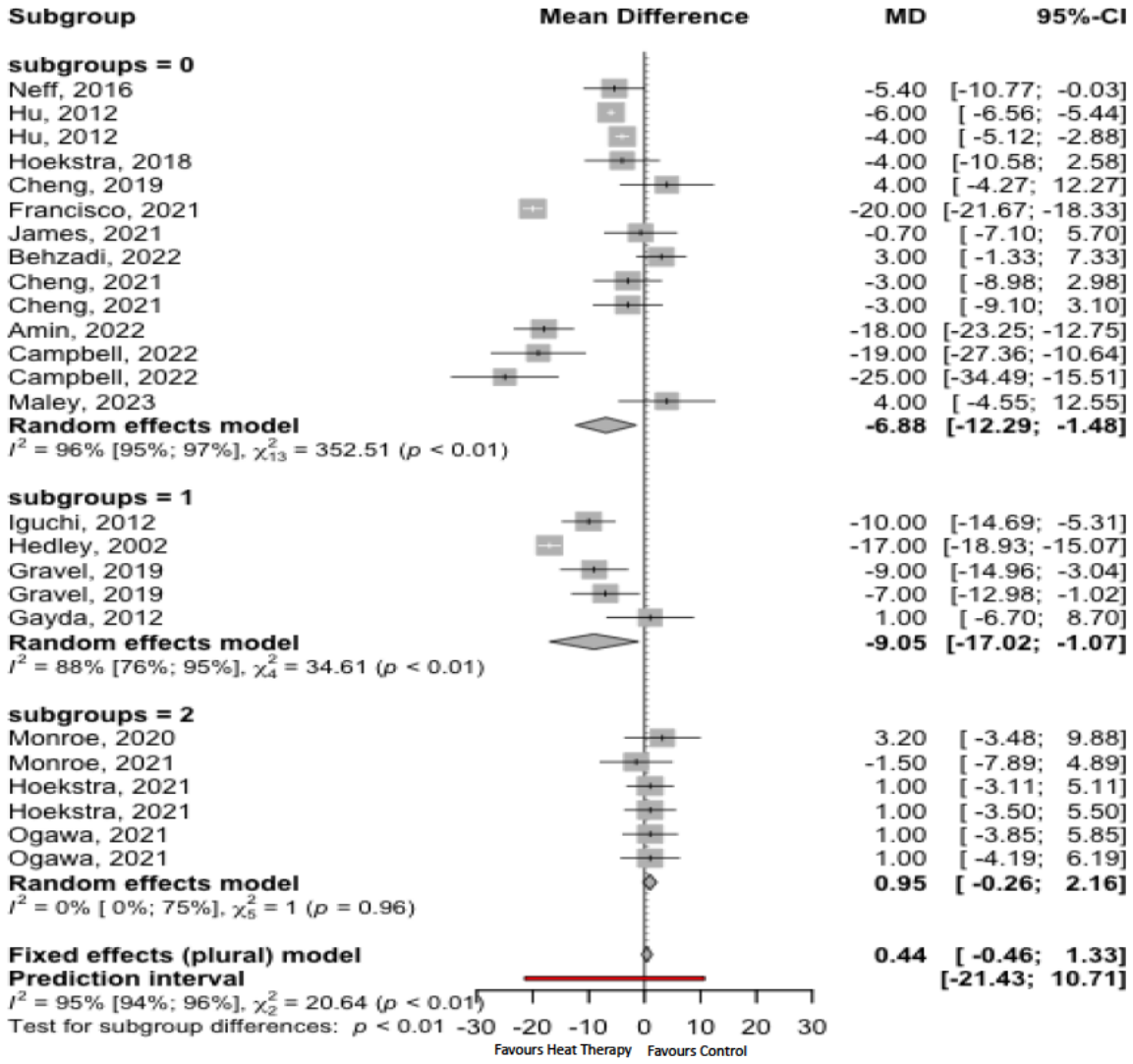

Figure 2.5. Subgroup analysis, assessing the impact of heating modality for diastolic BP (DBP) and flow-mediated dilation (FMD) from a heat thermotherapy (HT) single bout. Subgroup 0: hot water immersion, subgroup 1: sauna bathing; subgroup 2: water-perfused suit. A) DBP heating modality subgroup analysis. B) FMD heating modality subgroup analysis.

2.4 DISCUSSION

2.4.1 Main findings

This systematic review and meta-analysis, consisting of 44 publications and 817 participants, aimed to investigate the potential CV and cardiometabolic health benefits of HT comprehensively and empirically, comparing how these benefits respond to acute versus multiple HT bouts. A single-bout of HT significantly reduced BP (DBP, MAP) and significantly increased peripheral artery shear rate and FMD, whilst multiple-bouts of HT significantly reduced resting DBP and MAP. Furthermore, participants' age or health status did not influence HT CV benefits. Therefore, this systematic review and meta-analysis has demonstrated that HT invokes a positive, acute CV response alongside improvements in long-term resting BP, regardless of an individuals' health status. The meta-regression was unable to reveal the most effective HT strategy (i.e., heating modality, duration of HT bout) to elicit positive CV and cardiometabolic responses, although there was substantial subgroup heterogeneity across publications. Therefore, which HT strategy best improves CV and cardiometabolic parameters remains unclear.

2.4.2 Blood pressure

To our knowledge, this is the first meta-analysis to investigate CV function after a single HT bout. Data from this meta-analysis indicates that a single HT bout significantly reduced DBP (drop of 2

mm Hg) and MAP (drop of 6 mm Hg) with no change in SBP. Meanwhile, multiple bouts of HT significantly reduced DBP (drop of 5 mm Hg) and MAP (drop of 4 mm Hg), with no significant change indicated for SBP (p=0.08). Importantly, small changes in DBP have clinically meaningful implications for normotensive and hypertensive individuals (Cook et al., 1995). For example, a 2 mm Hg reduction in DBP results in a 6% reduction in CVD risk for hypertensive individuals (Cook et al., 1995). This indicates that HT induces a hypotensive response, which, following multiple HT bouts, has demonstratively positive BP (and therefore CV health) benefits. Furthermore, acute reductions in BP may be requisite for an acute HT stimulus to affect positive CV changes chronically.

HT modality affected the magnitude of DBP reduction achieved by a single HT bout. The current subgroup analysis showed that after a single HT bout, sauna bathing resulted in the greatest DBP reduction followed by HWI (-9 vs. -7 mm Hg; (figure 2.5)). It is unclear what aspects of a sauna bathing stimulus drive this greater acute hypotensive effect. However, a potential underlying mechanism is the inverse relationship between T_c and CVP) (Crandall et al., 2008). During HWI, this relationship is modified to some extent as the hydrostatic pressure of water attenuates heat-related reductions in peripheral resistance, causing CVP to be higher during HWI than during sauna bathing (Tei et al., 1995). In addition, sauna bathing may promote greater sweat loss, leading to a larger reduction in plasma volume and subsequently a larger hypotensive effect relative to HWI (Akerman et al., 2017). It is important to highlight that the HT strategy influences these underlying mechanisms (i.e., air or water temperature, body immersion levels, HT

duration, etc.). Furthermore, given the heterogeneity of HT strategies used in the current meta-analysis, heat modality subgroup analysis should be interpreted cautiously. There were no subgroup effects after multiple bouts of HT; however, this is likely due to the limited number of included publications rather than a physiological adaptation (multiple bouts of HT publications: DBP; *n*=6, MAP; *n*=4).

The dynamic nature of the CV system is important to consider when interpreting CV measures, as BP responses can be affected by mild changes in air temperature (Lanzinger et al., 2014) or routine movements, such as lying or standing (Lucas et al., 2010) The current meta-analysis found that participant state (e.g., postural position) and environmental conditions (e.g., air temperature) were not consistently reported in the included publications. Unfortunately, it is also unclear whether all the included publications followed BP measurement guidelines (Stergiou et al., 2021). It is recommended that future studies standardise and report these BP measurement methodological considerations (postural position and environmental conditions). As a bare minimum, BP guidelines (Stergiou et al., 2021) must be followed for studies aiming to investigate the hypotensive effect of HT. Alongside the BP guidelines, to prevent postural related changes to BP affecting readings (Harris et al., 1991, Wald et al., 1937), a standardised resting period of 10 minutes should be applied when changing postural position. Additionally, morning BP is an independent CVD risk factor (Kario, 2010) with BP surges associated with increased CV events (Li et al., 2010, Booth et al., 2020). Therefore, interventions that can reduce morning BP are advantageous and should be sought after. Plasma-volume-driven hypotension has been

observed 24-h post-HT, via improved venous return and cardiac efficiency ((Akerman et al., 2017, Davies et al., 2023)), therefore researchers may wish to include this 24-h timepoint into their protocols.

2.4.3 Flow-mediated dilation and shear rate

To our knowledge, this is the first meta-analysis to investigate the acute SR and FMD response following HT. The meta-analysis has demonstrated that a single bout of HT substantially increases total SR (3.88 Hedge's G) with a small increase in FMD (0.31 Hedges G). The increase in total SR is not surprising as increases in T_c lead to the redistribution of blood flow to the peripheral vasculature (Rowell, 1974). Increased peripheral SR is associated with an increase in acute FMD responses (Carter et al., 2013b). Therefore, it is highly likely that the small, but significant, increase in FMD is driven by the substantial total SR elevation.

The meta-analysis FMD measurement timings varied following a single bout of HT (immediately to over 90 minutes post-HT). This variance in the measurement timings could have reduced the magnitude of the effect observed following HT. Measurement timing appears to be important when assessing SR and FMD post-HT. For example, previous studies have been shown that 10-20 minutes post-HT brachial artery baseline diameter and total SR remain elevated (Coombs et al., 2021, Cheng et al., 2021). By comparison, one study that examined FMD 60 minutes post-HT found brachial artery baseline diameter, total SR, and FMD measures had returned to pre-HT values (Engelland et al., 2019). Although, it should be noted that post-HT responses may be

affected by a potential upregulation of vasodilatory substances (i.e. nitric oxide) alongside an improved antioxidant status (Dawson et al., 2013). Therefore, the timing of the FMD measure is important to determine the physiological mechanisms driving potential increases in FMD following an acute HT session. Future studies should consider repeating FMD measurements (e.g. within 20 minutes and after 60 minutes, post-HT) to elucidate the physiological mechanisms responsible for the increased FMD response post-HT. Interestingly this repeated FMD protocol would establish whether the FMD response following bathing is similar to the bi-phasic response seen following exercise interventions (Dawson et al., 2013).

Despite an acute increase in FMD, FMD did not change (p=0.10) after multiple bouts of HT. There are two possible reasons for this occurrence. Firstly, there was significant participant heterogeneity (age and CV disease risk factors) across FMD publications. Individuals with CVD risk factors and older adults typically have less compliant arteries (Black et al., 2009). Therefore, it might be expected that a younger group would have a greater increase in FMD due to improved arterial compliance, as compared to a cohort with CVD risk factors or an older cohort (Black et al., 2009). Indeed, Brunt and colleagues demonstrated a 5% improvement in FMD in young, healthy adults (Brunt et al., 2016a) compared to no significant FMD change in elderly adults with PAD (Akerman et al., 2019). Thus, including a wide range of participant demographics (e.g. age and health status) may have prevented a significant FMD meta regression value. Alternatively, it may be that the multiple bouts of HT and subsequent FMD outcome analysis was underpowered due to a lack of publications (n=7).

2.4.4 Arterial stiffness

Due to a low sample size, changes in central and peripheral arterial stiffness following single and multiple HT bouts were pooled. Arterial stiffness changes are normally associated with a structural artery change, which can take 4-6 weeks (Brunt et al., 2016a). Thus, the pooling of acute and chronic responses in the current meta-analysis likely contributed to the insignificant effect of HT on arterial stiffness. Additionally, even when pooled, HT-induced arterial stiffness responses were underpowered. Therefore, further research is needed to identify how HT modulates arterial stiffness (and compliance) as this is a key indicator of CV health and CVD disease (Niiranen et al., 2016).

2.4.5 Cardiometabolic health

The findings from this meta-analysis demonstrate that it is unclear whether HT improves cardiometabolic health. The cause of this uncertainty is the lack of publications that include cardiometabolic variables. For example, the only significant change following HT was a long-term reduction in IL-6, which was reported in only two publications. Due to the lack of publications, compromises were made, such as the grouping of variables, including the combination of fasting glucose and postprandial glucose into one analysis and the combination of several heat shock proteins (HSP) into one analysis. This is problematic as the postprandial and fasting glucose response to HT could be remarkably different (Maley et al., 2019, Sebok et al., 2021). For HSPs,

there was no consistency in the HSPs reported (e.g., HSP70, HSP72 & HSP90), of which it would be expected that different HSPs would have different levels of proliferation after HT (Ely et al., 2018). Therefore, the lack of available publications in the meta-analysis for cardiometabolic variables and the grouping of certain variables (glucose and HSP) means that it is still uncertain whether HT improves cardiometabolic health. In future, researchers should consider including a battery of cardiometabolic measurements alongside CV measurements in their study protocols. To overcome potential financial challenges or a lack of expertise in measuring cardiometabolic variables, collaborations should occur with other research groups with expertise in this area.

2.4.6 Meta-regression

Due to the lack of publications reported in the meta-regression, it is not possible to identify the duration of HT required to elicit an optimal CV or cardiometabolic response or adaptation. Due to the lack of reported outcomes, the meta-regression combined all the publications for the particular variable in question. This means the meta-regression has substantial participant and method heterogeneity. This has several implications. Firstly, it is not possible to separate the effect of specific participant characteristics (e.g., young and healthy). As such, it is possible that there could be a significant effect of time for a specific participant characteristic, but this is diminished when all the other participant characteristics are collectively combined. Alternatively, an effect may not have been seen due to the cumulative minutes and thermal load heterogeneity. For example, a 2400-minute HT intervention may be the optimal dose if the method is a neck-level immersion at 41°C, however, a 37°C neck-level immersion may not be

effective, or *vice versa*. In future, researchers should consider the relationship between the thermal load and cumulative minutes. In other words, can a systematic approach be applied to decipher the optimal single HT bout method before then applying this to a multiple HT bouts strategy with a specific participant group in mind?

2.4.7 Strengths and limitations

This systematic review and meta-analysis are the most comprehensive assessment of HT literature to date, with 44 publications included. This meta-analysis only included publications with a control arm and therefore, greater confidence can be credited to the reported HT related values/comparisons. Most importantly, this meta-analysis is unique because it separately identified single-bout and multiple-bouts of HT. Therefore, acute responses and chronic adaptations of CV and cardiometabolic parameters to HT have been systematically assessed where possible.

Substantial heterogeneity (I^2>75%) was reported for most CV and cardiometabolic variables. This substantial heterogeneity was not surprising due to the method heterogeneity reported across publications. We attempted to explore some of this method heterogeneity through the subgroup analysis (i.e., heating modality), however, this subgroup analysis did not resolve the reported heterogeneity. Some subgroup analyses were limited because they included a small number of publications (<10; alongside the inclusion of outlier publications), which may have skewed the results.

The risk of bias assessment identified that all included publications had some concerns (38/44) or a high risk of bias. The primary cause for this was the randomisation of groups, with most included publications not stating their randomisation method. Other researchers have used a matched pairs design. A potential problem with this method is that it can lead to selection bias and was flagged in the risk of bias assessment as “high risk”. Another area of concern was the potential selective reporting of results, as most publications did not register their study (i.e., on a clinical trial website) before publication. Thus, publications may not have reported all recorded variables from the data collection stage. In summary, the risk of bias assessment indicates that HT research is at the proof-of-concept stage. In other words, small sample-sized study designs have been utilised to demonstrate the proof of concept and will, hopefully, in the future, provide a basis for large, randomised controlled trials. To conclude, the risk of bias assessment, the limited number of publications included in the meta-analysis for some CV/cardiometabolic variables, and the substantial heterogeneity reported indicate that some caution is needed when interpreting the results of this meta-analysis.

2.4.8 Future research recommendations

It is not possible at this stage to provide HT guidelines for the public, or to be sure of the HT intervention that will deliver the best CV function or cardiometabolic health outcomes. Future research should focus on refining HT protocols so that the optimal and minimal conditions needed to yield CV function and cardiometabolic health benefits are realised and safe for the

public to engage in. To do this, future research should focus on reducing the substantial method heterogeneity. This will allow a more straightforward interpretation of results across publications and begin to create a protocol that can then be tested across different participant characteristics. Researchers should start by identifying whether these CV benefits are seen due to the T_c gained or the prolonged elevation of T_c past a certain threshold.

More standardisation is needed when reporting the post-HT environmental conditions to reduce method heterogeneity further. By standardising these key, but easily missed details (i.e., the post-HT environmental conditions and collecting data using recommended guidelines (e.g. BP)), more confidence can be gained from the reported outcomes. These decisions should be reported within the methods sections of publications to help readers make informed decisions regarding the publication's results. Additionally, all dependent variables included in the publication should be registered in a public database before the HT sessions occur. This would address two problems. Firstly, it would eliminate the possibility of selectively reporting outcome variables, reducing the risk of bias. Secondly, it will allow a future meta-analysis to analyse more data, allowing a more informed decision on the efficacy of HT to improve CV and cardiometabolic health.

2.4.9 Conclusion

HT improves acute and chronic CV parameters, although the magnitude of these improvement was unaffected by an individual's health status or HT intervention duration. These data support

the use of HT to improve CV parameters. However, further research is required to establish how HT can improve cardiometabolic parameters.

3. GENERAL METHODS

3.1 Ethical approval

Each experimental study received ethical approval from the University of Birmingham's Science, Technology, Engineering, and Mathematics Ethical Review Committee (Chapter 4: (ERN_19-1491_AP1); Chapter 5 (ERN_19-1491_AP2)), which complied with the Declaration of Helsinki. All participants were briefed on the study's benefits and risks before providing verbal and written consent (Appendix A and C).

Experimental sessions were stopped if any of the following criteria were met:

1) The participant wished to stop participating in the study
2) The participant felt unwell or could not complete the maximal oxygen consumption ($VO_{2\ max}$) test or high-intensity interval exercise (HIIEx) cycling protocol
3) The participant reached a T_{rec} of 39.5°C, the safety limit set by the ethical review committee.

3.2 Participants

Young, healthy males and females were recruited to participate in Chapter 4 (January to June) and Chapter 5 (January to April). Participants were excluded if they had any history of CVD, were regular spa users (>1 per week), or were above the age of 50 years. Recruited female participants were eumenorrheic or using hormonal contraceptives. The menstrual cycle was tracked using a commercialised digital tracking app (e.g. Flo Period & Cycles Tracker, Flo Health UK Limited, London, UK) or a paper menstrual cycle diary. For both experimental studies,

naturally, menstruating females took part during the mid-luteal phase of their menstrual cycle (days 17-21), while females using contraceptives were tested during the active-pill phase. The rationale for selecting female participants during the mid-luteal phase is threefold. Firstly, during this phase, both the elevated oestrogen and progesterone levels are relatively stable, and similar to individuals using hormonal contraceptives (Schmalenberger et al., 2021). Secondly, females in the mid-luteal phase experience a heightened T_c akin to those using hormonal contraceptives (de Mouzon et al., 1984). Finally, compared to the potential participant discomfort and bleeding during menses, female participants will likely find the mid-luteal phase more comfortable for participation than the early follicular phase.

Before the main experimental trial visits, participants were told to refrain from vigorous exercise 24 hours prior, alongside caffeine and alcohol at least 12 hours prior. Additionally, participants were told to avoid eating 12 hours before experimental trials and were instructed to arrive in the laboratory fasted. Participants could drink water *ad libtum* during the 12 hours prior. Upon arriving at the laboratory, participants completed a 24-hour food recall diary to confirm that dietary controls were followed. If participants did not follow the restrictions, the session was rescheduled.

All participants completed a familiarisation session before experimental laboratory visits in Chapters 4 and 5. This session involved completing baseline mood, anxiety, and sleep quality assessments and familiarising themselves with all the equipment and measures to be used

during the experimental visits. Participants also underwent a brachial artery scan using a colour duplex ultrasound device (Terason uSmart 3000, Teratech, USA) to familiarise the participant with the procedure and the operator (B.P.) with their brachial artery.

3.3 Measurements

3.3.1 Flow-mediated dilation

The vascular endothelium is a monolayer of endothelial cells that maintains healthy vascular function (Krüger-Genge et al., 2019). For example, the endothelium cells regulate vascular tone by releasing vasoactive agents, including NO for vasodilation and endothelin-1 for vasoconstriction (Deanfield et al., 2007), and they also regulate leukocyte wall adhesion/migration (Ley et al., 2007). Vascular dysfunction is a precursor for atherosclerosis, plaque rupture, and CVD-related events (Ross, 1999). Therefore, examining endothelial function could improve the early detection of CVD and assess vascular responsiveness following an intervention (Flammer et al., 2012).

Poor endothelial function in the coronary artery is associated with a higher CVD incidence than in other locations, such as the brachial artery (Minhas et al., 2022). Therefore, the coronary artery is the ideal location to assess endothelial function. For example, repeated coronary artery endothelial disruption can lead to myocardial ischemia, small infarctions and, ultimately, heart failure (Lerman and Zeiher, 2005). The golden standard test for endothelial function is via quantitative angiography, which detects luminal changes after a pharmaceutical (e.g.,

acetylcholine) or physiological (e.g., exercise testing) stimulus (Minhas et al., 2022). This test, however, is invasive and requires specialist training. Non-invasive alternatives include magnetic resonance imaging and measurement of flow-mediated dilation of a conduit artery (e.g. brachial or femoral artery) via doppler ultrasound (Celermajer et al., 1992).

FMD is a popular and non-invasive method to assess endothelium-dependent dilation, first reported in 1992 (Celermajer et al., 1992). Typically, endothelial function is measured in the conduit arteries, such as the brachial artery, as it is easier to measure and less risky/expensive to access than the coronary arteries. FMD measures conduit artery NO-dependent endothelial function by measuring the change in brachial artery diameter after exposure to ischemia (Thijssen et al., 2019). FMD is a valuable tool for predicting CVD events; for example, small changes in the FMD response (e.g. 1% increase) can reduce the risk of a CV disease-related event by 8-13% (Green et al., 2011). FMD has a strong, positive correlation with quantitative angiography assessments (r = 0.77; (Broxterman et al., 2019)) and distinguishes between patients with and without coronary artery endothelial dysfunction (Broxterman et al., 2019, Anderson et al., 1995). Additionally, FMD is cheaper than magnetic resonance imaging and is not constrained by space, allowing for greater intervention variety. Therefore, this thesis utilised the FMD technique as it is cost-effective, non-invasive, and can be used consistently after training (Greyling et al., 2016).

In Chapters 4 and 5, endothelial function was assessed via FMD at the brachial artery using high-resolution duplex ultrasound (Terason uSmart 3000, Teratech, USA) with a 15–4 MHz linear array transducer (Terason 15L4 Smart Mark, Teratech, USA), set at a frequency of 4.5 MHz. In accordance with guidelines (Thijssen et al., 2019), the Doppler signal was set at an isolation angle of 60°, and the sample volume (1.5mm) was placed at the centre of the artery lumen. All FMD measures in the current thesis were taken following a 15-minute supine rest and consisted of imaging the brachial artery to obtain baseline diameter and blood velocity measurements before inflating a manual BP cuff (set to 220 mmHg) just below the elbow for 5 minutes. The peak diameter, shear rate, and blood velocity were recorded following cuff deflation. Before undertaking the repeatability study (see below), the PhD candidate (B.P.) underwent 3–4 months of ultrasound imaging training with supervision from a trained operator (A. Danielle).

All FMD data analysis was performed using a specialised custom-designed edge-detection and wall-tracking software (Cardiovascular Suite, Quipu, Italy). The video recordings were analysed multiple times with the region of interest moved across the artery until 2-3 similar and reliable values were recorded. Blood vessel diameter (mm) was determined automatically along the highest quality portion of the B-mode image. Baseline diameter was calculated as the average diameter during the 1-minute baseline. Peak diameter was the highest value recorded once the cuff was deflated. FMD was calculated as follows:

$$\text{FMD (\%)} = \frac{Peak\ diameter - baseline\ diameter}{baseline\ diameter} \times 100$$

Doppler flow velocity waveform envelopes were automatically traced, and mean blood flow velocity (cm/s) was calculated as half of this time-averaged peak envelope velocity. Shear stress was estimated using shear rate (SR (Pyke and Tschakovs.ky, 2005)), calculated as:

$$\text{SR} = \frac{4 \times Baseline\ mean\ positive\ blood\ velocity}{Baseline\ mean\ diameter}$$

Inter-participant baseline diameter can account for a substantial proportion of FMD variance (Atkinson et al., 2013). Therefore, the allometric scaling technique (Atkinson et al., 2013) was applied to the FMD results to account for the variation in baseline diameter across participants. Subsequently, FMD results are presented as traditional and allometrically scaled in Chapters 4 and 5.

3.3.2 Reliability of the flow-mediated dilation measure

The main limitation of the FMD measure is the variance that can occur between repeat measures. For example, reproducibility studies for brachial FMD report a coefficient of variation (CV) of more than 9% (intra-day) and 14% (inter-day) even when the same trained operator performs measures (Charakida et al., 2013, Craiem et al., 2007)). Using a probe holder for inexperienced operators (Donald et al., 2008), following the expert FMD guidelines (Greyling et al., 2016), and extensive practice before completing a research study (Thijssen et al., 2019) reduces intra-day and inter-day CV. Therefore, the PhD candidate completed extensive practice using a probe holder (FMD-probe-holder-X-Y-Z, Quipu, Italy) and followed the FMD guidelines (Thijssen et al., 2019). To test the operator's FMD technique consistency, a repeatability study was conducted before data collection in Chapters 4 and 5.

Ten participants (21±2 years; 4 female) were recruited, gave written consent (ethics code ERN_18-1707), and completed an FMD repeatability study. Participants visited the laboratory on three separate occasions to assess brachial artery FMD (visit one – familiarisation), inter-day variability (visit two, scan 1 (2a) vs. visit three, scan 1 (3a)) and intra-day variability (visit three, scan 1(3a) vs. visit three, scan 2 (3b)). In total, six scans (two per visit) were completed.

During the study, participants refrained from alcohol, caffeine, and extensive exercise for at least 12 hours before visiting the laboratory, and lab visits were at least 24 hours apart. Once at the laboratory, participants lay supine for 15 minutes before the FMD scan began, following the processes outlined above. During a laboratory visit, participants were allowed to complete light physical activity tasks (i.e. walking to the bathroom) after the first FMD scan before starting the supine rest period for scan 2. Once the FMD scan was completed, participants exited the laboratory.

FMD reliability was assessed by calculating the CV between intra-day and inter-day measurements. The calculation for CV was:

$$\mathrm{CV} = \left(\frac{\sigma}{\bar{x}}\right) \times 100$$

where $\bar{x}$ is the sample mean, and σ is the standard deviation.

As per previous research, an inter-day CV of ≤2% for baseline brachial diameter and ≤15% for brachial FMD was deemed an agreeable standard for the current thesis (Greyling et al., 2016, Charakida et al., 2013). The intraclass coefficient (ICC) was also used to assess the PhD candidate's overall reliability (excellent reliability: >0.90; good reliability: 0.75 – 0.90; moderate reliability: 0.50 – 0.75; and poor reliability <0.50 (Liljequist et al., 2019)). ICC was calculated using a two-way mixed-effects model. As shown in Table 3.1 and Figures 3.1 and 3.2, the ICC, CV, and mean difference values for the intra-day and inter-day FMD measurements indicate the operator has sufficient measurement agreement within and between days to produce reliable data from brachial FMD measurements.

Table 3.1. Coefficients of variation and inter-class correlation values for brachial flow-mediated dilation and brachial artery baseline diameter.

	Intra-day CV (3a to 3b)	Inter-day CV (2a to 3a)	Intra-day ICC (3a to 3b)	Inter-day ICC (2a to 3a)
Brachial FMD	8.4%	6.9%	0.78	0.68
Brachial artery baseline diameter	2.7%	2.5%	0.94	0.90

Note. CV, coefficient of variation; ICC, intra-class correlation; FMD, flow-mediated dilation. Inter-day variability (visit two, scan 1 (2a) vs. visit three, scan 1 (3a)) and intra-day variability (visit three, scan 1(3a) vs. visit three, scan 2 (3b)).

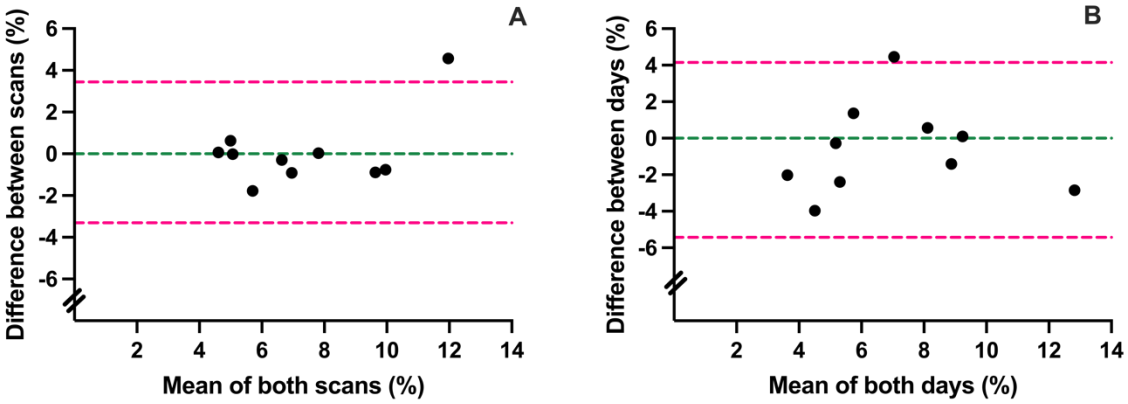

Figure 3.1. Bland-Altman plots of the intra-day and inter-day repeatability of brachial FMD measurements. A) intra-day; B) inter-day; FMD, flow-mediated dilation. Inter-day variability (visit two, scan 1 vs. visit three, scan 1) and intra-day variability (visit three, scan 1 vs. visit three, scan 2).

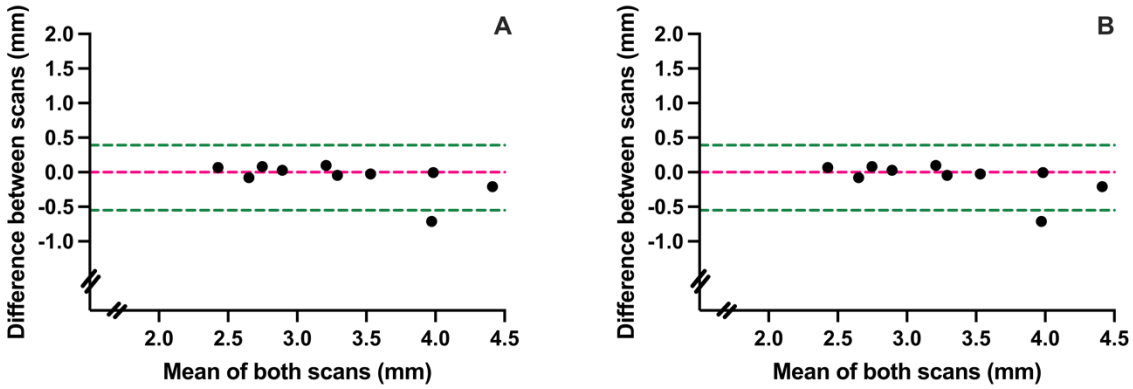

Figure 3.2. Bland-Altman plots of the intra-day (3a-3b) and inter-day repeatability of the brachial artery diameter at baseline. A) intra-day; B) inter-day. Inter-day variability (visit two, scan 1 vs. visit three, scan 1) and intra-day variability (visit three, scan 1 vs. visit three, scan 2).

3.3.3. Blood pressure

BP for Chapters 4 and 5 was measured using an automated oscillation or auscultation device. Both techniques are based on the detection of changes in cuff pressure or the detection of Korotkoff sounds. The process used to measure BP was as follows: the cuff was placed on the arm above the elbow and inflated to restrict brachial artery blood flow. When slowly reducing the cuff pressure, a Korotkoff sound is detected by the microphone within the cuff (auscultation) or by detecting a vibration (oscillation). Eventually, by slowly reducing the cuff pressure, the Korotkoff sound reaches a peak loudness/vibration, which correlates with the MAP (Alpert et al., 2014). Automated devices then use the predicted MAP to calculate SBP and DBP (Alpert et al., 2014). It is worth noting that BP devices do not use uniform calculations; therefore, it is possible to see differences in BP results between devices.

The Tango device is widely regarded as the "gold standard" for automated BP measurement, especially during exercise when movement artefacts can impact the accuracy of results (Cameron et al., 2004). Additionally, the Tango is reported to be at least as accurate as the manual BP technique (Cameron et al., 2004). This is due to its use of a microphone in the BP cuff to detect Korotkoff sounds and its inclusion of a 3-lead electrocardiogram (ECG). Due to the availability of devices and safety restrictions with a main power supply within the hydrotherapy facility, different BP devices were used in Chapters 4 and 5. For Chapter 4, an automated oscillometric device measured arterial BP from the right arm (Vicorder®, SMT Medical, Bristol, UK). For Chapter 5, the Vicorder device was used during and immediately post-bathing; for all

other measurements, including during HIIEx, the automated auscultation device, Tango (M2; SunTechMedical Instruments Inc., United States) was used. Measures were taken in duplicate to make an average. However, if the first two values were not within 5 mm Hg of each other, a third measurement was taken, and the median number was selected.

Using SBP and DBP readings from BP devices, MAP was calculated as follows:

$$\mathrm{MAP} = \mathrm{DBP} + \frac{1}{3}(\mathrm{SBP} - DBP)$$

Chapters 4 and 5 aimed to measure BP during bathing and to keep participants' immersion level to the suprasternal notch to maintain the hydrostatic effect of the water bathing condition. Participants moved so they were seated upright and semi-recumbent, with their shoulders out of the water. Both arms were taken out of the water (if applicable), and the right arm was dried and placed on an inflatable float. Then, the BP cuff was applied to the right arm, and the Vicorder was activated to record the measurement. All other BP measurements were taken in the semi-recumbent position to match the postural position of water bathing.

3.3.4 Heart rate

Heart rate was measured throughout testing in Chapters 4 and 5 via short-range telemetry at a sampling range of 1Hz (H10, Polar, Kempele, Finland). Data were captured using the Polar Beat Application (Polar, Kempele, Finland) via an Apple iPad device (iPad 8th Edition, Apple, USA).

3.3.5 Arterial stiffness

Pulse wave velocity (PWV) was measured using the Vicorder® system device (SMT Medical, UK) in both central (carotid to femoral) and peripheral (femoral to ankle) areas. This non-invasive oscillometric method was performed with the patient in a semi-recumbent position after 10 minutes of rest. A 100-mm cuff was placed around the right upper thigh and above the right ankle, while a 30-mm plethysmographic partial inflatable sensor was placed over the carotid region to detect the carotid pulse wave. The cuffs (carotid and femoral or femoral and ankle) were simultaneously inflated to 65 mm Hg. The oscillometric signals from each cuff were analysed to determine the pulse time delay and PWV. The first few pulse waves were omitted, and at least five pulse waves of high quality over 10-15 heartbeats were analysed. PWV was calculated as D_{time} travelled (TT)/D distance between the cuffs. For central PWV, this distance was between the neck cuff and the top of the thigh cuff. For peripheral PWV, the distance was between the top of the thigh cuff and the top of the ankle cuff. Pulse Pressure Index (PPI) was calculated as (SBP – DBP)/SBP (Peng-Lin and Yue-Chun, 2009). TT was calculated as the pulse wave's time to reach the distal BP cuff. PWV measurements taken with the Vicorder® device has been validated against applanation tonometry systems (Sphygmocor®, Cardiex, USA) and invasive central BP measurements (Pucci et al., 2013, Hickson et al., 2009).

3.3.6 Core body temperature

T_c is important for understanding thermoregulation and evaluating the degree of thermal stress caused by an intervention. The most accurate measure of T_c is hypothalamic temperature (Miller

et al., 2017). However, due to accessibility, core temperature is often estimated using body locations such as oesophageal, rectal, and gastrointestinal areas. The choice of body location and temperature measurement tool influences T_c readings, impacting the interpretation of intervention effects on thermoregulation (Notley et al., 2023).

Rectal temperature is a stable and reliable assessment of T_c (Moran and Mendal, 2002a). Due to its practicality and measurement accuracy, rectal temperature assessment is regarded as a "golden standard" for temperature assessment in different settings, including in hospitals (El-Radhi, 2014) and research settings (Moran and Mendal, 2002a). Despite its prolonged response time, which can be problematic when recording temperature change in a thermally transitioning environment (i.e. high-intensity exercise bouts) (Notley et al., 2023), T_{rec} is preferred to oesophageal and gastrointestinal temperature measurements (Moran and Mendal, 2002b). Mainly, this is due to improved participant comfort when fitting the thermistor and the fact that the temperature reading is not impacted by ingesting fluids (as occurs with oesophageal and gastrointestinal measures). Additionally, rectal thermistors are cheaper than gastrointestinal pills that can only be used once and must be swallowed at least one hour before the intervention study begins to ensure proper and consistent positioning in the intestinal tract (D'Souza et al., 2019). Therefore, for Chapters 4 and 5, T_c was measured using a rectal thermistor at a depth of ~10cm past the anal sphincter (Mon-a-Therm, Covidien, Mansfield, MA, United States) and continuously logged at 60-second intervals (Squirrel 2010 series, Eltek Ltd, UK).

3.4 Mood and sleep measurements

Questionnaires and visual analogue scales were used to capture the participants' mood, anxiety, and sleep quality before and during experimental trials in Chapters 4 and 5 (Appendix E-I). All questionnaires and scales during the familiarisation stage and the experimental laboratory visits were repeated in the same order and frequency. Recording participants' mood states and sleep quality provides insight into whether HWI or post-exercise HWI was well tolerated by participants and ultimately has any acute benefits for mood and sleep quality.

3.4.1 Mood state questionnaire

The Mood States Questionnaire (Liao et al., 2017a) consisted of 8 questions on a scale of 1 (not at all) to 5 (extremely) and enquired about mood sensations such as happiness and frustration (Appendix E). The questionnaire focuses on two fundamental dimensions (valence and arousal) for affect states, as the circumplex model indicates (Posner et al., 2005). The fundamental dimensions are bidirectional (positive and negative); hence, the questionnaire investigates positive and negative affect alongside additional factors (i.e. energy and fatigue). The mood state questionnaire was chosen for Chapters 4 and 5 as it provides a quick, multiple assessment of mood state that is not burdensome and unlike the Profile of Mood States Questionnaire (Pollock et al., 1979). Additionally, the Mood States Questionnaire assesses positive and negative mood states, unlike other questionnaires in the literature that focus only on negative mood states (e.g., Brunel Mood State Questionnaire (Terry et al., 1999).

3.4.2 Sleep quality

The Groningen Sleep Quality Scale (Meijman et al., 1990) consists of 17 yes or no questions that relate to the previous night's sleep quality (Appendix F). Scores that are above 6 indicate a poor night's sleep. The Hollands Sleep Disorder Questionnaire (Mulder-Hajonides Van Der Meulen, 1981) (Appendix G) is a sleep disorder screening tool with 16 items. For any question, a score ≥3/5 for any item indicates that a participant may have insomnia. The Pittsburgh Sleep Quality Index (Appendix G (Buysse et al., 1989)) consists of 10 items, each producing a score of 0 for no sleep difficulty and 3 for severe sleep difficulty. Higher overall scores indicate worse sleep quality.

3.4.5 Anxiety and stress

The Perceived Stress Scale (Cohen et al., 1994) has ten items scaled from 0 (never) to 5 (very often) (Appendix G). Overall scores of 0-13, 14-26 and 27-40 indicate low, moderate and high perceived stress, respectively. The Hospital and Anxiety Disorder Scale (Zigmond and Snaith, 1983); (Appendix G)) measures anxiety and depressive symptoms, consisting of 10 items. An overall score of 0-7, 8-10 and 11-21 indicates typical, borderline abnormal and abnormal anxiety/depressive symptoms, respectively.

3.4.6 Physical activity

The International Physical Activity Questionnaire (Booth, 2000) was completed in the long-form version. Participants were asked to quantify their total physical activity: light, moderate, and vigorous. The questionnaire is divided into four categories: leisure, work, commuting and domestic tasks. The duration of physical activity is converted into metabolic equivalents of a task per week. Higher metabolic equivalents of a task per week scores indicate more physical activity is conducted by the participant than a lower metabolic equivalents of a task per week score. The International Physical Activity Questionnaire (Appendix G) is a validated tool with acceptable measurement properties like other self-report questionnaires (Craig et al., 2003).

3.4.7 Bathing preference questionnaire

The Bathing Preference Questionnaire aimed to understand participants' perceptions of a bathing/post-exercise bathing experience and whether they enjoyed bathing conditions (Appendix H). Additionally, the Bathing Preference Questionnaire provided insight into whether a participant would be willing to repeat any of the conditions, albeit unsupervised. The questionnaire had four items that asked which condition was their favourite, least favourite, which condition a participant would complete again, and any other comments. Participants were encouraged to write why they made that decision for each question.

3.4.8 Thermal sensation and thermal comfort

Thermal Sensation and Comfort were measured throughout the experimental trials using modified 13- and 10-point scales (Gagge et al., 1967). Participants were asked to comment on "how does the temperature of your body feel?" and "how comfortable do you feel with the temperature of your body?" (Appendix I).

3.5 Intervention techniques

3.5.1 Hot water immersion

In the hydrotherapy room, participants bathed in a jacuzzi bath (Dura Spa S380, Rotospa, Birmingham, UK; see Figure 3). Immersion levels were selected to mirror the most popular method of HWI as derived from the systematic review in Chapter 2. This method involves 30 minutes of shoulder-level immersion at ~41°C to rapidly increase T_c, followed by 30 minutes of chest-level immersion (arms out of the water) to maintain T_c at ~38.5-39°C.

In Chapter 4, the HWI30 condition involved 30 minutes of bathing in ~41°C water, submerging participants to the top of their shoulders. The HWI60 condition involved 60 minutes of bathing in ~41°C water, with participants submerged to the top of their shoulders for the first 30 minutes, followed by submersion to the base of their sternum and with arms out of the water for the last 30 minutes. The TWI30 condition involved 30 minutes of bathing in ~34°C water, submerging participants to the top of their shoulders. This water temperature was selected to

match the the skin temperature and prevent an increase or decrease in T_c or skin temperature. For Chapter 5, participants in ExHWI completed the same bathing conditions as HWI30; in ExTWI, participants completed the same bathing conditions as TWI30 post-exercise, albeit at 35°C rather than 34°C to improve participant comfort and thermal sensation when leaving the bath.

Figure 3.3. A demonstration of the set-up in the hydrotherapy room where bathing sessions were conducted.

3.6 Statistical analysis

For Chapters 4 and 5, the statistical package JAMOVI 2.3 (The JAMOVI Project 2023 - Computer Software, Sydney, Australia) was used for all analyses, and statistical significance was set at $P \leq 0.05$. The statistical package GraphPad, version 10.0, generated the figures. Repeated-measures outcome data were compared using linear mixed-effects models (LMEM), with time and bathing

conditions included as fixed factors and participants and/or bathing conditions as random factors. All other data were analysed using linear models. The normality of data assumption was assessed visually using a quantile-quantile plot, a residual histogram, and the Kolmogorov-Smirnov test (if ≤0.05, this indicates the data are skewed; (Berger and Zhou, 2014). When appropriate, outlying data points were identified and removed. Post hoc analysis was carried out using Bonferroni corrections for pairwise comparisons.

4. THE ACUTE EFFECT OF 30 MINUTES VS. 60 MINUTES OF HOT WATER IMMERSION ON CARDIOVASCULAR AND PSYCHOLOGICAL FACTORS IN YOUNG, HEALTHY ADULTS

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Contributions:

B.P led this chapter's conception, interpretation, drafting and revision

S.L. & R.L. contributed to this chapter's conception, interpretation, drafting and revision.

B.P. completed this chapter's data extraction process and data analysis.

4.1 INTRODUCTION

Cardiovascular disease (CVD) costs the UK economy approximately £15.8 billion per year (Waterall, 2019), with over 480 deaths per day ((ONS), 2023). As the leading cause of premature death in the UK ((ONS), 2023), strategies to reduce CVD risk are imperative. HT is a passive heating (non-exercising) stimulus, with typical HT interventions including sauna visits and bathing in hot water (i.e. HWI). HT is associated with reduced CVD risk, as shown by reduced resting BP (Akerman et al., 2019) and improved artery endothelial function (Brunt et al., 2016a). HT is particularly useful for physically inactive individuals who subsequently have a higher CVD risk and may also have difficulties engaging in exercise interventions (Thomas et al., 2017, Hoekstra et al., 2018, Akerman et al., 2019).

A key mechanism purported to drive HT-related improvements in CV function is exposure to an uncompensable heat stimulus, which leads to sharp elevations in T_c and skin temperatures (Larson et al., 2021, Minson et al., 1998). Such elevations in body temperature increase cutaneous blood flow (Rowell, 1974) and elevate antegrade shear rate (SR (Larson et al., 2021)). This elevation in antegrade SR is postulated to increase the proliferation of vasodilatory substances such as NO (Laughlin et al., 2008, Ando and Yamamoto, 2013). NO is associated with antiatherogenic properties and can help regulate vascular tone and arterial wall stress (Moncada, 1992). Over time, repeated episodes of antegrade shear stress lead to positive structural artery remodelling (Kang et al., 1999) that can lower BP (Newcomer et al., 2011). Consequently, repeat exposure to HT-related increases in antegrade SR has been shown to

improve CV function by reducing resting BP (Brunt et al., 2016a) and improving endothelial function (Coombs et al., 2020).

Alongside CV function, HT may be able to improve positive mood state and alleviate depressive symptoms, both of which are linked to a heightened risk of CVD (Krittanawong et al., 2023). HT can improve nighttime body temperature regulation, especially in populations that have increased risk of circadian abnormalities (e.g. major depressive disorder (Duncan, 1996), night shift workers (Jin et al., 2017)), which may lower depressive symptoms and improve mood state (Avery et al., 1982, Naumann et al., 2018). Indeed, for patients with major depressive disorder, biweekly, 20-minute HWI sessions significantly reduced depressive symptoms (Naumann et al., 2020). Research is limited in young adults. However, in a recent study, an acute, 20-minute, 80°C sauna session in young females increased vigor and reduced tension, depression, anger, fatigue, and confusion, whilst a 120°C sauna session resulted in the opposite effect (Podstawski et al., 2024). Thus, despite the potential, it remains uncertain if HT can improve mood state in healthy young males and females or how this might evolve over 24 hours.

HWI is a common HT heating modality as it is relatively straightforward and has advantages over sauna bathing. For example, HWI can increase body temperature quickly due to the thermal convection of water (Parsons, 2019) and by reducing the effectiveness of sweat evaporation for heat loss (Cramer and Jay, 2016). Additionally, the hydrostatic pressure of the water immersion improves venous return, and improves arterial compliance (Arborelius et al., 1972). Therefore,

HWI is an effective heating modality for a positive HT-related CV response. However, the substantial methodological heterogeneity across HT publications currently prevents evidence-based HWI prescription for CVD prevention (Chapter 2). Therefore, for HWI to be a successful intervention to reduce CVD risk and improve CV health, it is necessary to identify a safe and effective HWI intervention that can be prescribed to the public.

The most common HWI approach used within HT literature is a 60-minute HWI protocol (Chapter 2). This 60-minute HWI approach has been shown to increase T_c above 38.5°C, reduce BP and improve endothelial artery function in those with CVD risk factors (Ely et al., 2019b). Despite 60-minute HWI's apparent effectiveness in reducing CVD risk, the extended duration (and associated prolonged thermal discomfort) makes it a less appealing or pragmatic HWI intervention for public use. Alternatively, a shorter HWI method may be more feasible. For example, a 30-minute HWI protocol (tympanic temperature rise from 35.4±0.4°C to 37.2±0.5°C) has been shown to reduce post-bathing mean arterial BP (MAP) in elderly, healthy participants, and elderly participants with peripheral arterial disease (Thomas et al., 2017). Additionally, 20 minutes of neck level HWI (42°C; (Kojima et al., 2018)) has been demonstrated to increase serum brain-derived neurotrophic factor, which is associated with CV function (e.g. coronary vessel development (Pius-Sadowska and Machaliński, 2017)).

Therefore, the purpose of this study was to investigate whether a short, hot, neck-level, 30-minute HWI session (30-min HWI) can elicit the same, meaningful CV and cardiometabolic

health benefits as the 60-minute, shoulder/waist level immersion HWI intervention. The secondary aim was to investigate how HWI affects mood, stress, anxiety, and sleep quality over 24 hours post-HWI. It was hypothesised that whilst both HWI strategies would improve CV, mood and sleep outcomes to a greater extent than a thermoneutral water immersion (TWI30) condition, they will not significantly differ from each other.

4.2 METHODS

4.2.1.1 Study design

Seventeen participants (see Table 1) completed three bathing conditions in a randomised crossover study design, separated by a minimum of 48 hours. The three conditions were: 30 minutes of hot water immersion (HWI30); 60 minutes of hot water immersion (HWI60), and 30 minutes of thermoneutral water bathing (TWI30). Recruited participants were healthy individuals, assigned to conditions in a randomised, counterbalanced order using a computer-generated randomiser.

4.2.1.2 Ethical approval and consent

The study was approved by the University of Birmingham Ethics Committee (ERN_19-1491) and all participants gave written, informed consent before taking part in the study, in adherence with the Declaration of Helsinki.

4.2.1.3 Participants

In total, 26 participants were recruited, and 17 participants (8 males and 9 females) completed all the bathing conditions (see figure 4.1). Participants who completed the study were aged 23±4 years; had a body mass index value of 22.9±1.94kg/m^2; had not recently visited a hot climate and were not regular jacuzzi/sauna users (>1 session per week). Female participants

were naturally menstruating (n=2) or using hormonal contraceptives (n=7). Naturally menstruating females took part during the late luteal phase of their menstrual cycle, while females using contraceptives were tested during the active-pill phase.

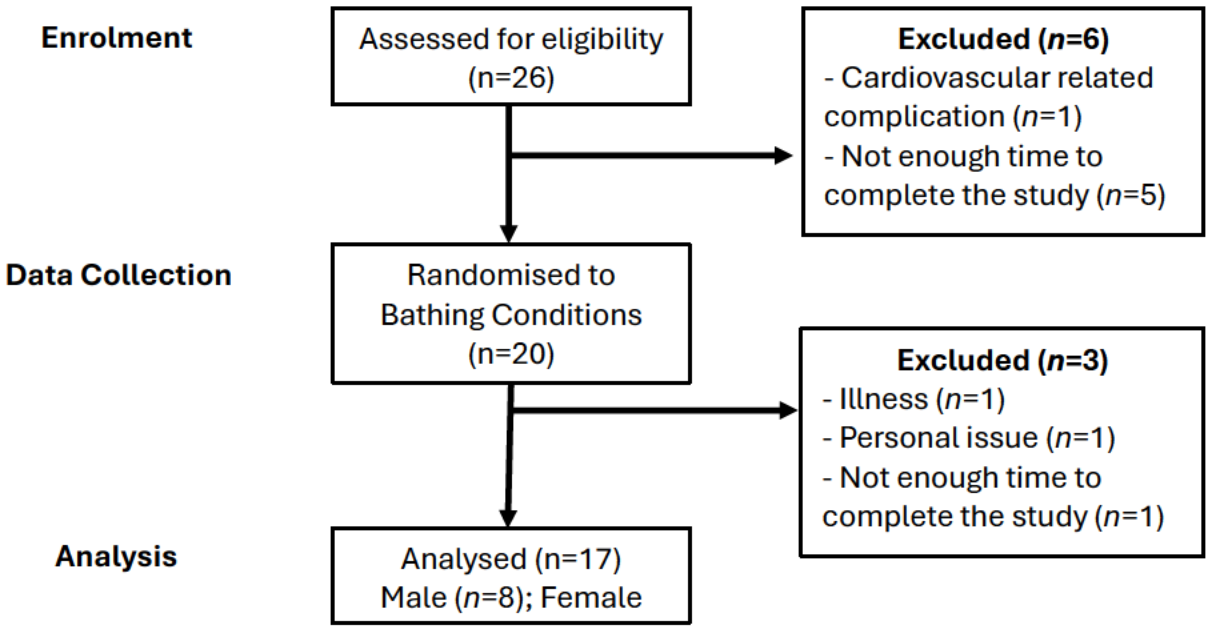

Figure 4.1. Flowchart of the participant recruitment, data collection and analysis of results.

4.2.1.4 Experimental visits

Familiarisation Session: Participants visited the lab and were briefed on the study design and requirements. During the familiarisation session, anxiety and depression were measured via the Perceived Stress Scale (Cohen et al., 1994), the Multidimensional Fatigue Scale (Smets et al., 1995) and the Hospital Anxiety and Depression Scale (Zigmond and Snaith, 1983). Participants' sleep quality for the night prior to this session was measured using the Pittsburgh Sleep Quality Index (Buysse et al., 1989) and the Holland Sleep Disorders Questionnaire (Kerkhof et al., 2013); (Appendix G)). Physical activity was measured via the International Physical Activity Questionnaire (Booth, 2000); (Appendix G). Finally, a brachial artery ultrasound scan was conducted to improve the researcher's scanning consistency during the bathing conditions and to familiarise participants with the FMD procedure.

4.1.2.5 Experimental conditions

All experimental conditions entailed the same pre- and post-bathing measures. Participants arrived fasted in the morning between 07:00-10:00 and were weighed (Seca Alpha Model 770, Seca Scales) upon entering the laboratory and after the water immersion. Participants then completed a food diary (to replicate diet for subsequent visits), a mood state questionnaire, a sleep quality questionnaire, thermal sensation and thermal comfort Scales (Gagge et al., 1967). Following this, participants were fitted with a HR monitor (H10, Polar, Kempele, Finland) and rested supine for 15 minutes before Brachial FMD was recorded. Following this, participants were moved into a semi-recumbent position for 10 minutes before HR, BP at rest and arterial

stiffness were recorded. Following resting measures, participants changed into swimming gear. They fitted a rectal thermistor (T_{rec}) at a depth of ~10cm past the anal sphincter (Mon-a-Therm, Covidien, Mansfield, MA, United States).

4.1.2.6 Bathing

The HWI30 condition involved 30 minutes of bathing in ~41°C water, with participants submerged to the top of their shoulders. The HWI60 condition involved 60 minutes of bathing in ~41°C water, with participants submerged to the top of their shoulders for the first 30 minutes followed by submersion to the base of their sternum and with arms out of the water for the last 30 minutes. The TWI30 condition involved 30 minutes of bathing in ~34°C water, with participants submerged to the top of their shoulders. HR and T_{rec} were continuously measured throughout bathing. Questionnaire and BP measurements were recorded every 15 minutes throughout and during the last minute of bathing. For BP measures, if necessary, participants moved so that the water level was at the base of the sternum for the duration of BP measurement (~2 min).

4.1.2.7 Post-immersion: BP and HR were recorded within the first 1-5 minutes of exiting the bath in a seated position. Following this, participants dried themselves and changed into comfortable clothing before post-immersion measures were recorded. Before changing clothes, at 2-5 minutes post-bathing (immediately post-bathing), between 30-55 minutes post-bathing (post-1) and 55-90 minutes post-bathing (post-2) pre-bathing measures were repeated in the same order

as described for pre-immersion, alongside an additional FMD measure at 90 minutes (post-2; see figure 4.2). In the evening, before participants were about to sleep, participants were instructed to complete another mood state questionnaire.

4.1.2.8 24-h Post: Participants returned to the laboratory between 07:00 and 10:00, 24 hours after baseline measures. Vascular measurements, the Groningen Sleep Quality Questionnaire, and the Mood State Questionnaire were completed in the same order. A bathing preference questionnaire was also completed during this visit.

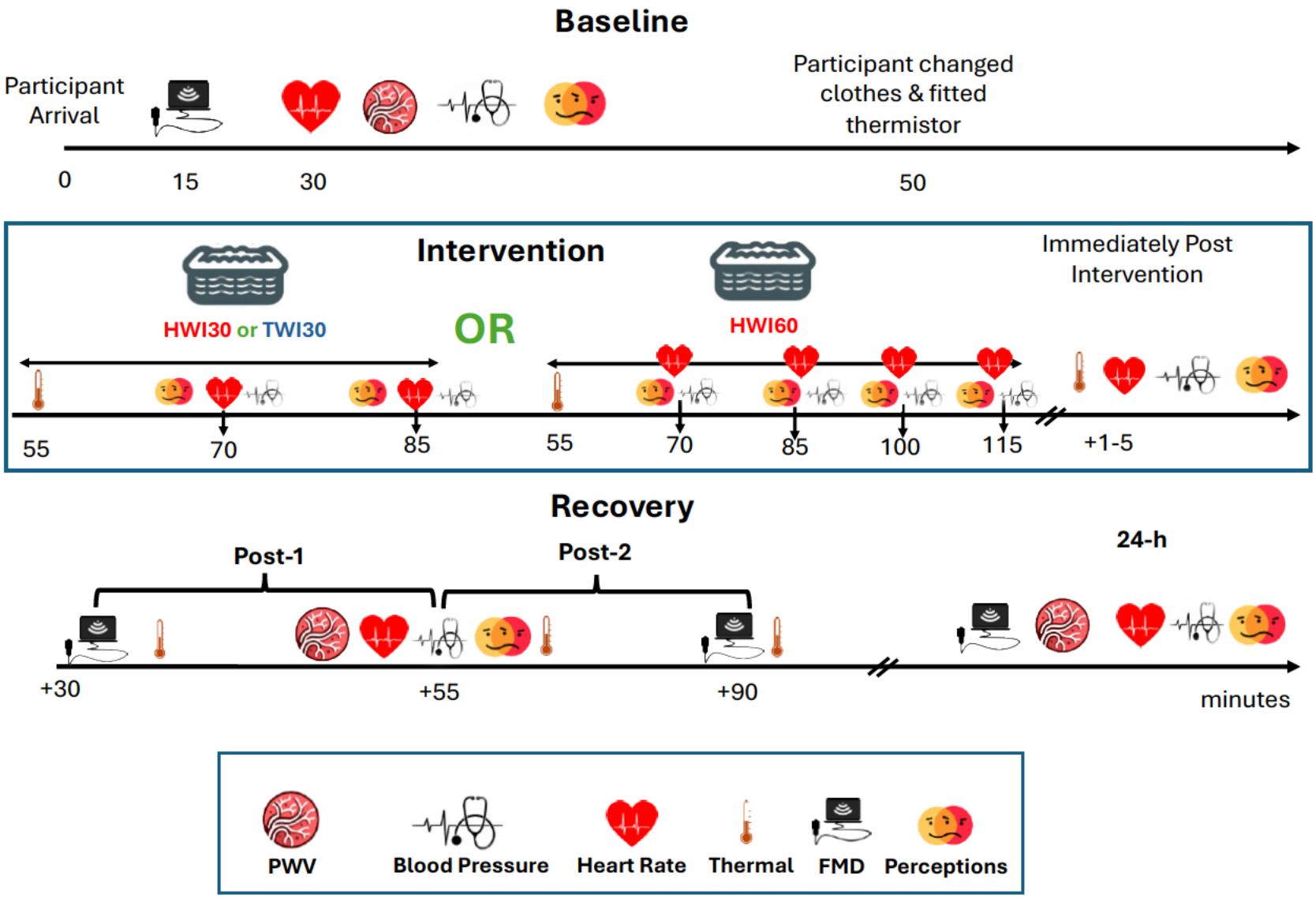

Figure 4.2. Schematic of the study protocol. HWI30; 30 minutes of bathing at 41°C, HWI60; 60 minutes of bathing at 41°C, TWI30; 30 minutes of bathing at 34°C. FMD; flow-mediated dilation; PWV, pulse wave velocity.

4.2.2 Measurements

4.2.2.1 Thermal measures

All T_{rec} measures were collected and stored every 60 seconds on a portable logger for later analysis (Squirrel 2020 series, Eltek, Ltd., United Kingdom).

4.2.2.2 Endothelial function

Endothelial function was assessed via FMD at the brachial artery using high-resolution duplex ultrasound (Terason uSmart 3000, Teratech, USA) as per the guidelines (Thijssen et al., 2019). More information on the technique is available in Section 3.3.1. All measurements were taken by the same experienced operator (B.P.). All FMD data analysis was performed by the same researcher (B.P.), using specialised custom-designed edge-detection and wall-tracking software (Cardiovascular Suite, Quipo, Italy). Further details on the calculation of SR, velocity, and diameter are available in section 3.3.1. To account for the variability in baseline diameter between participants, the allometric scaling technique (Atkinson et al., 2013) was applied to the FMD results. Subsequently, FMD results are presented as traditional and allometrically scaled.

4.2.2.3 Blood pressure

Arterial BP was measured from the right arm by electrosphygmomanometry (Vicorder®, SMT Medical, Bristol, UK). Measures were taken in duplicate to make an average. However, if the first two values were not within 5 mm Hg of each other, then a third measurement was taken, and

the median value was selected. Using SBP and DBP readings from BP devices, MAP was calculated as follows:

$$\mathrm{MAP} = \mathrm{DBP} + \frac{1}{3}(\mathrm{SBP} - DBP)$$

4.2.2.4 Arterial stiffness

Central (carotid to femoral) and peripheral (femoral to ankle) pulse wave velocity (PWV) were measured via a non-invasive oscillometric method using the Vicorder® system device (SMT Medical, UK; see Section 3.3.5.). The Vicorder® device has been validated against applanation tonometry systems (Sphygmocor®) and invasive central BP measurements (Pucci et al., 2013, Hickson et al., 2009).

4.2.2.5 Mood and sleep outcomes

Sleep quality was assessed with the validated Groningen Sleep Quality Scale (Meijman et al., 1990). Mood state was assessed via the Mood State Questionnaire (Liao et al., 2017a). For thermal perception, thermal comfort and thermal sensation were recorded (Gagge et al., 1967, Cotter and Taylor, 2005). For further information on the sleep, mood, and thermal perception questionnaires, please see Section 3.4. The bathing preference questionnaire was used to decipher the participants' perception of each bathing condition, the order of preference for bathing conditions, and their willingness to repeat a particular bathing experience (see Appendix H and Q for questionnaire and results).

4.2.3 Statistical analysis

A prior power analysis was calculated using statistical software (G*Power, Germany) with FMD as the primary outcome measure. At the time of the sample size calculation (2022), a limited number of studies had investigated shorter 20–30-minute HWI on CV function (Thomas et al., 2017, Kojima et al., 2018). However, none of those studies had reported an FMD test. Instead, the operator's coefficient of variation and inter-class correlation values (Chapter 3.3.2) were considered alongside the knowledge that a 1% change in FMD can reduce CVD risk by 8-13% (Green et al., 2011). Therefore, to detect a change in FMD (baseline vs. post-immersion) with a=0.05 and β=0.8, we calculated that 17 participants were needed to achieve a large effect size (Cohen's D = 0.8 (Cohen, 2013)).

Repeated-measures outcome data were compared using linear mixed-effects models (LMEM), with time and bathing conditions included as fixed factors and participants and/or bathing conditions as random factors. All other data were analysed using linear models. The focus of this analysis was to compare bathing conditions at pre, as well as changes from pre to bathing, pre to post-bathing, and pre to 24-h post, across bathing conditions. Posthoc pairwise comparison, if appropriate, were performed using the Bonferroni post hoc procedure. The statistical package JAMOVI 2.3 (The JAMOVI Project 2023 - Computer Software, Sydney, Australia) was used for all analyses, and statistical significance was set at $P \leq 0.05$. The statistical package Graphpad, version 10.0 was used to generate the figures. Unless otherwise stated, all results are presented as mean ± standard deviation (mean ± SD).

4.3 RESULTS

4.3.1. Baseline

Across all bathing conditions, there were no differences for any haemodynamic, thermal, or body mass variable at baseline (all $p>0.05$; Table 4.1).

4.3.2. Bathing stimulus

4.3.2.1 Rectal core temperature

There was a significant time-by-condition effect for T_{rec} (Figure 4.3). During bathing (up to 30 minutes), T_{rec} increased to a similar extent in HWI30 and HWI60 from pre-bathing values (HWI30 vs. HWI60, $p=1.00$; pre vs. 30-min bathing $p<0.01$; see Figure 2, Table 1). For TWI30, there was no change in T_{rec} during (30 minutes) or post-bathing compared to pre-bathing values (pre vs. 30-min bathing, $p=1.00$; pre vs. immediately post-bathing, $p=1.00$). Peak rectal temperature (T_{peak}) was highest for HWI60 (HWI60 vs. HWI30 and TWI30, $p<0.001$) followed by HWI30 (HWI30 vs. TWI30, $p<0.001$) with no change for TWI30 ($p=1.000$; Table 4.1). After HWI60 and HWI30, T_{rec} remained elevated until 55 minutes post-bathing (pre vs. +5 minutes (both $p<0.001$); pre vs. +30 minutes (both $p<0.001$); pre vs. +55 minutes (both ($p<0.001$)), before returning to pre-bathing values at 90 minutes post-bathing (pre vs. +90 minutes (both $p=1.000$)). After HWI60, T_{rec} was higher than HWI30 at post +5 minutes ($p=0.050$), but there was no difference between HWI60 and HWI30 at post +30 minutes ($p=1.000$). After HWI60 and HWI30, T_{rec} was higher than TWI30 until 55 minutes post-bathing (HWI60 and HWI30 vs. TWI30:

+5 minutes (both $p<0.001$), +30 minutes (both $p<0.001$); HWI60 vs. TWI30: +55 minutes (both $p=0.179$)).

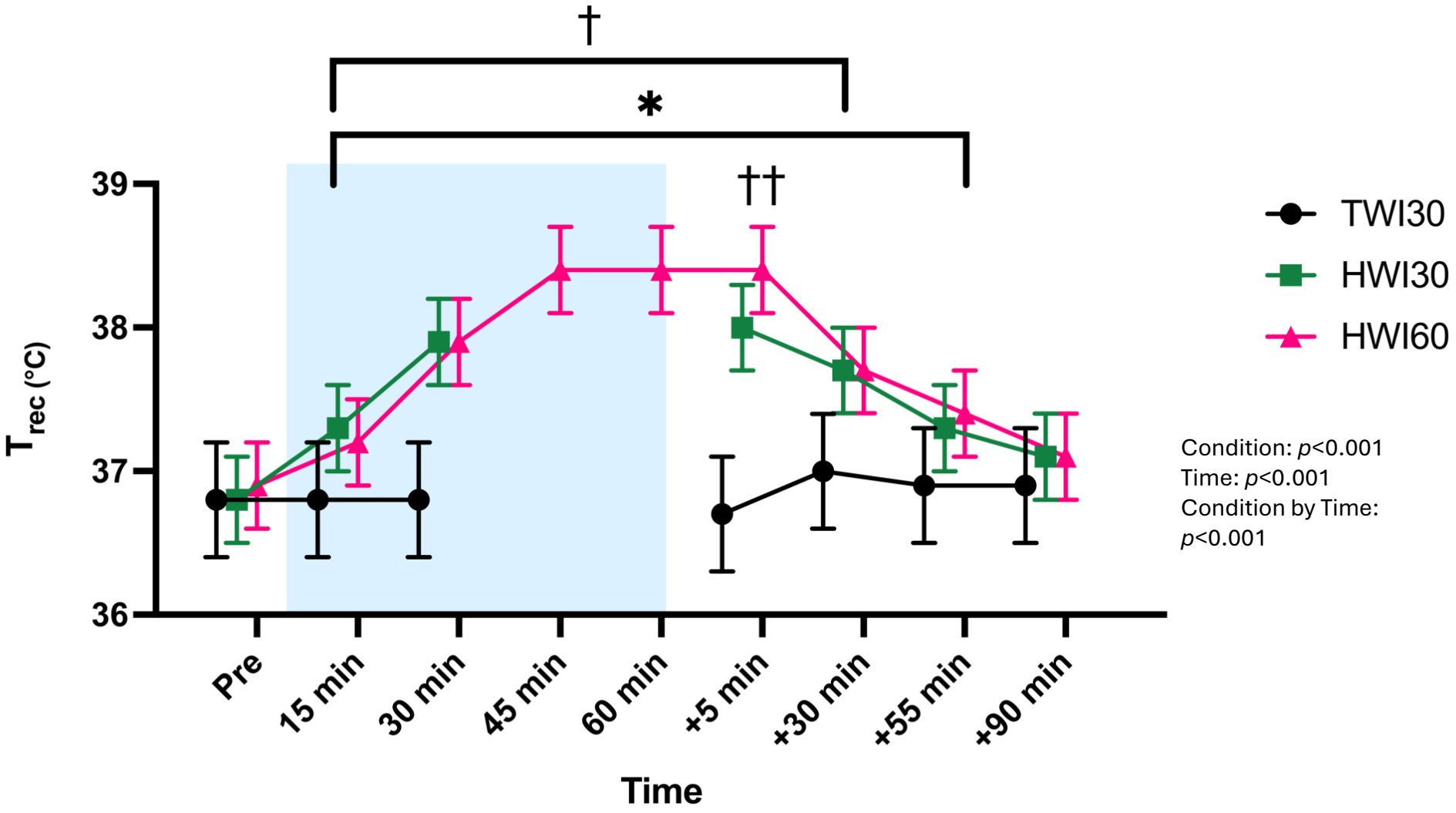

Figure 4.3. Rectal temperature (T_{rec}) responses during and post-bathing. TWI30, 30 minutes of thermoneutral water bathing; HWI30, 30 minutes of hot water immersion; HWI60, 60 minutes of hot water immersion; *($p< 0.05$), significantly different from pre-bathing value (Pre) in HWI30 and HWI60; †($p< 0.05$), HWI60 and HWI30 significantly different from TWI30; ††HWI60 significantly different from HWI30 ($p< 0.05$).

4.3.2.2 Thermal sensation and comfort

There was a time-by-condition interaction effect for thermal sensation and thermal comfort (Figure 4.4). Thermal sensation and comfort peaked after 30 minutes of bathing in both HWI conditions (HWI30 vs. HWI60 (p=1.000); pre vs. 30-min bathing (p<0.001). However, only HWI30 remained elevated immediately post bathing relative to baseline (HWI30 pre vs. immediately post-bathing (p<0.001)). During bathing, thermal sensation did not change in TWI30 ("neutral"; pre vs. 30-min bathing (p=1.000)), but dropped immediately after exiting the bath (dropping from "neutral" to "slightly cool"; pre vs. immediately post-bathing (p<0.001)).

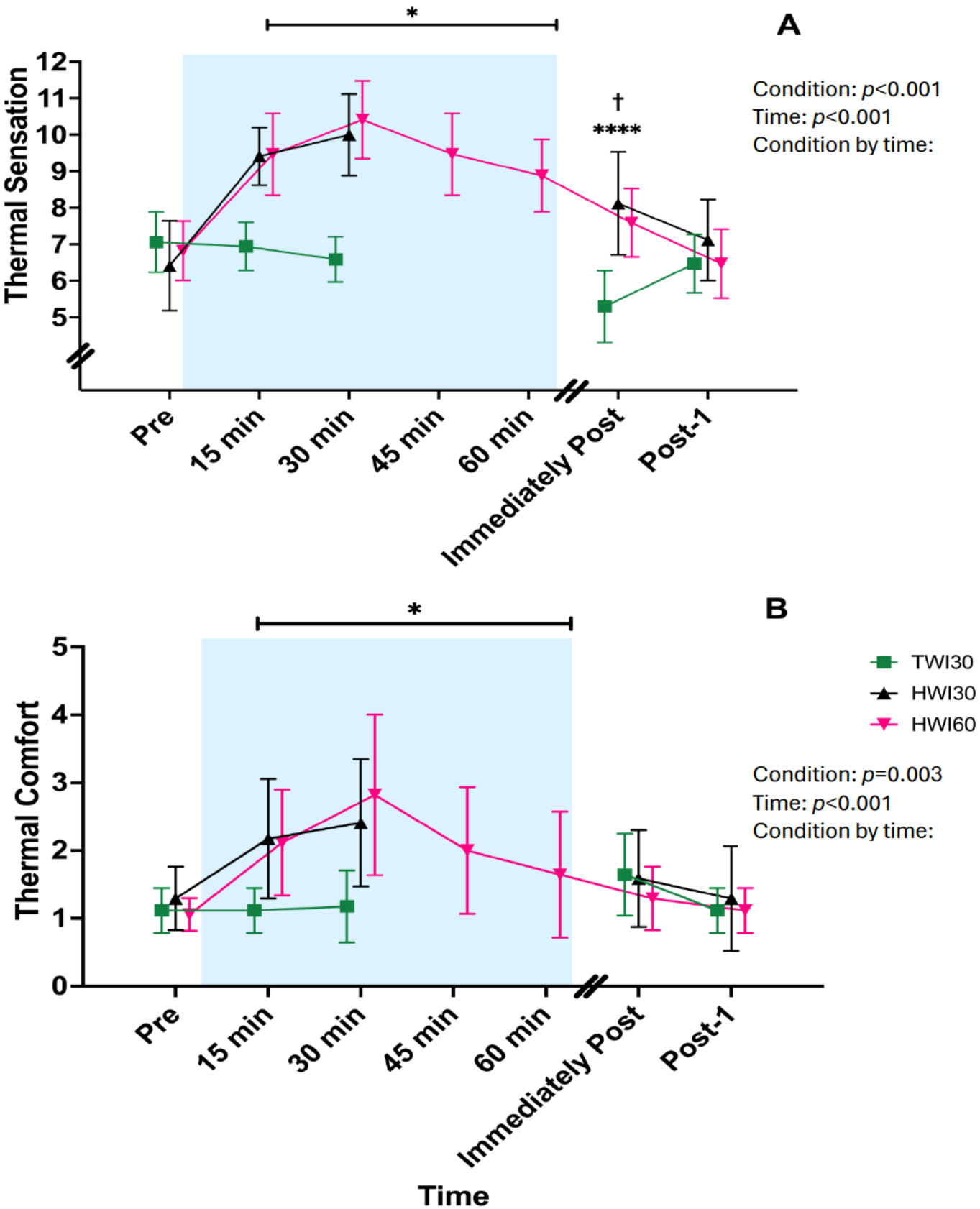

Figure 4.4. The thermal perception response during and post-bathing. *($p< 0.05$), significantly different from pre-bathing value (Pre) in HWI30 and HWI60; **($p< 0.05$) significantly different from pre-bathing value in HWI30, HWI60 and TWI30; ***($p< 0.05$), significantly different from pre-bathing value in HWI60 and TWI30; ****($p< 0.05$), significantly different from pre-bathing value in HWI30; †($p< 0.05$), HWI60 and HWI30 significantly different from TWI30. A) thermal sensation; B) thermal comfort.

Table 4.1. T_{rec}, HR, sweat loss, and thermal perception responses during and immediately post-water immersion.

	TWI30	***30HWI***	***60HWI***
T_{rec} (°C)			
ΔT_{rec} at 30 min	0.03±0.04	1.17±0.37†	1.09±0.17†
ΔT_{rec} at Peak T_{rec}	0.01±0.06	1.31±0.36†	1.66±0.24†, ††
Peak T_{rec}	36.87±0.35	38.12±0.41*†	38.52±0.27*†, ††
Heart Rate ($b \cdot min^{-1}$)			
ΔHR at 30 min	1±6	37±14†	40±12†
ΔHR at Peak HR	5+8	41±10†	45±12†
Peak HR	69±14	105±13*†	111±13*†
Pre-bathing NBM (kg)	67.5±9.1	67.9±9.3	67.4±8.7
Sweat Loss (Δkg)	0.29±0.38	1.02±0.82†	1.32±0.88†
Sweat Loss (Δ%)	0.42±0.53	1.50±1.16†	1.96±1.20†

All data are expressed as mean ± SD. TWI30, 30 minutes of thermoneutral water bathing; HWI30, 30 minutes of hot water immersion; HWI60, 60 minutes of hot water immersion; HR, Heart Rate, T_{rec}; rectal temperature. *($p<0.05$), significantly different from pre-bathing value within the same bathing condition; †($p<0.05$), significantly different from TWI30; ††($p<0.05$), significantly different from HWI30.

4.3.2.3 Sweat loss

There was no difference in nude body mass pre-bathing between conditions ($p<0.001$; Table 4.1). There was a main effect of condition for sweat loss ($p<0.001$; see Table 4.1). Sweat loss was greater following HWI30 and HWI60 bathing sessions, as compared to TWI30 (HWI30 vs. TWI30 ($p=0.015$); HWI60 vs. TWI30 ($p<0.001$)). There was no difference in sweat loss between HWI30 and HWI60 ($p=0.708$).

4.3.2.4 Heart rate

There was a time-by-condition interaction effect for HR (Table 4.2). During bathing (up to 30 minutes), HR increased to a similar extent in HWI30 and HWI60 from pre-bathing values (HWI30

vs. HWI60 (p=1.000); pre vs. 30-min bathing (both p<0.001); see Table 4.1 & 4.2). For TWI30, there was no change in HR during (up to 30 minutes) or post bathing compared to pre-bathing values (pre vs. 30-min bathing, p=1.000; pre vs. immediately post-bathing, p=1.00).

After HWI30 and HWI60, HR remained elevated immediately post-bathing (HWI30 vs. HWI60: (p=1.000); pre vs. immediately post-bathing (p<0.001)), returning to pre-bathing resting values at post-1 (HWI30 vs. HWI60 (p=1.000); pre vs. post-1 (p=1.000)). Immediately after TWI30, HR remained similar to pre-bathing values (pre vs. immediately post-bathing (p=1.000)). Peak HR increased to a similar extent for HWI30 and HWI60 (Peak HR (both p<0.001), (HWI30 vs. HWI60 (p=0.885)). HWI30 and HWI60 were higher than TWI30 (HWI30 and HWI60 vs. TWI30 (both p<0.001)).

4.3.2.5 Arterial stiffness

After HWI30, HWI60 and TWI30, there was no change in central PWV, peripheral PWV, central TT, peripheral TT and central PPI values (pre vs. post-1 (p=1.000), see Table 4.2). There was a main effect of time for peripheral PPI (Table 4.2). Across bathing conditions, peripheral PPI increased post-bathing (pre vs. post-1 (p=0.026)).

4.3.3 Brachial flow-mediated dilation

4.3.3.1 Flow-mediated dilation (%)

Across all bathing conditions, brachial FMD (allometrically scaled) did not change post-bathing compared to pre-bathing values, all $p>0.05$; see Table 4.2).

4.3.3.2 Baseline diameter

There was a time-by-condition interaction effect for baseline diameter (Table 4.2). After HWI30, baseline diameter increased at post-1 (pre vs. post-1, $p<0.01$) but returned to pre-bathing values by post-2 (pre vs. post-2, $p=1.000$; Table 2). In TWI30 and HWI60, baseline diameter remained similar post bathing relative to pre-bathing values (pre vs. post-1, both $p=1.000$; pre vs. post-2, both $p=1.000$). After HWI30 and HWI60, baseline diameter was higher than TWI30 at post-1 (HWI30 vs. HWI60: ($p=1.000$); HWI30 and HWI60 vs. TWI30 (both $p>0.044$)). Across all bathing conditions, there was no change in baseline diameter between pre and 24-h time points (pre vs. 24-h: (all $p=1.000$)).

4.3.3.3 Peak diameter

There was a time-by-condition interaction effect for peak diameter (Table 4.2). After HWI30, baseline diameter increased at post-1 (pre vs. post-1, $p<0.01$) but returned to pre-bathing values by post-2 (pre vs. post-2, $p=1.000$; Table 2). In TWI30 and HWI60, baseline diameter remained similar post bathing relative to pre-bathing values (pre vs. post-1, both $p=1.000$; pre vs. post-2, both $p=1.000$). After HWI30 and HWI60, baseline diameter was higher than TWI30 at post-1

(HWI30 vs. HWI60: (p=1.000); HWI30 and HWI60 vs. TWI30 (both p>0.039)). Across all bathing conditions, there was no change in baseline diameter between pre and 24-h time points (pre vs. 24-h: (all p=1.000)).

4.3.3.4 Shear rate

There was a main effect of time for antegrade and total SR (Table 4.2). Across bathing conditions, total SR did not initially change post-bathing (pre vs. post-1 (p=0.656)), however, total SR decreased by post-2 (pre vs. post-2 (p=0.008); see Table 4.2)). Across bathing conditions, antegrade SR did not initially change post-bathing, (pre vs. post-1 (p=0.975), however, antegrade SR decreased by post-2 (pre vs. post-2 (p=0.006)).

4.3.3.5 Total velocity

There was a main effect of time for total velocity. Across bathing conditions, total velocity did not initially change post-bathing (pre vs. post-1 (p=0.819)), however, total velocity decreased by post-2 (pre vs. post-2 (p=0.013); see Table 4.2).

Table 4.2. Hemodynamic variables and thermal perception post bathing and 24-h post bathing.

	Condition	Time Points					*p* value and η^2p		
		Pre	Immediately Post	Post-1	Post-2	24-h	Condition	Time	Condition-by-Time
Brachial FMD									
FMD (%)	TWI30	7.6±1.6	n/a	7.7±2.4	7.5±2.1	7.1±1.8	*p*=.354	*p*=.456	*p*=.521
	HWI30	7.5±1.7	n/a	7.6±3.1	7.5±2.6	7.1±2.1	η^2p=.012	η^2p=.015	η^2p=.029
	HWI60	7.4±2.0	n/a	7.3±2.5	6.3±2.2	7.5±2.6			
Allometrically Scaled FMD (%)	TWI30	7.3±2.0	n/a	7.4±2.0	7.3±2.0	7.0±2.0	*p*=.771	*p*=.936	*p*=.546
	HWI30	7.3±2.0	n/a	7.6±2.1	7.9±2.1	6.8±2.0	η^2p=.034	η^2p=.003	η^2p=.034
	HWI60	7.2±2.0	n/a	7.6±2.0	6.4±2.0	7.5±2.0			
Baseline Diameter (mm)	**TWI30**	3.6±0.5	n/a	3.5±0.6	3.4±0.6	3.5±0.6	*p*=.075	*P*=.005	*p*<.001
	HWI30	3.4±0.5	n/a	3.7±0.6*†	3.5±0.5	3.5±0.5	η^2p=.260	η^2p=.086	η^2p=.185
	HWI60	3.5±0.5	n/a	3.6±0.5†	3.6±0.5	3.5±0.5			
Peak Diameter (mm)	TWI30	3.8±0.6	n/a	3.7±0.5	3.7±0.6	3.7±0.6	*p*=.170	*p*<.001	*p*<.001
	HWI30	3.7±0.5	n/a	4.0±0.5*†	3.7±0.5	3.7±0.5	η^2p=.211	η^2p=.124	η^2p=.201
	HWI60	3.7±0.5	n/a	3.9±0.5†	3.8±0.5	3.8±0.5			
Antegrade SR (s^{-1})	**TWI30**	267.6±144.7	n/a	257.2±166.0	204.0±130.7*	278.9±166.2	*p*=.352	*p*<.001	*p*=.234
	HWI30	245.6±160.3	n/a	320.9±144.6	185.8±114.0*	229.8±148.5	η^2p=0.012	η^2p=0.124	η^2p=0.158
	HWI60	279.0±165.4	n/a	290.4±161.2	212.4±118.9*	294.1±174.6			
Retrograde SR (s^{-1})	TWI30	-20.4±22.4	n/a	-17.5±13.5	-18.2±13.3	-18.3±18.5	*p*=.176	*p*=.063	*p*=.648
	HWI30	-12.6±11.2	n/a	-9.0±7.7	-16.8±8.8	-16.0±10.9	η^2p=.207	η^2p=.049	η^2p=.029
	HWI60	-14.4±11.3	n/a	-11.3±10.6	-18.7±10.3	-15.1±12.4			
Total SR (s^{-1})	**TWI30**	247.2±159.6	n/a	239.7±175.0	185.7±135.0*	260.6±178.3	*p*=.362	*p*<.001	*p*=.243
	HWI30	233.1±165.5	n/a	311.9±148.6	169.0±116.6*	213.8±156.2	η^2p=.012	η^2p=.123	η^2p=.044
	HWI60	264.6±172.5	n/a	279.1±167.6	193.7±123.1*	279.0±180.1			
Total Velocity ($cm{\cdot}s^{-1}$)	TWI30	21.9±13.6	n/a	18.7±11.8	14.1±10.1*	22.8±14.8	*p*=.191	*p*<.001	*p*=.183
	HWI30	18.9±13.6	n/a	26.7±14.2	14.3±9.6*	21.9±13.8	η^2p=.019	η^2p=.135	η^2p=.049
	HWI60	22.3±14.1	n/a	25.3±15.8	17.3±10.9*	24.0±14.3			
HR &									

Arterial Stiffness									
HR ($b \cdot min^{-1}$)	**TWI30**	64±12	64±14	62±11	n/a	n/a	p<.001	p<.001	p<.001
	HWI30	63±10	97±14*†	65±12	n/a	n/a	η^2p=.912	η^2p=.694	η^2p=.370
	HWI60	67±12	104±13*†	69±10	n/a	n/a			
Central PWV (m/sec)	TWI30	6.3±0.8	n/a	6.3±0.7	n/a	6.6±1.1	p=.827	p=.804	p=.146
	HWI30	6.5±1.4	n/a	6.6±1.5	n/a	6.3±0.7	η^2p=.025	η^2p=.005	η^2p=.068
	HWI60	6.3±0.6	n/a	6.3±0.6	n/a	6.5±0.6			
Central PPI	**TWI30**	1.4±0.4	n/a	1.4±0.4	n/a	1.3±0.2	p=.839	p=.900	p=.872
	HWI30	1.4±0.4	n/a	1.4±0.3	n/a	1.4±0.3	η^2p=.003	η^2p=.002	η^2p=.010
	HWI60	1.4±0.5	n/a	1.4±0.3	n/a	1.4±0.4			
Central TT (m/s)	TWI30	96±9	n/a	98.9±9	n/a	93±13	p=.971	p=.114	p=.667
	HWI30	94±15	n/a	94±14	n/a	96±10	η^2p=.000	η^2p=.033	η^2p=.018
	HWI60	94±11	n/a	95±13	n/a	95±10			
Peripheral PWV (m/sec)	**TWI30**	11.4±1.6	n/a	11.4±1.6	n/a	11.2±1.4	p=.961	p=.766	p=.753
	HWI30	11.5±1.3	n/a	11.1±1.3	n/a	11.4±1.7	η^2p=.005	η^2p=.006	η^2p=.019
	HWI60	11.4±1.6	n/a	11.5±2.0	n/a	11.3±1.7			
Peripheral PPI	TWI30	1.1±0.1	n/a	1.2±0.2*	n/a	1.2±0.1	p=.929	p=.024	p=.840
	HWI30	1.1±0.1	n/a	1.2±0.1*	n/a	1.2±0.1	η^2p=.001	η^2p=.056	η^2p=.011
	HWI60	1.1+0.1	n/a	1.2±0.2*	n/a	1.2±0.1			
Peripheral TT (s)	**TWI30**	55±8	n/a	55±9	n/a	56±8	p=.362	p=.385	p=.504
	HWI30	56±8	n/a	57±8	n/a	57±8	η^2p=.127	η^2p=.020	η^2p=.034
	HWI60	55±8	n/a	53±8	n/a	56±10			

TWI30, 30 minutes of thermoneutral water bathing; HWI30, 30 minutes of hot water immersion; HWI60, 60 minutes of hot water immersion; DBP, Diastolic Blood Pressure; SBP, Systolic Blood Pressure; MAP, Mean Arterial Pressure; PWV, Pulse Wave Velocity; PPI, Pulse Pressure Index; TT, Time travelled; HR, Heart Rate; SR, Shear Rate; η^2p, patrial eta squared. *(p<0.05), significantly different from pre-bathing value within the same bathing condition; †(p<0.05), significantly different from TWI30. All data are expressed as mean ± SD.

4.3.4 Blood pressure

4.3.4.1 Systolic blood pressure

There was a time-by-condition interaction effect for SBP (Figure 4.5). During bathing (up to 30 minutes), SBP decreased to a similar extent in HWI30 and HWI60 (HWI30 vs. HWI60, p=1.000; pre vs. bathing, p<0.001). For TWI30, DBP remained similar to pre-bathing values (pre vs. 30-min bathing, p=0.140). After HWI60, SBP remained lower immediately-post bathing before returning to pre-bathing values by post-1 (pre vs. immediately post-bathing, p<0.001; pre vs. post-1, p=1.000); however, for HWI30, SBP returned to pre-bathing values by immediately post-bathing (HWI30 vs. HWI60, p=1.000; pre vs. immediately post-bathing, p=1.000). After TWI30, SBP was higher immediately-post bathing (pre vs. immediately post-bathing, p=0.024), returning to pre-bathing values at post-1 (pre vs. post-1, p=1.000); however, SBP was lower in HWI30 and HWI60 than TWI30 (HWI vs. TWI30, both p<0.001).

4.3.4.2 Diastolic blood pressure

There was a time-by-condition interaction effect for DBP (Figure 4.5). During bathing (up to 30 minutes), DBP decreased to a similar extent in HWI30 and HWI60 (HWI30 vs. HWI60, p=1.000; pre vs. bathing, p<0.001). For TWI30, DBP decreased to a lesser extent during bathing (up to 30 minutes) than HWI30 and HWI60 (HWI30 and HWI60 vs. TWI30, both p<0.001; pre vs. 30-min bathing, p=0.001). After HWI30 and HWI60, DBP remained lowered immediately post-bathing, compared to pre-bathing values (HWI30 vs. HWI60, p=1.000; pre vs. immediately post-bathing,

p<0.001) and returned to pre-bathing resting values at post-1 (pre vs. post-1, both *p*=1.000). After TWI30, DBP returned to pre-bathing values immediately post-bathing (pre vs. immediately post-bathing, *p*=1.000). Across all bathing conditions, there was no change in DBP between pre and 24-h time points (pre vs. 24-h (all *p*=1.000)).

4.3.4.3 Mean arterial pressure

There was a time-by-condition interaction effect for MAP (Figure 4.5). During bathing (up to 30 minutes), MAP decreased to a similar extent in HWI30 and HWI60 (HWI30 vs. HWI60, *p*=1.000; pre vs. bathing, *p*<0.001). For TWI30, MAP decreased to a lesser extent during bathing (up to 30 minutes) than HWI30 and HWI60 (HWI30 and HWI60 vs. TWI30, both *p*<0.001; pre vs. 30-min bathing, *p*=0.001). After HWI30 and HWI60, MAP remained lowered immediately post-bathing (HWI30 vs. HWI60, *p*=1.000; pre vs. immediately post-bathing, *p*<0.001) and returned to pre-bathing resting values at post-1 (both *p*=1.000). After TWI30, MAP returned to pre-bathing values immediately post-bathing (pre vs. immediately post-bathing, *p*=1.000). Across all bathing conditions, there was no change in MAP between pre and 24-h time points (pre vs. 24-h (all *p*=1.000).

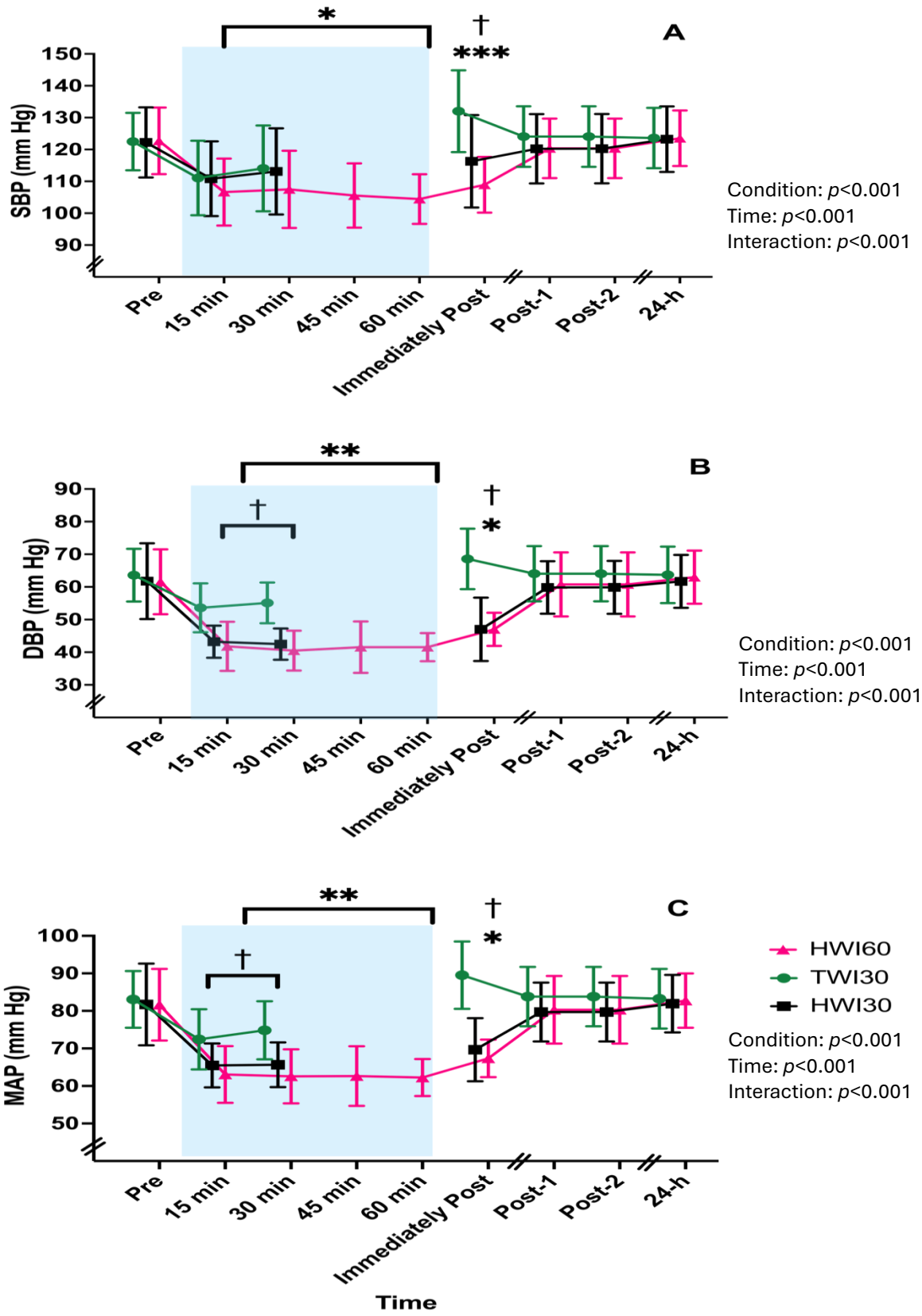

Figure 4.5. Blood pressure responses during, post and 24-h after bathing. A: Systolic Blood Pressure (SBP); B: Diastolic Blood Pressure (DBP); C: Mean arterial pressure (MAP). TWI30, thermoneutral water bathing for 30 minutes; HWI30, hot water bathing for 30 minutes; HWI60, hot water bathing for 60 minutes. *($p< 0.05$), significantly different from pre-bathing value (Pre) in HWI30 and HWI60; **($p< 0.05$) significantly different from pre-bathing value in HWI30, HWI60 and TWI30; ***($p< 0.05$), significantly different from pre-bathing value in HWI60 and TWI30; †($p< 0.05$), HWI60 and HWI30 significantly different from TWI30.

4.4.4 Mood measurements

4.4.4.1 Happiness

There was a condition-by-time interaction effect for happiness (Table 4.3). During bathing (up to 30 minutes), happiness ratings in HWI30, HWI60 and TWI30 were similar to pre-bathing values (pre vs. 30-min bathing: (all p=1.000). However, during bathing (at 30 minutes), happiness ratings were higher in TWI30 than in HWI60 (TWI30 vs. HWI60: “moderately” to “a little”, p=0.007). After HWI30, HWI60 and TWI30, post-bathing happiness ratings were similar to pre-bathing values (pre vs. immediately post-bathing: all p=1.000). Across all bathing conditions, there was no change in happiness ratings between pre and 24-h time points (pre vs. 24-h, all p=1.000).

4.4.4.2 Cheerfulness

There was a condition-by-time interaction for cheerfulness (see Table 4.3); however, the post hoc analysis did not demonstrate any significant differences (all p>0.05).

4.4.4.3 Calmness

There was a condition-by-time interaction effect for calmness (Table 4.3). During bathing (up to 30 minutes), calmness ratings were lower in HWI60 (pre vs. 30-min bathing: (p<0.001)) whilst for HWI30 and TWI30, calmness ratings remained unchanged (pre vs. 30-min bathing: (all p=1.000)).

However, during bathing (at 30 minutes), HWI60 calmness ratings were lower than TWI30 (HWI60 vs. TWI30: “moderately” vs. “a little”, (p<0.001)). There was no difference during bathing (30 minutes) between HWI30 and HWI60 (HWI30 vs. HWI60 (p=1.000)) or HWI30 and TWI30 (HWI30 vs. TWI30 (p=1.000)). After HWI30, HWI60 and TWI30, post-bathing calmness ratings were similar to pre-bathing values (pre vs. immediately post-bathing: (all p=1.000)). Across all bathing conditions, there was no change in calmness ratings between pre and 24-h time points (pre vs. 24-h: (all p=1.000)).

4.4.4.4 Anxious

There was a condition-by-time interaction for anxiety (see Table 4.3); however, the post hoc analysis did not demonstrate any significant differences (all p>0.05).

4.4.4.5 Stress

There was a condition-by-time interaction effect for calmness (Table 4.3). During bathing (up to 30 minutes), stressfulness ratings across bathing conditions were similar to pre-bathing values (pre vs. 30-min bathing: (all p=1.000). However, during bathing (at 30 minutes), stressfulness ratings were higher in HWI60 than in TWI30 (HWI60 vs. TWI30: “moderately” vs. “a little”, p=0.033). After HWI30, HWI60 and TWI30, post-bathing stressfulness ratings were similar to pre-bathing values (pre vs. immediately post-bathing, all p=1.000). Across bathing conditions, there

was no change in frustration ratings between pre and 24-h time points (pre vs. 24-h, all p=1.000).

4.4.4.6 Frustration

There was a condition-by-time interaction effect for calmness (Table 4.3). During bathing (up to 30 minutes), frustration ratings were higher in HWI60 (pre vs. 30-min bathing, p<0.001), while for HWI30 and TWI30, frustration ratings remained similar to pre-bathing values (pre vs. 30-min bathing, both p=1.000). After HWI30, HWI60 and TWI30, post-bathing frustration ratings were similar to pre-bathing values (pre vs. immediately post-bathing, all p=1.000). Across bathing conditions, there was no change in frustration ratings between pre and 24-h time points (pre vs. 24-h, all p=1.000).

4.4.4.7 Sadness

There was a condition-by-time interaction for sadness (see Table 4.3); however, the post hoc analysis did not demonstrate any significant differences (all p>0.05).

4.4.4.7 Sleep quality

There was a main effect of condition for sleep quality (Table 4.3). Sleep quality was higher for HWI60 than TWI30 (HWI60 vs. TWI30, p=0.039). For HWI30, sleep quality was similar to HWI60 and TWI30 (HWI30 vs. HWI60; p=1.000; HWI30 vs. TWI30, p=0.101).

Table 4.3. Sleep quality and mood variables during, post-bathing and 24-h post-bathing

	Condition							*p* value and η^2p		
		Pre	30min	Immediately Post	Post-1	Evening	24-h	Condition	Time	Condition -by-Time
Groningen Sleep Scale	**TWI30**	3.9±3.3	n/a	n/a	n/a	n/a	4.7±3.6	*p*=.028	*p*=.401	*p*=.848
	HWI30	4.1±3.3	n/a	n/a	n/a	n/a	3.2±2.9	η^2p=.086	η^2p=.009	η^2p=.004
	HWI60	2.3±2.5†	n/a	n/a	n/a	n/a	3.0±2.4†			
Happiness	TWI30	3.2±0.8	3.4±0.8	3.4+0.9	3.2±0.8	3.1±0.7	3.0±0.9	*p*<.001	*p*=.075	*p*=.021
	HWI30	3.5±0.8	3.1±0.9	3.1±0.9	3.1±0.8	3.3±0.8	3.4±0.9	η^2p=.476	η^2p=.034	η^2p=.082
	HWI60	3.2±0.5	2.6±0.7†	3.1±0.9	3.1±0.9	3.2±0.5	3.1±0.8			
Cheerfulness	**TWI30**	3.2±0.9	3.3±1.0	3.2±1.0	3.1±0.8	2.7±0.8	3.1±1.0	*p*=.209	*p*=.141	p<.001
	HWI30	3.4±0.7	3.0±0.9	2.7±1.0	3.0±0.7	3.1±0.8	3.2±0.8	η^2p=.178	η^2p=.037	η^2p=.104
	HWI60	2.9±0.6	2.5±0.9	3.0±0.9	3.0±1.0	3.1±0.7	3.0±0.9			
Calmness	TWI30	3.7±1.0	3.8±0.9	3.3±1.1	3.4±0.9	3.4±0.7	3.8±1.0	*p*=.009	p<.001	*p*<.001
	HWI30	3.6±1.1	2.9±1.1	3.0±1.2	3.5±1.2	3.4±1.1	3.5±1.2	η^2p=.206	η^2p=.127	η^2p=.119
	HWI60	3.9±0.7	2.5±1.0*†	3.5±0.8	3.6±1.1	3.6±0.9	3.8±0.9			
Energetic	**TWI30**	2.5±1.2	2.7±1.0	2.7±1.0	2.2±0.9	2.1±0.9	2.4±1.2	*p*=.596	p=.155	*p*=.065
	HWI30	2.8±1.0	2.2±0.9	2.2±1.0	2.4±1.1	2.3±1.0	2.3±0.8	η^2p=.064	η^2p=.036	η^2p=.060
	HWI60	2.3±0.8	2.1±1.0	2.4±0.9	2.2±1.0	2.0±0.8	2.5±1.1			
Stressed	TWI30	1.5±0.8	1.2±0.7	1.6±1.1	1.5±0.7	1.5±0.5	1.5±0.6	*p*=.251	*p*=.201	*p*=.004
	HWI30	1.5±0.9	1.6±0.7	1.4±0.6	1.3±0.5	1.3±0.5	1.2±0.4	η^2p=.158	η^2p=.034	η^2p=.084
	HWI60	1.6±0.8	2.1±1.2†	1.4±0.6	1.3±0.6	1.5±0.9	1.3±0.6			
Frustrated	**TWI30**	1.0±0	1.0±0	1.0±0	1.1±0.5	1.0±0	1.0±0	*p*=.381	*p*=.094	*p*=.010
	HWI30	1.0±0	1.0±0	1.0±0	1.0±0	1.2+0.4	1.0±0	η^2p=.115	η^2p=.041	η^2p=.078
	HWI60	1.0±0	1.3±0.8*	1.1±0.2	1.1±0.2	1.1±0.2	1.0±0			
Anxious	TWI30	1.2±0.6	1.1±0.2	1.1±0.3	1.2±0.4	1.4±0.5	1.2±0.4	*p*=.341	p=.159	*p*=.024
	HWI30	1.2±0.4	1.2±0.4	1.3±0.6	1.1±0.3	1.2±0.4	1.1±0.2	η^2p=.127	η^2p=.036	η^2p=.070
	HWI60	1.4±0.5	1.6±1.1	1.1±0.3	1.1±0.3	1.3±0.6	1.2±0.4			
Sadness	**TWI30**	1.3±0.5	1.1±0.3	1.1+0.3	1.1±0.3	1.2±0.6	1.1±0.2	*p*=.398	*p*=.135	*p*=.243
	HWI30	1.1±0.3	1.1±0	1.0±0	1.0±0	1.1±0.2	1.1±0.2	η^2p=.104	η^2p=.038	η^2p=.045

HWI60	1.0±0	1.1±0.5	1.1±0.2	1.0±0	1.1±0.2	1.1±0.2

TWI30, 30 minutes of thermoneutral water bathing; HWI30, 30 minutes of hot water immersion; HWI60, 60 minutes of hot water immersion; η^2p, partial eta squared. *($p<0.05$), significantly different from pre-bathing value within the same bathing condition; †($p<0.05$), significantly different from TWI30; ††($p<0.05$), significantly different from HWI30. All data are expressed as mean ± SD.

4.4 DISCUSSION

4.4.1 Main Findings

The primary aim of this study was to investigate if HWI30 elicits the same acute CV response as HWI60. The study results revealed that HWI30 and HWI60 had a similar hypotensive effect on MAP and DBP during and immediately post-bathing. However, neither HWI condition caused a change in FMD post-bathing, either from baseline (pre) or compared to TWI30. The secondary aim of the study was to examine the effects of HWI on mood state and sleep quality. During bathing, there was an increase in negative mood state for HWI60 but not in HWI30 (or TWI30), as reflected by the decrease in happiness and calmness ratings.

4.4.2 Thermal response to bathing

HWI60 was a more potent thermal stimulus than HWI30. This was evident by the near 0.5 °C higher peak T_{rec} reached (reached at 54± 6 min), and the extended duration T_{rec} was elevated for in HWI60, as compared to HWI30. This larger heat stress stimulus with a longer bathing protocol is unsurprising, however, this is the first study to formally compare 30 minutes of neck-level HWI to 60 minutes of shoulder/waist-level HWI and thus identify the magnitude of the difference. The T_{rec} response reported in the current study is similar to other 60-minute HWI protocols, in young (Campbell et al., 2022) and elderly participants (Behzadi et al., 2022); T_{rec} rise ~1.4-1.7°C)) while the HWI30 T_{rec} response appears consistent with other studies using a similar HWI stimulus for the first 30 minutes (Campbell et al., 2022; Behzadi

et al., 2022; James et al., 2021). Given that water immersion effectively clamps skin temperature and removes the efficacy of physiological heat loss responses for the submerged body's surface area (Cramer et al., 2022, Coombs et al., 2020), similar T_c responses might be expected across studies using similar HWI protocols and between populations.

4.4.3 Cardiovascular response to bathing

4.4.3.1 Heart Rate

HR increases during passive heating via mechanisms including increased conductivity of cardiomyocytes due to heightened sympathetic activity (Crandall and Wilson, 2015), increased metabolic activity of the cardiomyocytes due to the Q_{10} coefficient (Commission, 2001, Jose et al., 1970) and, most notably, a compensatory reflex to maintain cardiac output in the face of thermoregulatory cutaneous blood flow demands (Rowell, 1974). Notably, HR increases incrementally with passive heat-related elevations in T_c, even when an individual remains euhydrated (Lucas et al. 2018). However, despite differences in peak T_{rec} observed in the current study, peak HR remained similar between HWI60 and HWI30 during and immediately post-bathing. Like other studies using this protocol, the current study required participants to have their upper body and arms exposed to ambient conditions from the 31st to 60th minute of HWI60. This exposure of wet skin to ambient air may have resulted in some skin cooling and, as a result, reduced cutaneous blood flow (Wilson et al., 2007), which in turn would have reduced cardiac output (and subsequently HR) demands (Raven et al., 1981, Lucas et al., 2010). Interestingly, ΔHR for the current study (40±12 b·min^{-1} at 60

minutes of bathing for HWI60) was similar to that reported by others using a similar 60-minute HWI protocol (~40 b·min^{-1}); (Campbell et al., 2022)).

4.4.3.2 Blood pressure

Despite the increased CV strain for HWI60 than HWI30, both conditions had a similar hypotensive effect (DBP and MAP) during and immediately post-bathing. Crandall et al. (2008) demonstrated that increased T_c (>1.0°C) alongside high skin temperatures (~38°C) reduces CVP from 5.5 ±0.7 to 0.2 ±0.6 mm Hg in young healthy adults. In the current study, HWI increased T_{rec} by ~1-1.5°C and presumably clamped skin temperatures at ~41°C; with no change in body temperature in TWI30. Therefore, the additional heat stimulus of the HWI30 and HWI60 protocols presumably lowered CVP to a greater extent than TWI30, resulting in a lower DBP and MAP immediately post-bathing.

DBP reductions with passive heat stress are limited after a 1°C increase in T_c, as CVP reaches 0 mm Hg (Crandall et al., 2008). This likely explains why the additional T_{rec} rise during HWI60 did not further reduce DBP as compared to HWI30. Furthermore, as DBP is the primary driver in the MAP equation and there was no difference in SBP values (for HWI30 or HWI60) during or post-bathing, it is unsurprising to see a similar reduction in MAP for HWI30 and HWI60. The HWI DBP change reported in the current study is similar to previous literature (pre-bathing vs. last minute of bathing, HWI30: -20±10 mm Hg vs. HWI60: -24±10 mm Hg vs. (Campbell et al., 2022): HWI60 ~-20 mm Hg). Therefore, the decrease in DBP while bathing was consistently similar for both the HWI30 and HWI60 protocols.

During bathing, the hydrostatic pressure of water lowered DBP and MAP in TWI30. Hydrostatic pressure reduces peripheral vasculature resistance by 31%, resulting in a reduction in DBP of 9 mm Hg (Weston et al., 1987). This reduction in peripheral vasculature resistance has been observed in other TWI studies (Boussuges, 2006a, Park et al., 1999). Alternatively, increased peripheral resistance (via skin cooling) can increase CVP and MAP under normothermic conditions (Keller et al., 2011, Cui et al., 2005). As there is a relationship between peripheral vasculature resistance and DBP (Sprangers et al., 1991), hydrostatic-related decreases in peripheral resistance in TWI30 likely lowered DBP and MAP during thermoneutral bathing. This is further supported by MAP and DBP returning to pre-bathing values immediately post-bathing, when the hydrostatic pressure was removed. The TWI30 DBP change reported in the current study is similar to previous literature (pre-bathing vs. 30 minutes of bathing, TWI30: (drop of 9±8 mm Hg) vs. (Campbell et al., 2022): TWI60 drop of 11 mm Hg)). Therefore, TWI, independent of heat stress, appears to have a hypotensive effect on DBP and MAP during bathing.

Morning BP is an independent CVD risk factor (Kario, 2010) with BP surges associated with increased CV events (Li et al., 2010, Booth et al., 2020). Therefore, interventions that can reduce morning BP are advantageous and should be sought after. In the current study there was no change in resting BP 24-h post-bathing for HWI30, HWI60 or TWI30. These results are similar to Campbell et al. (2022) wherein young, healthy participants showed no change in MAP, DBP or SBP 24 hours following a session of either sauna, HWI or exercise in the heat. Therefore, it may be that passive heating alone, albeit acutely, is insufficient to result in the hypervolemia response needed to reduce morning resting BP.

4.4.4 Flow-mediated dilation

For HWI30, HWI60 and TWI30, FMD did not change post-bathing. An FMD change is associated with several factors, including SR (Carter et al., 2013b), nitric oxide (NO) (Green et al., 2014) and oxidative stress (Gurovich et al., 2014). Passive heating increases antegrade SR in the brachial artery (Larson et al., 2021) due to increased blood flow to the peripheral vasculature (Rowell, 1974), subsequently increasing NO availability (Hoekstra et al., 2018). This increase in NO availability is associated with increased FMD (Green et al., 2014). Indeed, our recent meta-analysis showed that a single bout of HT substantially increases total SR, producing a small but significant increase in FMD (Chapter 2). The current study, however, showed no increase in antegrade SR at post-1 for HWI30, HWI60 or TWI30, despite an increase in baseline and peak diameter for HWI30 and HWI60.

The timing of the FMD measurement after an intervention can be critical when assessing endothelial function (Dawson et al., 2013), due to factors such as NO availability (Cosio-Lima et al., 2006). Thus, differences in the time taken before post-bathing FMD measures in the current study may explain the observed differences in FMD responses compared to previous studies that measured the FMD response sooner (Cheng et al. 2021), given that SR and FMD changes post-HT are likely due to thermoregulatory-related increases in conduit artery blood flow (Larson et al., 2021). Notably, included publications in our systematic review and meta-analysis (Chapter 3) assessed FMD and SR either from immediately to >1 hour following HT, with no publication reported an increase in FMD >1 hour following HT. Conversely, the current study examined FMD approximately 35-39- and 90-95-minutes post-bathing and found no change in FMD relative to pre in any of the three experimental conditions. Therefore, our study results indicate that acute passive heat stress-related increases in

brachial antegrade SR and FMD may be diminished by 35 minutes post-bathing in young adults.

In the current study, total and antegrade SR decreased across all bathing conditions at post-2, driven by the reduction in total velocity (Table 4.3). This indicates that bathing and its hydrostatic pressure effects, independent of heat stress, was the principal mechanisms driving this reduction in total velocity at ~90 minutes post bathing. All conditions included some sedentary time, which is relevant as sedentary behaviour is associated with a decrease in FMD and SR (Daniele et al., 2022). Previous studies with young, healthy individuals have seen a reduction in superficial femoral artery SR after one and two hours (Thosar et al., 2014)), and a reduction in popliteal FMD and SR after three hours of sitting (O'Brien et al., 2019)). For the brachial artery, a reduction in antegrade SR has been seen after three hours (Thosar et al., 2014) and a reduction in mean SR, and mean velocity after four hours of sitting (Restaino et al., 2015). During the experimental lab visit, the participants did not undertake any physical activity (apart from getting changed and going from the hydrotherapy room to the CV laboratory (~30 m away). Therefore, the >1 hour of sedentary time post-immersion may have reduced antegrade SR and total velocity at post-2.

4.4.5 Mood responses to heat thermotherapy

Mood state post-bathing was unaffected by any of the bathing conditions. However, the HWI60 condition saw reduced happiness and calmness ratings after 30 minutes of bathing, which did not match the HWI30 condition. This was surprising as up to this 30-minute mark, both HWI30 and HWI60 protocols were identical, and both conditions showed similar

thermal and CV responses. One possible reason for the change in mood state for HWI60 alone was that participants were aware that another 30 minutes of bathing remained in this condition. Thus, the current study indicates that a more extended 60-minute bathing protocol may increase negative mood state during bathing. However, the increase in negative mood state does not persist after bathing. HWI60 appeared to improve sleep quality in comparison to the TWI30 group. This change, however, is likely due to the difference between scores at baseline, where TWI30 was higher than HWI60 (2.3±2.5 vs. 3.9±3.3), rather than a reflection of the difference in bathing protocols.

4.4.6 Experimental considerations and perspectives

This was the first study to compare the CV and mood responses of HWI30 to HWI60, and TWI30. By measuring BP whilst the participants are immersed, this study has investigated the effect of hydrostatic pressure alongside heat stress during bathing. Therefore, this study can confirm that whilst TWI30 does reduce DBP and MAP, this is further lowered by HWI. Meanwhile, the measurement of thermal sensation/comfort as well as mood during and post-bathing has provided further insight into the perception of bathing and its enjoyability. The second key strength of this study was the inclusion of two FMD measurements post-bathing. We are unaware of any previous study that has attempted to separate the effects of residual elevations in body temperature following HT on FMD responses. Therefore, for post-1, the aim was to complete the FMD scan as soon as possible so that the body temperature was still elevated. Thus, FMD was conducted as quickly as possible after participants had left the hydrotherapy room. For post-2, it was important to assess FMD once T_c was back to baseline levels to separate the effects of residual elevations in body temperature. In

previous studies, T_c had returned to baseline values one hour after HWI60 (Brunt et al., 2016b). Therefore, to ensure that body temperature had returned to baseline levels and that there was sufficient time to prevent a “knock-on effect” from the previous FMD scan (Inaba et al., 2014), post-2 was completed at ~90 minutes post-bathing. The study has shown no change in FMD at post-2. Therefore, it appears in young adults that HWI is insufficient to increase FMD 90 minutes post-bathing.

Despite this study's promising results, several factors must be considered. First, the Tango (M2; SunTechMedical Instruments Inc., United States) could not be used in the hydrotherapy room due to electrical power restrictions. As automated BP devices use a predicted MAP to calculate SBP and DBP (Alpert et al., 2014), BP values can vary between devices. Therefore, a single BP device (which could be powered by a laptop) was used for this study. Whilst Tango is considered the "gold standard" for automated BP measurement (Cameron et al., 2004), the Vicorder has been validated as a reliable device for assessing BP (Pucci et al., 2013, Hickson et al., 2009). Second, there may be variability in the FMD results due to the skill of the ultrasound operator (B.P.). However, by the data collection phase, the operator had conducted two repeatability studies, plus additional training. Therefore, confidence can be found in the operator's ability to undertake brachial artery FMD scans. Third, this study did not measure peripheral vasculature resistance directly. Therefore, we cannot be sure that reduced peripheral vasculature resistance did reduce DBP during TWI30. Finally, this study did not collect venous blood samples from participants. In the future, venous blood samples will allow for the analysis of cardiometabolic factors and investigate whether there was a CV function or cardiometabolic health response following HT.

Whilst it is hoped that repeated HWI bouts will lead to long-term CV adaptations, this study has only recorded the acute CV response to HWI. It is recommended that future research examines whether HWI30 leads to functional and structural improvements in CV health. Future studies should also investigate if the acute HWI30 effect is consistent across different population groups with CV disease risk factors such as obesity or hypertension to ensure its effectiveness.

4.4.7 Conclusion

The current study shows that although HWI60 created a greater hyperthermic impulse than HWI30, the CV strain appeared to be similar between conditions, albeit maintained for longer in HWI60. Furthermore, HWI30 and HWI60 acutely reduced MAP and DBP to a similar extent during and immediately after bathing, despite HWI60 causing a higher T_{rec}. However, neither HWI condition acutely changed endothelial function (as measured via FMD) ~35, ~90 minutes or 24-h post bathing. Therefore, HWI30 appears to be as effective as HWI60 in inducing a positive, acute BP post-bathing response, with the added benefit of being more time-efficient and a more positive experience for participants.

5. THE EFFECT OF HIGH-INTENSITY EXERCISE AND HOT WATER IMMERSION ON CARDIOVASCULAR AND PSYCHOLOGICAL FACTORS IN YOUNG, HEALTHY ADULTS

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S.B., J.C. & A.P. contributed to this chapter's conception

B.P. led this chapter's data extraction process and data analysis.

G.P. & S.B contributed to this chapter's data collection

5.1 INTRODUCTION

Exercise is an effective method to improve CV function and attenuate the risk of CVD; (Fiuza-Luces et al., 2018)). HIIEx is an effective exercise approach to enhance CV function by reducing resting BP and improving endothelial function, as well as cardiorespiratory fitness (Ramos et al., 2015). HIIEx can be completed in low-volume sessions lasting as little as 15 minutes (Taylor et al., 2019), and thus, is considered a more time-efficient and enjoyable exercise protocol than longer-duration, moderate-intensity exercise (Thum et al., 2017). Due to increased metabolic activity from active skeletal muscle during exercise, T_c rises are a key stressor (Lafrenz et al., 2008, Wingo and Cureton, 2006) and form part of exercise-induced CV adaptations (Romero et al., 2017b). By comparison, HT mimics some mechanisms by which exercise improves CV function, i.e., elevations in skin and T_c. For example, research has shown HT-induced increases in cardiac output and reductions in BP post-HT (Crandall and Wilson, 2015, Campbell et al., 2022, Roxburgh et al., 2023)). HT has also been reported to be more popular than traditional exercise programmes (Akerman et al., 2019, Naumann et al., 2018), and may appeal to those who have difficulty engaging with physical activity interventions.

Hot water immersion (HWI) is a type of HT and has been demonstrated to improve CV function by reducing resting BP and arterial stiffness, as well as improving endothelial function (Brunt et al., 2016a). Some studies have also shown that chronic use of HWI (4-5 sessions a week for 8-10 weeks) causes structural changes to conduit arteries, as reflected by an increase in brachial artery diameter (Brunt et al., 2016a, Ely et al., 2019b).

Furthermore, improvements in CV function following HWI have previously been demonstrated in young adults (Brunt et al., 2016a), older adults (Akerman et al., 2019), and those with CVD risk factors (Ely et al., 2019b). Thus, HWI is a promising strategy for improving CV function.

While HWI mimics some benefits of exercise, there are fundamental thermoregulatory, CV and metabolic differences between these modalities. For example, different pathways drive reductions in BP and increases in vascular compliance. With exercise, peripheral vasodilation is primarily driven by increased blood flow to active skeletal muscle beds via increased histamine production from the skeletal muscle (Lockwood et al., 2005). HIIEx also increases thermoregulatory demands (due to metabolic heat production) increasing cutaneous blood flow. By comparison, peripheral vasodilation during HWI is primarily mediated by a thermoregulatory-driven increase in blood flow to the cutaneous vascular beds, due to increased acetylcholine production (Kellogg Jr et al., 1995). Therefore, combining both HIIEx and HWI stressors could be complementary and result in a more potent CV response via flow-mediated adaptations. For example, single HIIEx and HWI bouts have independently been shown to acutely increase antegrade SR and lower blood pressure immediately post (within 60 min) interventions (Thomas et al., 2017, Liu et al., 2025). Furthermore, repeated bouts of HIIEx or HWI lead to long-term CV adaptations such as lower resting blood pressure, increased FMD and positive arterial structural changes (Ely et al., 2019b, Ramos et al., 2015). Repeated bouts of HWI or HIIEx can also result in cardiometabolic adaptations such as increased heat shock protein proliferation plus lowered fasting glucose and insulin levels (Ely et al., 2019a, Edwards et al., 2023). Thus, combining HIIEx and HWI may lead to a stronger CV and cardiometabolic response, resulting in a greater positive long-term CV and

cardiometabolic adaptations than HWI or HIIEx alone. However, whether it is feasible to combine both interventions in young, healthy adults is still to be determined.

Alongside CV function, HT may improve mood state and alleviate depressive symptoms, both of which are linked to a heightened risk of CVD (Krittanawong et al., 2023). HT may improve nighttime body temperature regulation, especially in populations which have increased risk of circadian abnormalities (e.g. major depressive disorder (Duncan, 1996), and lifestyle choices such as night shift working (Jin et al., 2017)), which may lower depressive symptoms and improve mood state (Avery et al., 1982, Naumann et al., 2018). In Chapter 4, it was demonstrated that there was no positive mood state increase after HWI in young adults. However, regular exercise is associated with improved mood state and reduced depressive symptoms in young men and women (Glavin et al., 2022). Therefore, combining HWI and HIIEx may lead to an improvement in positive mood state for young adults.

To date, few studies have investigated the combined effects of exercise and HT on CV function. One previous study has shown that an acute cycling session (15 minutes at 75% HR maximum) followed by a 15-minute sauna session lowered MAP and pulse pressure in young adults 30 minutes after the intervention (Lee et al., 2020). In a multiple-session study, middle-aged participants with at least one CVD risk factor completed either: i) 60 minutes of resistance and aerobic exercise (65-80% HR maximum), ii) 60 minutes of resistance and aerobic exercise (65-80% HR maximum) training followed by 15 minutes of sauna, or iii) a resting control (Lee et al., 2022). The findings of this study showed that the exercise and sauna group had a higher cardiorespiratory fitness and lower systolic BP (SBP; (~8 mm Hg)) than the exercise-only and the control groups following the intervention. These recent

findings of Lee and colleagues indicate that the combination of moderate-intensity exercise and HT may elicit an enhanced CV response and adaptation compared to HT or exercise alone (Lee et al., 2022). However, further work in this area is required as it is unclear how combining alternative exercise (such as HIIEx) and HT (such as HWI) modalities affects CV responses when measured against appropriate experimental controls or comparisons.

Therefore, this study aimed to examine the acute CV response to a combined HIIEx and HWI protocol compared to a combined HIIEx and TWI protocol. It was hypothesised that ExHWI (HWI + HIIEx) would result in lower BP, a greater FMD increase and plasma blood volume (PV) expansion than ExTWI (TWI + HIIEx).

5.2 METHODS

5.2.1.1 Study design

Six participants completed two conditions in a pseudo-randomised crossover study design, separated by a minimum of 48 hours. The two conditions were: i) HIIEx (high-intensity interval exercise) followed by 30 minutes of hot water immersion (41°C; ExHWI); and ii) HIIEx followed by 30 minutes of thermoneutral water immersion (35°C; ExTWI). Recruited participants were healthy individuals assigned to conditions in a randomised, counterbalanced order using a computer-generated randomiser.

5.2.1.2 Ethical approval and consent

The study was approved by the University of Birmingham Ethics Committee (ERN_19-1491_AP2), and all participants gave written, informed consent before participating in the research study, in accordance with the Declaration of Helsinki.

5.2.1.3 Participants

Participants (see Table 5.1 for participant characteristics) had not recently visited a hot climate and were not regular jacuzzi/sauna users (>1 session per week). Female participants were naturally menstruating (*n*=1) or using hormonal contraceptives (*n*=2). Naturally menstruating females took part during the late luteal phase of their menstrual cycle, while females using contraceptives were tested during the active-pill phase.

Table 5.1. Participant baseline characteristics

Variable	*N*=6
Sex (male/female)	3/3
Age (years)	25±7
Body Mass Index (kg/m^2)	25±2
VO_{2peak} (ml/kg/min)	38±9
W_{peak} (W)	288±50

VO_{2peak}, peak oxygen consumption; W_{peak}, peak power output.

5.2.1.4 Experimental visits

Familiarisation Session: Participants visited the lab and were briefed on the study design and requirements. During the familiarisation session, anxiety and depression were measured via the Perceived Stress Scale (Cohen et al., 1994), the Multidimensional Fatigue Scale (Smets et al., 1995) and the Hospital Anxiety and Depression Scale (Zigmond and Snaith, 1983). Participants' sleep quality for the night/month before this session was measured using the Pittsburgh Sleep Quality Index (Buysse et al., 1989) and the Holland Sleep Disorders Questionnaire (Kerkhof et al., 2013). Physical activity was measured via the International Physical Activity Questionnaire (Booth, 2000). Finally, a brachial artery ultrasound scan was conducted to improve the researcher's scanning consistency during the bathing conditions and to familiarise participants with the FMD procedure.

5.2.1.5 VO_{2peak} visit

The participant's peak oxygen consumption (VO_{2peak}) visit was completed within a week of the first experimental trial. VO_{2peak} was assessed via a ramp-incremental cycling protocol using a stationary cycle ergometer (Excalibur Sport, Lode, Netherlands). During the protocol, HR, rating of perceived exertion (RPE (Borg, 1982)), respiratory exchange ratio and oxygen

consumption (Vyntus CPX, Vyaire Medical, United Kingdom) were measured. After a 5-minute warmup at 35 watts (W), resistance was increased by 35W every two minutes until volitional fatigue was achieved. Volitional fatigue was defined as an respiratory exchange ratio >1.10 or the cycling cadence reducing below 60 revolutions per minute. VO_{2peak} was calculated as the highest 30-second average achieved during the protocol. Peak power output (W_{peak}) was calculated using the equation below (Andersen, 1995).

$$peak\ power\ output\ (W) = previous\ stage\ wattage + \left(35 \times \left(\frac{final\ stage\ exhaustion\ time}{120} \times 100\right)\right)$$

5.2.1.6 Experimental conditions

All experimental conditions entailed the same pre- and post-bathing measures (Figure 5.1). Participants arrived between 07:00 and 09:30 in a fasted state and rested supine for 10 minutes before a "pre" blood sample was taken. Following venipuncture, participants were given a self-selected breakfast (avoiding high-fat food products (Baynham et al., 2023)), which was recorded and repeated across both experimental conditions. Alongside food, participants were allowed to drink water *ad libitum.* Participants then completed a food diary (to replicate diet for subsequent visits), a Mood State Questionnaire, a Groningen Sleep Quality Questionnaire and Thermal Comfort Scales. Following this, participants were fitted with an HR monitor (H10, Polar, Kempele, Finland) and rested supine for 15 minutes before a brachial FMD test via ultrasound was conducted. Following this, participants were moved into a semi-recumbent position for 10 minutes before baseline HR and BP were recorded. Following baseline measures, participants emptied their bladder, took a nude body mass measure (Seca Alpha Model 770, Seca Scales), changed into exercising kit, and

fitted a rectal thermistor at a depth of ~10cm past the anal sphincter (Mon-a-Therm, Covidien, Mansfield, MA, United States) for the measurement of rectal temperature (T_{rec}). From this point, participants were fluid-restricted, with a maximum of 100 mL of water given to participants.

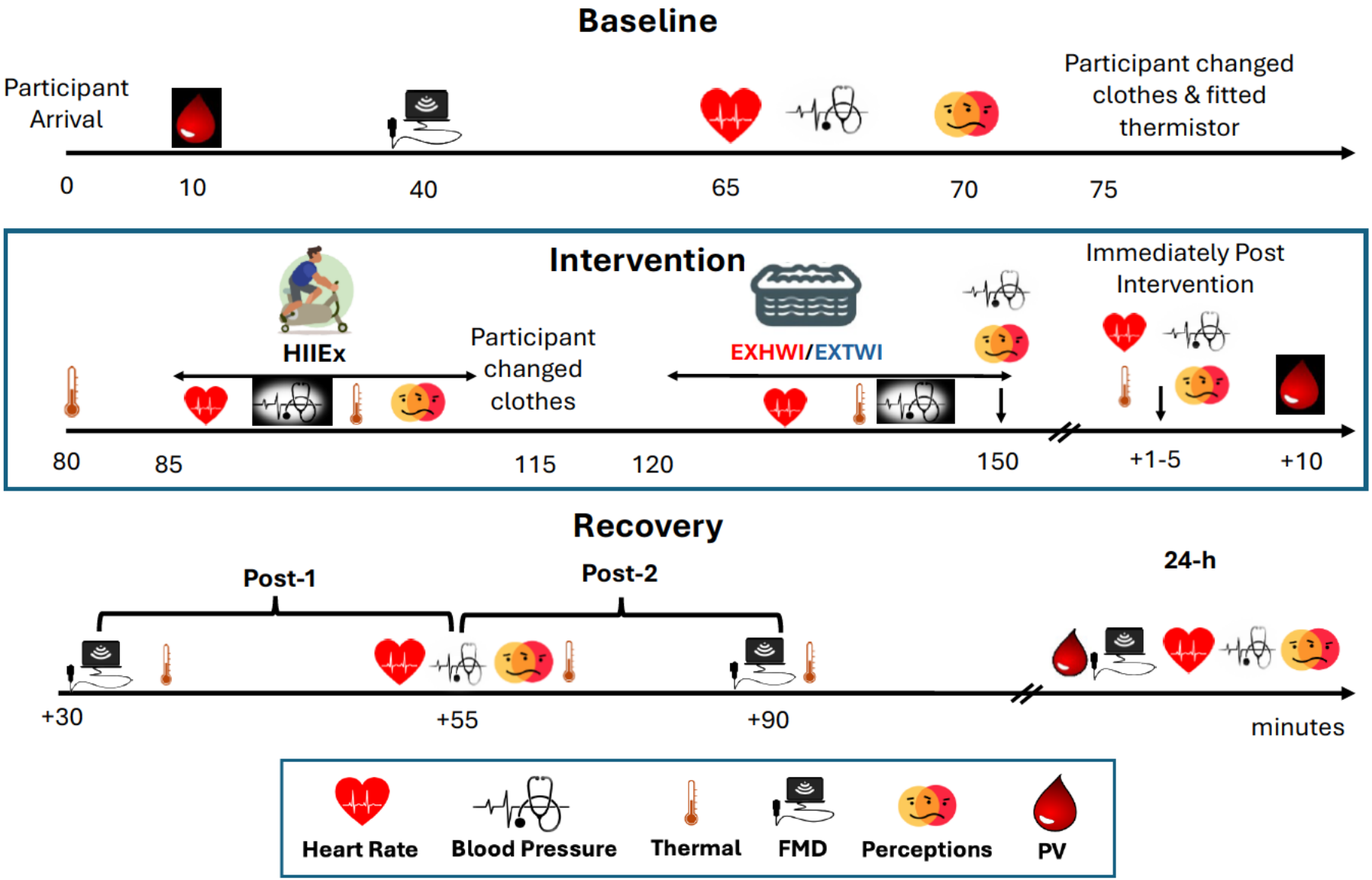

Figure 5.1 Schematic of the study protocol. ExHWI: 5x1 minute bouts of 100% W_{peak} followed by 30 minutes of bathing at 41°C; ExTWI: 5x1 minute bouts of 100% W_{peak} followed by 30 minutes of bathing at 35°C. FMD, flow-mediated dilation; W_{peak}, peak power output; PV, plasma blood volume.

5.2.1.7 High-intensity interval exercise

Both experimental visits followed the same cycling protocol, as shown in Figure 5.2. The protocol began with a 5-minute warm-up at 35% W_{peak}, followed by five sets of 1-minute intervals at 100% W_{peak}, interspersed with 4 minutes of recovery at 35% W_{peak} (totalling 29 minutes, including warm up). BP was recorded at the end of the first, third, and final interval bout, while HR and T_{rec} were continuously measured. RPE were recorded at the end of each 1-minute HIIEx bout. Verbal encouragement and music were provided to support the participant's performance. After completing the final recovery period, participants changed into swimwear and entered the hydrotherapy bath, approximately 10 yards away.

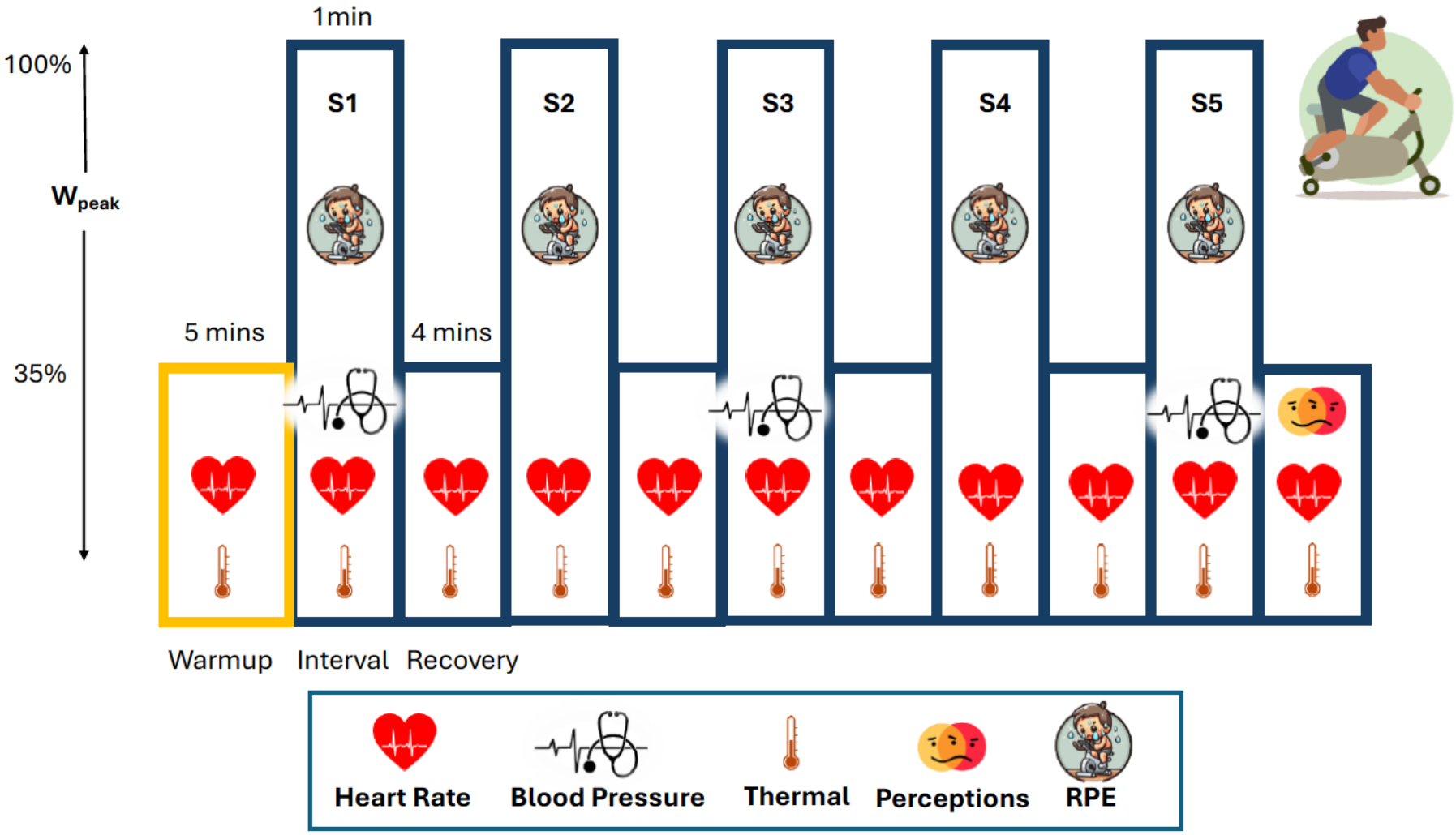

Figure 5.2. Schematic of the HIIEx session during both experimental conditions. The HIIEx session took place on a cycle ergometer with a 5-minute warmup at 35% W_{peak}, followed by 5 x 1-minute bouts at 100% W_{peak} interspersed with 4-minute recovery sessions at 35% W_{peak}. HIIEx, high-intensity interval exercise, W_{peak}, peak power output; S, HIIEx rep.

5.2.1.8 Bathing

Participants in the hydrotherapy room used a jacuzzi bath (Dura Spa S380, Rotospa, Birmingham, UK) for both the ExHWI and ExTWI conditions. HR and T_{rec} were continuously monitored during the bath. The water level was to the sternal notch with the participant's arms submerged. Questionnaires and BP measurements were taken after 15 minutes and during the last minute of bathing. During BP measurements, if needed, participants adjusted the water level to reach the base of the sternum (Xiphoid process) for ~2 minutes before returning to their original position.

5.2.1.9 Post-intervention

BP and HR were recorded immediately upon exiting the bath once in a seated position. After these initial post-immersion measurements, participants dried themselves and changed into comfortable clothing. At 2-5 minutes post-bathing (immediately post-bathing), between 30-55 minutes post-bathing (post-1) and 55-90 minutes post-bathing (post-2) pre-bathing measures were repeated in the same order as described pre-immersion, alongside an additional FMD measure at 90 minutes (post-2; see Figure 5.1). Before bed, participants were instructed to complete another Mood State Questionnaire.

5.2.1.10 24-h post

Participants returned to the laboratory between 07:00 and 09:30, 24 hours after baseline measures. Breakfast, vascular measurements, the Groningen Sleep Quality Questionnaire, and the Mood State Questionnaire were completed in the same order as the pre-bathing measurements.

5.2.2 Measures

5.2.2.1 Core body temperature

All T_{rec} measures were collected and stored every 60 seconds on a portable logger for later analysis (Squirrel 2020 series, Eltek, Ltd., United Kingdom).

5.2.2.2 Endothelial function

Endothelial function was assessed via FMD at the brachial artery using high-resolution duplex ultrasound (Terason uSmart 3000, Teratech, USA) as detailed previously (Section 3.3.1). All measurements were taken by the same experienced operator (B.P.). All FMD data analysis was performed by the same researcher (B.P.), using specialised custom-designed edge-detection and wall-tracking software (Cardiovascular Suite, Quipo, Italy). The allometric scaling technique (Atkinson et al., 2013) was applied to the FMD results. Subsequently, FMD results are presented as traditional and allometrically scaled.

5.2.2.3 Blood pressure

Arterial BP was measured from the right arm by automated devices (during bathing: Vicorder® (oscillometric), SMT Medical, Bristol, UK; all other time points: Tango (auscultatory), M2; SunTechMedical Instruments Inc., United States)). Measures were taken in duplicate to make an average. However, if the first two values were not within 5 mm Hg of each other, a third measurement was taken, and the median number was used. MAP was calculated as:

$$MAP\ (mm\ Hg) = DBP\ + \frac{1}{3}\ (SBP - DBP)$$

5.2.2.4 Plasma volume and sweat loss

Due to device availability, PV was analysed using two approaches. For approach one, blood was collected into a 3 mL vacutainer (BD vacutainer, (Medisave, Dorset, UK). Within 30-40 minutes, a whole blood count was obtained using the Yumizen H500 cell counter (Horiba Medical, Montpellier, France). For approach two, 3 mL of blood was collected using an aspirator arterial blood gas syringe (safePICO, Radiometer UK Limited, Crawley, UK). Within 5 minutes, the blood was measured by a blood gas analyser (ABL90 FLEX PLUS, Radiometer UK Limited, Crawley, UK). In total, 18/36 blood analyses were completed with approach one, which was inconsistent across participants. PV was computed using the calculations proposed by Dill and Costill (1974) as shown below:

$$DPV = PV_{post} - PV_{pre} \,/\, PV_{pre}$$

Sweat loss (Δ) was calculated as:

$$\Delta\ Sweat\ loss\ (kg) = Baseline - immediately\ post\ intervention$$

5.2.2.5 Psychological outcomes

During the bathing conditions, sleep quality was assessed using the validated Groningen Sleep Quality Scale (Meijman et al., 1990). Mood State was assessed using a modified questionnaire (Liao et al., 2017a) as detailed previously. For thermal perception, thermal comfort and thermal sensation were recorded (Gagge et al., 1967, Cotter and Taylor, 2005) as detailed previously.

5.2.3 Statistical analysis

The six participants in this Chapter were part of a pilot/feasibility study. Repeated-measures outcome data were compared using linear mixed-effects models (LMEM), with time and bathing conditions included as fixed factors and participants as random factors. All other data were analysed using a Two-way ANOVA. Follow-up tests, if appropriate, were performed using the Bonferroni post hoc procedure. The statistical package JAMOVI 2.3 (The JAMOVI Project 2023 - Computer Software, Sydney, Australia) was used for all analyses, and statistical significance was set at $P \leq 0.05$. The statistical package Graphpad, version 10.0 was used to generate the figures. Unless otherwise stated, all results are presented as mean ± standard deviation (mean ± SD).

5.3 RESULTS

5.3.1 Baseline (pre)

There were no differences for ExHWI or ExTWI for any haemodynamic or thermal variable at baseline (p<0.05). Please see Appendix (R – X) for the baseline sleep, anxiety, stress, physical activity scores and the 24-hour food recall results.

5.3.2 Thermal measures

5.3.2.1 Rectal core temperature

There was a significant time-by-condition effect for T_{rec} (Figure 5.3). After HIIEx, T_{rec} increased to a similar extent in ExHWI and ExTWI from baseline values (ExHWI vs. ExTWI, p=1.000; pre vs. end-HIIEx, p<0.001). During bathing (up to 30 minutes), T_{rec} remained elevated in ExHWI compared to baseline values but did not further increase from HIIEx (pre vs. 30-min bathing, p<0.001; end-HIIEx vs. 30-min bathing, p=0.977). For ExTWI, T_{rec} returned to near baseline values after 30 minutes of bathing (pre vs. 30-min bathing, p=0.105). After ExHWI, T_{rec} remained elevated and returned to near baseline values by 90 minutes post-intervention (pre vs. +5 minutes, p<0.001; pre vs. +30 minutes, p<0.001; pre vs. +55 minutes, p=0.037; pre vs. 90 minutes, p=1.000). After ExHWI, T_{rec} was higher than ExTWI until 30 minutes post-intervention (ExHWI vs. ExTWI: +5 minutes, p<0.001; ExHWI vs. ExTWI: +30 minutes, p=0.067). T_{peak} was higher in ExHWI than ExTWI (p<0.001).

5.3.2.2 Sweat loss

At baseline, there was no difference in nude body mass between conditions (ExHWI: (75.9±10 kg) vs. ExTWI (75.3±11.4); (p=1.000)). After bathing, sweat loss (Δ) was greater in ExHWI than in ExTWI, with participants losing 1.5±0.2 and 0.7±0.2 kg, respectively (p<0.001).

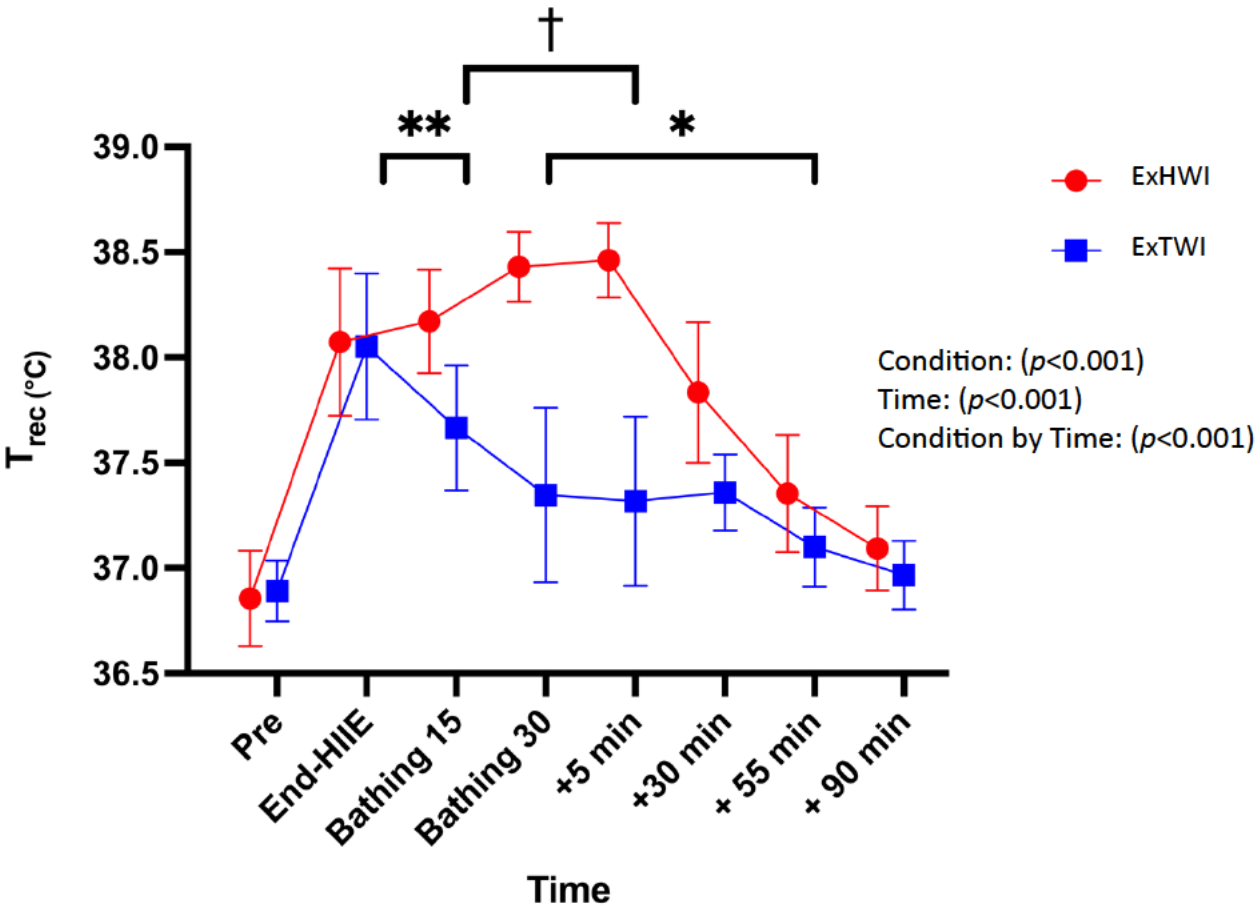

Figure 5.3. Rectal temperature (T_{rec}) responses at baseline (Pre), during HIIEx and bathing and post-intervention. ExTWI, a 5x1-min HIIEx cycle bout (100% W_{peak}) followed by 30 minutes of hot water bathing at 41°C; ExHWI, a 5x1-min HIIEx bout (100% W_{peak}) followed by 30 minutes of hot water bathing at 35°C; HIIEx, high-intensity interval exercise. *(p< 0.05), significantly different from pre-bathing value (Pre) in ExHWI alone; **(p< 0.05) significantly different from pre-bathing value in ExHWI and ExTWI; †(p< 0.05), ExHWI significantly different from EXTWI. Presented as means ± SD.

5.3.2.3 Ratings of perceived exertion

There was a significant main effect for time and condition for RPE (condition (p=0.008; η^2p = 0.147); (time: p<0.001; η^2p = 0.743); (condition-by-time: p=0.655; η^2p = 0.055)). During HIIEx, RPE for intervals 4 and 5 was higher than that reported for interval 1 in both ExHWI and ExTWI (interval 1 (13) vs. interval 4 (17): (p<0.001) and interval 1 vs. interval 5 (18): (p<0.001)).

5.3.2.4 Thermal sensation/thermal comfort

There was a time-by-condition interaction effect for thermal sensation (Table 5.3). Thermal sensation increased during HIIEx for both ExHWI and EXTWI (”neutral” to “warm”, both <0.001). During bathing, thermal sensation remained elevated for EXHWI and was higher than ExTWI immediately post bathing (“warm”, pre vs. 30-min bathing, p=0.002; immediately post: ExHWI vs. ExTWI, p=0.010). For ExTWI, thermal sensation returned to near baseline values by the end of bathing (at 30 minutes; p=0.108).

There was a main effect of condition for thermal comfort, such that ExHWI remained higher than ExTWI across timepoints (p=0.036). There was a main effect of time for thermal comfort (p=0.013) however, the post hoc analysis did not indicate any change post-HIIEx or during/post-bathing, compared to baseline values (all p>0.05).

5.3.2.5 Plasma volume

There was a main effect of time for PV (Figure 5.4). After the intervention, PV decreased to a similar extent in ExHWI and ExTWI from baseline values (ExHWI vs. ExTWI, p=1.000; pre vs.

end-intervention, $p<0.001$). 24-h post-baseline, PV was restored to a similar extent in ExHWI and ExTWI (ExHWI vs. ExTWI, $p=1.000$; pre vs. 24-h, $p<0.001$).

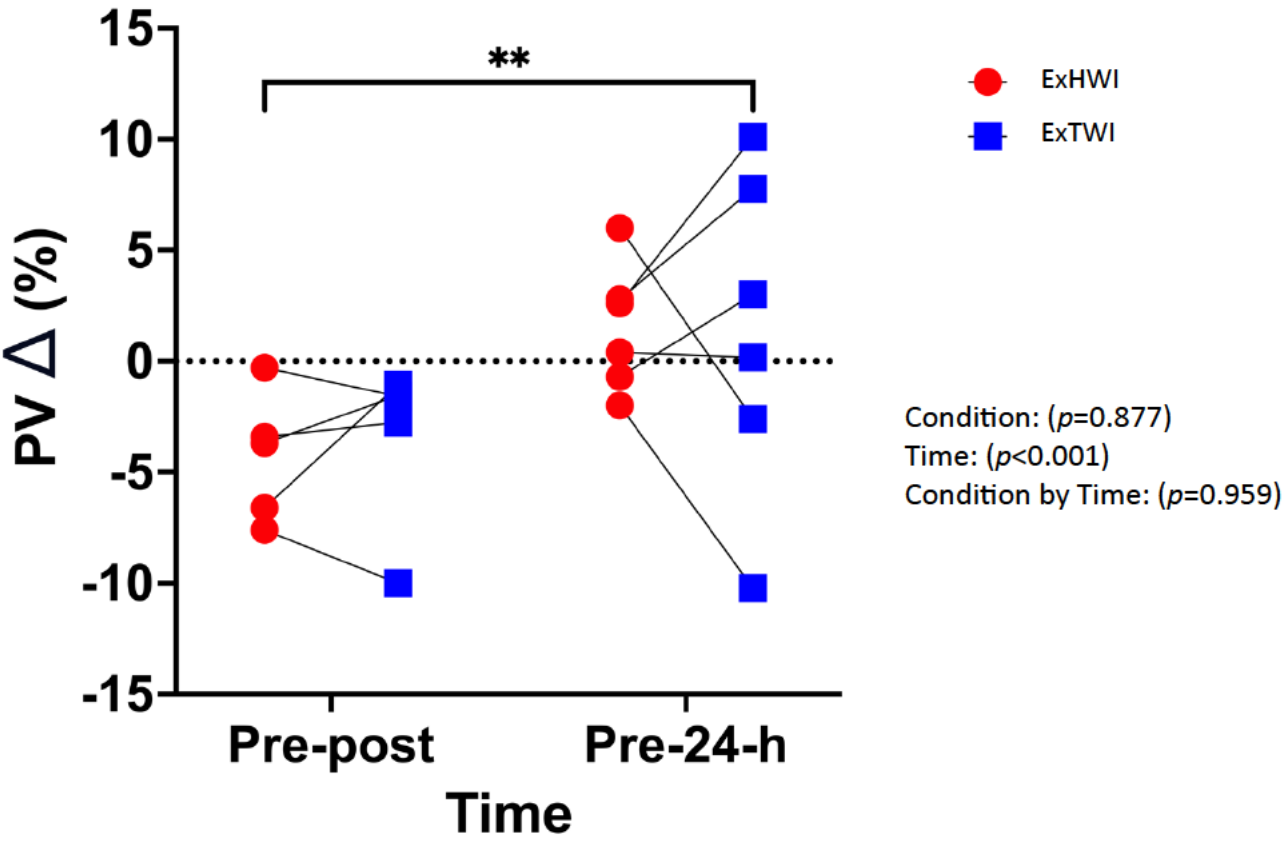

Figure 5.4. Plasma volume change (Δ) between baseline (pre) and post-intervention vs. baseline and 24-h post-baseline. PV, plasma volume. Each marker represents individual data for the six included participants and the lines represent the difference for each participant between conditions. **(p< 0.05) significant difference from pre-post to pre-24-h in ExHWI and ExTWI.

5.3.3 Cardiovascular variables

5.3.3.1 Systolic blood pressure

There was a condition-by-time interaction effect for SBP (Figure 5.5). Relative to baseline values, SBP increased to a similar extent during the HIIEx intervals in ExHWI and ExTWI (ExHWI vs. ExTWI: (p=1.000); pre vs. I1; pre vs. I3; pre vs. I5: (all p<0.001). SBP returned to baseline values in ExHWI and ExTWI during bathing (at 30 minutes of bathing, both

p=1.000). There was no difference in SBP between baseline, post-1 and 24-h time points for ExHWI or ExTWI (pre vs. post 1, both *p*=1.000; pre vs. 24-h, both (*p*=1.000).

5.3.3.2 Diastolic blood pressure

There was a condition-by-time interaction effect for DBP (Figure 5.5). Relative to baseline values, DBP did not change during HIIEx for ExHWI or ExTWI (both *p*=1.000). During bathing (at 30 minutes), DBP decreased in ExHWI and ExTWI from baseline values (EXHWI vs. EXTWI, *p*=1.000; pre vs. 30-min bathing, *p*<0.001). Immediately post-bathing, DBP remained lower than baseline values for ExHWI whilst immediately post-bathing DBP returned to near baseline values for ExTWI (ExHWI vs. ExTWI, p<0.001; ExHWI: pre vs. immediately post-bathing, *p*<0.001; ExTWI" pre vs. immediately post-bathing, *p*=1.000). There was no difference in DBP between baseline, post 1 and 24-h time points for ExHWI and ExTWI (pre vs. post-1, both *p*=1.000; pre vs. 24-h, both *p*=1.000).

5.3.3.3 Mean arterial pressure

There was a condition-by-time interaction effect for MAP (Figure 5.5). Relative to baseline values, MAP increased during HIIEx for ExHWI and ExTWI (ExHWI vs. ExTWI, p=1.000; pre vs. I1; pre vs. I3; pre vs. I5, all *p*<0.001). During bathing (at 30 minutes), MAP decreased in ExHWI and ExTWI from baseline values (EXHWI vs. EXTWI, *p*=1.000; pre vs. 30-min bathing, *p*<0.001). Immediately post-bathing, MAP remained lower than baseline values for ExHWI whilst immediately post-bathing MAP returned to near baseline values for ExTWI (ExHWI vs. ExTWI, p<0.001; ExHWI: pre vs. immediately post-bathing, *p*<0.001; ExTWI: pre vs. immediately post-bathing, *p*=1.000). There was no difference in MAP between baseline, post

1 and 24-h time points for ExHWI and ExTWI (pre vs. post-1, both p=1.000; pre vs. 24-h, both p=1.000).

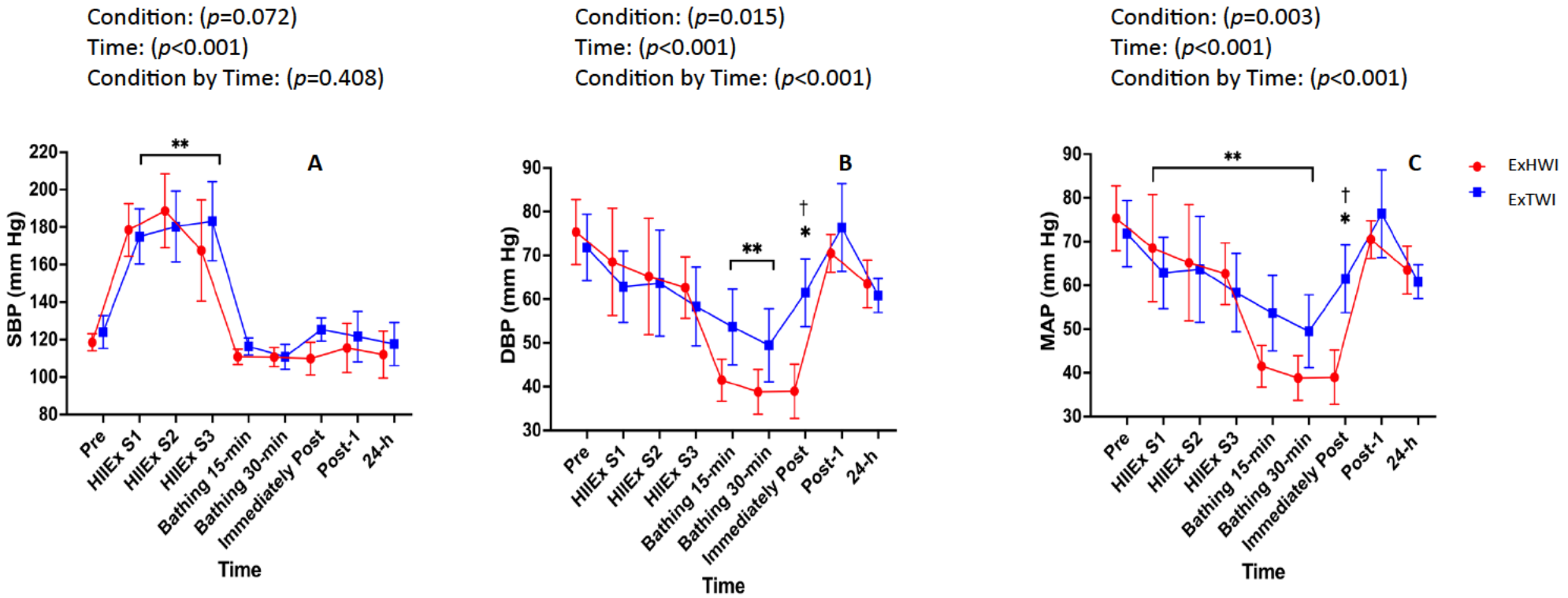

Figure 5.5. Blood pressure responses at baseline (Pre), during HIIEx and bathing, and post-intervention. ExTWI, a 5x1-min HIIEx cycle bout (100% W_{peak}) followed by 30 minutes of hot water bathing at 41°C; ExHWI, a 5x1-min HIIEx bout (100% W_{peak}) followed by 30 minutes of hot water bathing at 35°C. A) SBP, systolic blood pressure; B) DBP, diastolic blood pressure; C) MAP, mean arterial pressure. HIIEx, high-intensity interval exercise; W_{peak}, peak power output. *($p< 0.05$), significantly different from baseline in ExHWI alone; **($p< 0.05$) significantly different from baseline in ExHWI and ExTWI; †($p< 0.05$), ExHWI significantly different from ExTWI. Data presented as means ± SD.

5.3.3.4 Heart rate

There was a condition-by-time interaction effect for HR (Figure 5.6). During HIIEx, HR increased in ExHWI and ExTWI, reaching a peak HR during the final HIIEx interval (peak HR EXHWI vs. EXTWI, p=1.000; pre vs. interval 5, Table 5.2 and Figure 5.6). During bathing (at 30 minutes), HR remained elevated in ExHWI and to a lesser extent in ExTWI (ExHWI vs. ExTWI, p<0.001; pre vs. 30-min bathing, both p<0.001). Immediately post-bathing, HR remained elevated compared to baseline values for ExHWI and ExTWI before returning to baseline values by post-1 (ExHWI vs. ExTWI, p<0.001; pre vs. immediately post-bathing, both p<0.005; pre vs. post-1 (both p=1.000).

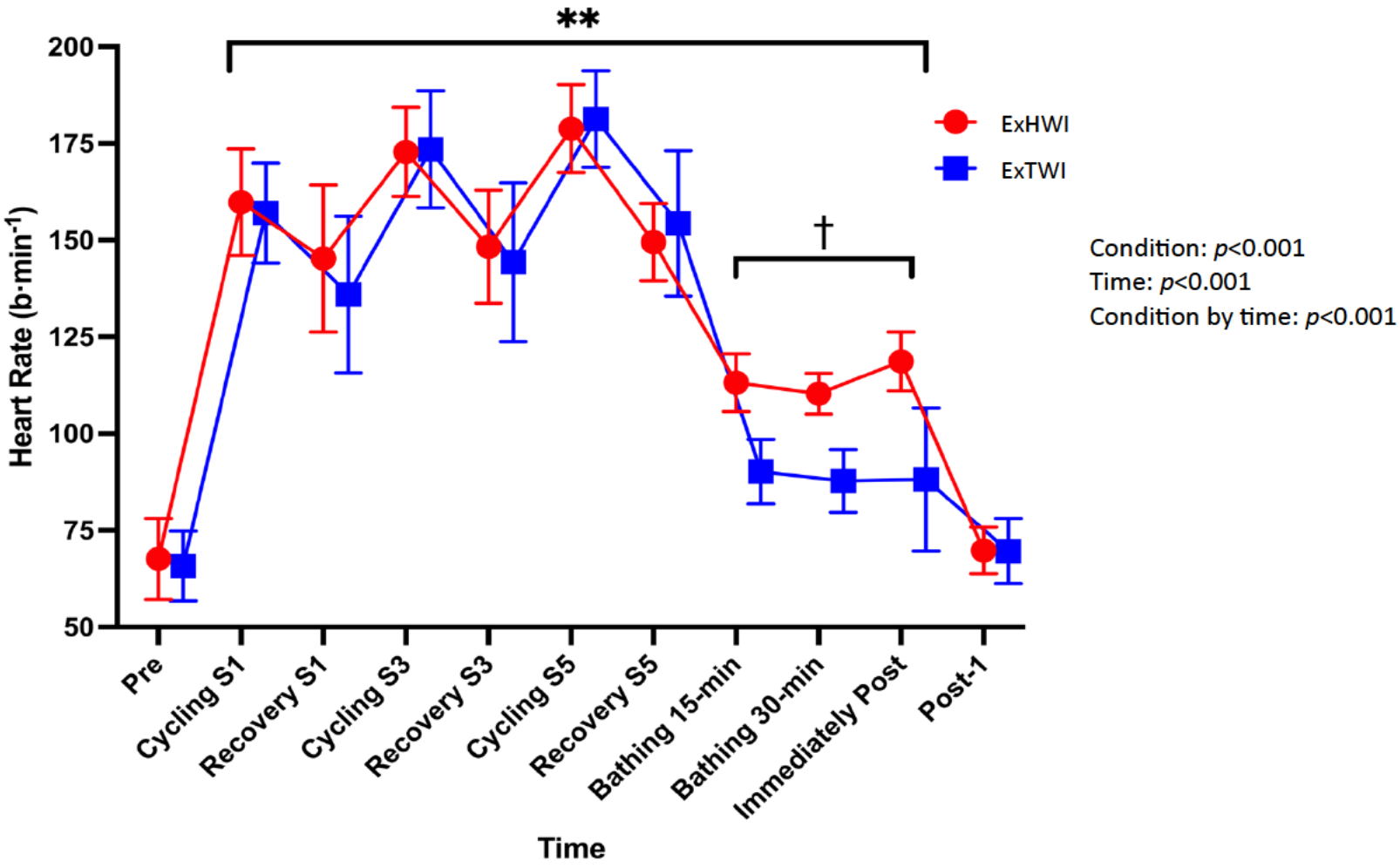

Figure 5.6. Heart rate responses at baseline (Pre), during HIIEx and bathing, and post-intervention. ExTWI, a 5x1-min HIIEx cycle bout (100% W_{peak}) followed by 30 minutes of hot water bathing at 41°C; ExHWI, a 5x1-min HIIEx bout (100% W_{peak}) followed by 30 minutes of hot water bathing at 35°C. HIIEx, high-intensity interval exercise; W_{peak}, peak power output. **($p< 0.05$) significantly different from baseline in ExHWI and ExTWI; †($p< 0.05$), ExHWI significantly different from ExTWI. Data presented as means ± SD.

5.3.4 Brachial endothelial function

5.3.4.1 Flow-mediated dilation

Across all bathing conditions, brachial FMD (allometrically scaled) did not change post-bathing compared to pre-bathing values, all $p>0.05$; see Table 5.2).

5.3.4.2 Diameter

There was a main effect of time for baseline diameter and peak diameter (Table 5.2). After ExHWI and ExTWI, baseline diameter increased at post-1 before returning to baseline values by post-2 (pre vs. post-1, $p=0.026$; pre vs. post-2, $p=1.000$). Similarly, after ExHWI and ExTWI, peak diameter increased at post-1 before returning to baseline values by post-2 (pre vs. post-1, $p=0.010$; pre vs. post-2: $p=1.000$).

5.3.4.3 Shear rate

There was a time-by-condition interaction effect for antegrade and total SR (Table 5.2). After ExHWI, antegrade SR increased at post-1 before returning to baseline values by post-2 (pre vs. post-1, $p<0.001$; pre vs. post-2, $p=1.000$). After ExTWI, antegrade SR was similar to baseline values (pre vs. post-1, $p=1.000$). At post-1, antegrade SR was higher in ExHWI than ExTWI (ExHWI vs. ExTWI, $p<0.001$).

After ExHWI, total SR increased at post-1 before returning to baseline values by post-2 (pre vs. post-1, $p<0.001$; pre vs. post-2, $p=1.000$). After ExTWI, total SR was similar to baseline

values (pre vs. post-1, p=1.000). At post-1, total SR was higher in ExHWI than ExTWI (ExHWI vs. ExTWI, p<0.001).

Table 5.2. Brachial flow-mediated dilation (FMD) and heart rate measures at baseline (Pre) and after an exercise and water immersion intervention.

	Condition	Time Points				*p* value and η^2p		
		Pre	Immediately Post-bathing	Post-1	Post-2	24-h	Condition	Time	Condition-by-Time
Brachial FMD									
Brachial FMD (%)	**EXTWI**	7.6±2.3	n/a	8.4±2.0	7.9±1.8	6.6±1.4	*p*=0.294	*p*=0.266	*p*=0.669
	EXHWI	7.7±2.3	n/a	7.4±1.6	6.5±2.3	6.7±2.9	η^2p=0.032	η^2p=0.108	η^2p=0.044
Allometrically scaled FMD (%)	**EXTWI**	6.9±2.9	n/a	7.1±3.0	6.3±3.1	6.5±2.9	*p*=0.070	*p*=0.285	*p*=0.744
	EXHWI	7.4±2.9	n/a	8.4±2.9	7.5±2.9	6.7±2.9	η^2p=0.094	η^2p=0.102	η^2p=0.035
Baseline diameter (mm)	**EXTWI**	4.0±0.7	n/a	4.1±0.6**	3.9±0.7	4.0±0.4	*p*=0.304	*P*=0.017	*p*=0.223
	EXHWI	4.0±0.7	n/a	4.3±0.6**	4.2+0.8	4.0+0.6	η^2p=0.304	η^2p=0.017	η^2p=0.223
Peak diameter (mm)	**EXTWI**	4.3±0.7	n/a	4.4±0.6**	4.2±0.7	4.3±0.5	*p*=0.652	*p*=0.004	*p=0.389*
	EXHWI	4.2±0.7	n/a	4.5±0.6**	4.4±0.8	4.3±0.6	η^2p=0.652	η^2p=0.004	η^2p=0.389
Antegrade SR (s^{-1})	**EXTWI**	114±30	n/a	132±33	120±68	144±33	*p*=0.002	*p*=0.002	*p*=0.014
	EXHWI	125±49	n/a	248±65**†	150±50	165±87	η^2p=0.259	η^2p=0.346	η^2p=0.265
Retrograde SR (s^{-1})	**EXTWI**	-28±15	n/a	-19±9	-17±10	-21±26	*p*=0.854	*p*=0.062	*p*=0.495
	EXHWI	-32±20	n/a	-9±11	-15±9	-33±24	η^2p=0.001	η^2p=0.191	η^2p=0.067
Total SR (s^{-1})	**EXTWI**	86±39	n/a	113±40	103±74	123±51	*p*=0.005	*p*=0.001	*p*=0.012
	EXHWI	94±58	n/a	249±59**†	135±47	132±81	η^2p=0.212	η^2p=0.368	η^2p=0.271
Total velocity ($cm \cdot s^{-1}$)	**EXTWI**	8.7±4.0	n/a	11.7±5.0	10.5±9.4	11.3±5.7	*p*=0.003	*p*<0.001	*p*=0.013
	EXHWI	8.8±4.4	n/a	26.0±5.3**†	13.7±4.7	12.3±6.1	η^2p=0.238	η^2p=0.410	η^2p=0.276
Heart rate									
HR ($b \cdot min^{-1}$)	**EXTWI**	66±9	88±8**	70±6	n/a	n/a	*p*<0.001	*p*<0.001	*p*<0.001
	EXHWI	68±11	111±8**†	70±8	n/a	n/a	η^2p=0.959	η^2p=0.086	η^2p=0.281

ExTWI, a 5x1-min HIIEx cycle bout (100% W_{peak}) followed by 30 minutes of hot water bathing at 41°C; ExHWI, a 5x1-min HIIEx bout (100% W_{peak}) followed by 30 minutes of hot water bathing at 35°C; HIIEx, high-intensity interval exercise; W_{peak}, peak power output; HR, Heart Rate; SR, Shear Rate; η^2p, partial eta squared. **(p<0.05), significantly different from baseline (pre) values; †(*p*< 0.05), ExHWI significantly different from ExTWI. All data are expressed as mean ± SD.

5.3.4.4 Total blood velocity

There was a time-by-condition interaction effect for total blood velocity (Table 5.2). After ExHWI, total blood velocity increased at post-1 before returning to baseline values by post-2 (pre vs. post-1, $p<0.001$; pre vs. post-2, $p=1.000$). After ExTWI, total velocity was similar to baseline values (pre vs. post-1, $p=1.000$). At post-1, total blood velocity was higher in ExHWI than ExTWI (ExHWI vs. ExTWI, $p=0.001$).

5.3.5 Mood measurements

5.3.5.1 Calmness

There was a condition-by-time interaction effect for calmness (Table 5.3). After HIIEx, calmness ratings decreased from baseline values in both ExHWI and ExTWI (ExHWI vs. ExTWI $p=1.000$; pre vs. end-HIIEx: “moderately” to “a little”; $p=0.010$). During bathing (at 30 minutes), ExHWI and ExTWI calmness ratings returned to baseline values (pre vs. 30-min bathing, $p=1.000$)). For both ExHWI and ExTWI, there was no difference in calmness ratings between baseline and post-intervention time points (pre vs. immediately-post, $p=1.000$; pre vs. post-1, $p=0.938$; pre vs. 24-h, $p=1.000$).

5.3.5.2 Cheerfulness

There was a main effect of time for cheerfulness (Table 5.3), however, post hoc analysis did not indicate a change from baseline at end-HIIEx, during bathing or at post-intervention time points (all $p>0.05$).

5.3.5.3 Energetic

There was a main effect of time for energetics (Table 5.3); however, post hoc analysis did not indicate a change from baseline at end-HIIEx, during bathing or at post-intervention time points (all $p>0.05$). There was no other significant interaction or main effect for the remaining mood state variables or sleep quality (all $p>0.05$, Table 5.3.)

Table 5.3. Sleep quality, mood state variables and thermal perception at baseline (Pre), during, and after an exercise and water immersion intervention.

		Condition							*p* value and η^2p		
		Pre	End HIIEx	Bathing 30min	Immediately Post	Post-1	Evening	24-h	Condition	Time	Condition -by-Time
Groningen Sleep Scale	**EXTWI**	4.8±4.2	n/a	n/a	n/a	n/a	n/a	4.5±2.9	p=0.079	p=0.797	p=0.566
	EXHWI	6.2±3.8	n/a	n/a	n/a	n/a	n/a	6.2±3.7	η^2p=0.086	η^2p=0.009	η^2p=0.004
Happiness	**EXTWI**	2.8±1.0	3.5±0.8	3.5±0.5	3.3±0.8	3.2±1.0	2.6±0.9	3.0±1.0	p=0.234	p=0.191	p=0.416
	EXHWI	2.8±1.0	2.8±1.2	3.7±1.4	3.8±1.5	3.7±1.0	3.5±0.5	3.2±0.8	η^2p=0.203	η^2p=0.005	η^2p=0.024
Cheerfulness	**EXTWI**	2.5±1.0	3.3±1.2	3.5±0.5	3.3±0.8	3.0±1.1	2.2±1.3	3.0±1.0	p=0.676	p=0.080	p=0.377
	EXHWI	2.7±0.8	2.8±1.2	3.3±1.0	3.5±1.2	3.2±1.0	3.3±0.8	2.6±0.9	η^2p=0.037	η^2p=0.005	η^2p=0.024
Calmness	**EXTWI**	3.5±1.2	2.2±1.2**	4.2±0.4	3.3±1.2	4.2±0.4	3.4±0.9	3.8±0.8	p=0.442	p<0.001	p=0.577
	EXHWI	3.5±1.2	2.2±0.8**	4.2±1.2	4.5±0.8	4.3±0.5	3.7±0.8	3.8±0.4	η^2p=0.022	η^2p=0.140	η^2p=0.086
Energetic	**EXTWI**	2.3±0.8	2.8±0.8	2.3±1.0	2.7±1.0	2.0±0.9	1.2±0.4	2.8±0.8	p=0.326	p=0.050	p=0.339
	EXHWI	2.5±1.2	2.3±1.0	2.5±0.8	3.2±1.2	2.8±0.4	2.0±0.9	2.2+0.4	η^2p=0.015	η^2p=0.166	η^2p=0.060
Stressed	**EXTWI**	1.7±1.2	1.5±0.5	1.0±0	1.2+0.4	1.2+0.4	1.6±0.9	1.8±0.8	p=0.882	p=0.049	p=0.999
	EXHWI	1.7±1.2	1.5±0.5	1.2±0.4	1.2±0.4	1.2±0.4	1.7±0.8	1.6±0.9	η^2p=0.035	η^2p=0.125	η^2p=0.071
Frustrated	**EXTWI**	1.0±0	1.3±0.5	1.0±0	1.2±0.4	1.2±0.4	1.2±0.4	1.2±0.4	p=0.327	p=0.128	p=0.421
	EXHWI	1.2±0.4	1.5±0.8	1.2+0.4	1.0±0	1.0±0	1.0±0	1.2±0.4	η^2p=0.022	η^2p=0.125	η^2p=0.071
Anxious	**EXTWI**	1.3±0.5	1.2±0.4	1.0±0	1.2±0.4	1.0±0	1.4±0.9	1.2±0.4	p=0.271	p=0.187	p=0.613
	EXHWI	1.5±0.8	1.5±0.5	1.2±0.4	1.3±0.8	1.2±0.4	1.2±0.4	1.0±0	η^2p=0.035	η^2p=0.125	η^2p=0.071
Sadness	**EXTWI**	1.3±0.5	1.0±0	1.2±0.4	1.2±0.4	1.3±0.8	1.6±1.3	1.2±0.4	p=0.531	p=0.506	p=0.401
	EXHWI	1.3±0.8	1.2±0.4	1.3+0.8	1.3±0.8	1.3±0.8	1.2±0.4	1.4±0.9	η^2p=0.012	η^2p=0.076	η^2p=0.091
Thermal Perception											
Thermal Sensation	**EXTWI**	7±1	10+2**	7±2	6±1	7±1	n/a	n/a	p=0.002	p<0.001	p=0.005
	EXHWI	7±1	10±1**	10±1**	9±2†	7±0	n/a	n/a	η^2p=0.205	η^2p=0.561	η^2p=0.281
Thermal Comfort	**EXTWI**	1±0	2+1	1±1	1±0	1±0	n/a	n/a	p=0.036	p=0.013	p=0.135
	EXHWI	1±0†	2±1†	3±1†	2±1†	1+0†	n/a	n/a	η^2p=0.096	η^2p=0.246	η^2p=0.145

ExTWI, a 5x1-min HIIEx cycle bout (100% W_{peak}) followed by 30 minutes of hot water bathing at 41°C; ExHWI, a 5x1-min HIIEx bout (100% Wpeak) followed by 30 minutes of hot water bathing at 35°C; HIIEx, high-intensity interval exercise; W_{peak}, peak power output. **($p<0.05$), significantly different from baseline (pre); †($p< 0.05$), ExHWI significantly different from ExTWI. η^2p, patrial eta squared. All data are expressed as mean ± SD.

5.4 DISCUSSION

5.4.1 Main findings

This pilot study examined the acute CV response to a combined HIIEx and HWI protocol as compared to a combined HIIEx and TWI protocol. It was hypothesised that ExHWI would lower BP, increase FMD and cause a greater PV expansion than ExTWI. The study results revealed that ExHWI caused a more potent CV response, as shown by the higher HR and T_{rec} levels during bathing and post-intervention than ExTWI. ExHWI and ExTWI had a similar hypotensive effect on MAP and DBP during bathing; however, only ExHWI BP remained lower immediately post-intervention. Despite the BP response, neither condition caused a change in FMD post-intervention or increased plasma blood volume 24-h following the intervention. The secondary aim was to examine the effects of HIIEx and water immersion on mood state and sleep quality. In both conditions, HIIEx caused an increase in negative mood state (i.e., decreased calmness ratings); however, within 30 minutes of bathing, all mood state data were similar to baseline values. In summary, this pilot study has demonstrated that ExHWI is a feasible intervention in young adults that offers a greater hyperthermic response and CV strain than ExTWI.

5.4.2 Thermal response to high-intensity interval exercise and bathing

ExHWI resulted in a stronger and longer hyperthermic stimulus than ExTWI. This was evident by the higher peak T_{rec} reached and the extended duration T_{rec} was elevated for in ExHWI, as

compared to ExTWI (see Figure 5.3). In comparison, albeit in a different cohort of participants, the 30 minutes of HWI used in Chapter 4 resulted in a lower peak T_{rec} than the ExHWI intervention (i.e. 38.1±0.4°C vs. 38.4±0.2°C), whilst 60 minutes of HWI resulted in a similar peak T_{rec} (38.5±0.3°C) compared to ExHWI. The elevated T_{rec} achieved during HIIEx has meant ExHWI resulted in a stronger and longer hyperthermic stimulus than a 30-minute HWI intervention on its own. However, the hyperthermic response was similar in ExHWI and HWI60.

There are a limited number of exercise and HT studies. Therefore, it can be challenging to make T_C comparisons, especially as some studies did not include T_C measurements (e.g. (Lee et al., 2020)). However, Steward et al. (2024) demonstrated in middle-aged, inactive adults, 60 minutes of HWI had the highest peak $T_{rec,}$ compared to a combined 30-min of cycling (50% VO_{2peak}) and 30-min HWI condition (38.04°C vs. 37.77°C, respectively). Interestingly, the present data indicate that HIIEx and 30-min HWI (ExHWI) resulted in a stronger hyperthermic response than the combination of exercise and HWI used by Steward et al. (2024). This is likely due to two factors: HIIEx resulted in a higher T_{rec} rise than the moderate intensity exercise bout, and secondly, the difference in the HWI protocol (waist vs. neck level immersion).

5.4.3 Cardiovascular response to HIIEx and bathing

As predicted, these preliminary findings demonstrate that HIIEx resulted in a strong CV response, as shown by the elevated HR and SBP. For example, there was a sharp elevation in SBP (~174±24 mm Hg) and HR (~181 $b.min^{-1}$) during HIIEx, which peaked during the final

HIIEx interval. These results are unsurprising, as increased exercise intensity (as seen in HIIEx protocols) increases metabolic demand for active skeletal muscle (Romero et al., 2017b). Thus, cardiac output increases to increase skeletal muscle blood flow, supported by the observed increases in HR in the current study (and likely an increase in stroke volume, although this was not measured). These increases in HR are similar to other normotensive HIIEx studies. For example, Seeger et al. (2015) found that in young, healthy participants performing a HIIEx session of 10x1 minute cycle bouts at 100% maximum workload, HR reached 188 ± 11 $b \cdot min^{-1}$ during the final HIIEx interval. To our knowledge, we are the first study to assess BP during HIIEx; therefore, it is not possible to make a comparison during HIIEx. However, when BP was evaluated immediately following an HIIEx intervention (~1 minute) in 39 middle-aged, healthy men (6x1 minutes at 98% W_{peak} with 4-minute rest intervals), SBP was elevated to ~172 mm Hg (Ketelhut et al., 2016). This SBP elevation is similar to the SBP achieved at the final interval of HIIEx during EXHWI and EXTWI. In summary, HIIEx, has caused a strong CV response as shown by the elevated BP and HR.

From these preliminary data, ExHWI appears to cause a more potent CV response than ExTWI. This is demonstrated by the higher and prolonged elevation in HR and T_{rec} during bathing and post-intervention, alongside a reduction in DBP and MAP immediately post-intervention. In the current study, HWI prolonged the elevated HR response from HIIEx to a greater extent than TWI. An elevated HR with HWI is unsurprising given the thermal strain and subsequent cardiac output demands of HWI (Rowell, 1974). A key mechanism for a reduction in peripheral resistance during exercise is the increased active skeletal muscle bed vasodilation from increased histamine H_1 and H_2 receptor activation (Lockwood et al., 2005).

Additionally, HIIEx increases antegrade SR to the conduit arteries, increasing the production of vasodilators including NO (Dawson et al., 2013). This post-exercise reduction in vascular resistance (from increased active skeletal muscle bed vasodilation attenuates venous return, which is then compensated for with increased HR to maintain cardiac output (Crandall and Wilson, 2015). This chain of events may explain why HR remained slightly elevated (as compared to baseline) during TWI, despite the hydrostatic effects of water (Park et al., 1999). Interestingly, ExHWI had a higher HR response (~7-10 b.min^{-1}) and peak T_{rec} than the HWI30 and HWI60 conditions assessed in Chapter 4, suggesting that ExHWI may be a stronger CV stressor than HWI alone.

Following a bout of HIIEx, the arterial baroreflex shifts downwards and to the left, reducing sympathetic activity despite the CV system operating at lower BP (Romero et al., 2017b). This can result in an SBP reduction (down by ~8 mm Hg) up to 20 minutes post-HIIEx in normotensive individuals (Jones et al., 2021). For the current study, there was no SBP change during bathing or post-intervention for ExHWI or ExTWI. In contrast, recent studies investigating the effect of exercise (15 minutes of cycling at 75% HR maximum) followed by sauna bathing (15 minutes at 75°C) have seen reductions in SBP 30 minutes post-intervention (down by 2.7 mm Hg (Lee et al., 2021)). Similarly, 30 minutes of cycling (50% VO_{2peak}) followed by 30 minutes of HWI (waist level immersion) in young adults has been shown to reduce SBP (down by ~9 mm Hg) immediately post-intervention (Steward et al., 2024). Therefore, given the pilot study nature of this study, further research is needed to confirm the SBP profile following HIIEx and water immersion interventions, especially given the DBP and MAP observed effects as discussed below.

During bathing, DBP and MAP were lowered to a similar extent for ExHWI and ExTWI. As there was no change in SBP, it is clear that DBP primarily drove the reduction in MAP. Changes in DBP are typically due to an imbalance between cardiac output and vascular resistance (Crandall and Wilson, 2015, Sprangers et al., 1991). During bathing, the hydrostatic pressure of water lowered DBP and MAP in TWI30. This is likely due to a reduction in peripheral vascular resistance, as demonstrated in previous TWI literature (Weston et al., 1987, Boussuges, 2006b, Park et al., 1999) and supported by the fact that MAP and DBP returned to baseline values immediately post-intervention when the hydrostatic pressure was removed. The TWI DBP change observed in the current study at 30 minutes of bathing (drop of 22 mm Hg) is higher than HT (passive heating only) studies (Campbell et al., 2022): TWI60 (drop of 11 mm Hg) and TWI30 in Chapter 4 (drop of 9 mm Hg)). Therefore, TWI, independent of heat stress, appears to have a hypotensive effect on DBP and MAP during bathing in the ExTWI condition.

A high skin temperature (~38°C) and T_c that has increased above 1°C have been demonstrated to significantly lower CVP (5.5 ± 0.7 to 0.2 ± 0.6 mm Hg); (Crandall et al., 2008)). Therefore, it would be expected that the combination of the high skin temp and T_c achieved during ExHWI would have resulted in a reduced peripheral vasculature resistance and thus a lower DBP for ExHWI during bathing. In this current pilot study, the DBP values were not different at 30 minutes post-bathing (ExHWI (39±5 mm Hg) vs. ExTWI (50±8 mm Hg)). However, the on-average change score indicates a likely difference between the HWI and TWI conditions that should be seen with a larger sample size.

Interestingly, BP was not lowered at post-2 (~55 minutes post-intervention) for either condition. This is in contrast to other HIIEx studies, where reductions in SBP and MAP have been seen up to one-hour post-intervention (Pimenta et al., 2019, Quinn, 2000). By combining the hypotensive effect of HIIEx and HWI within one intervention, it was predicted that BP would remain lower for ExHWI at post-2. A possible explanation could be that this study has recruited normotensive individuals rather than hypertensive individuals. Hypertensive individuals' higher BP can be due to several factors, including elevated systemic vascular resistance (Renna et al., 2013). After HIIEx, vasodilation of the active skeletal muscle beds is prolonged, which causes a lowered systemic vascular resistance (Romero et al., 2017b). Therefore, this change in systemic vascular resistance (alongside other factors) can result in a more pronounced and prolonged reduction in BP than in normotensive individuals (Pescatello et al., 2004). In a recent meta-analysis that investigated the post-exercise hypotensive effect of HIIEx in normotensives and hypertensive individuals, there was no significant reduction in SBP or DBP 60 minutes post-intervention (Marçal et al., 2021). Interestingly, only 2/11 papers for SBP and DBP included in the analysis included hypertensive participants, which meant most participants were normotensive. Thus, it appears from the findings of this pilot study that ExHWI or ExTWI does not lower BP approximately one-hour post-intervention for young, healthy normotensive individuals.

A key independent CVD risk factor is morning BP, with BP surges associated with increased CV events (Booth et al., 2020, Li et al., 2010). Thus, interventions that can reduce morning BP are sought after. The current study showed no change in resting BP for ExHWI or ExTWI 24-h post-baseline measures. This is the first study to combine HIIEx and HWI; thus, it is hard to

find comparable studies. However, when used separately, HIIEx (Graham et al., 2016) and HWI (Campbell et al., 2022) did not lower 24-h BP in healthy, normotensive participants. One previous study has shown that reductions in resting BP after a moderate-intensity exercise session (50 minutes of cycling at 65% maximal oxygen uptake) were associated with an increased PV (Graham et al., 2016). If increased PV is the mechanism driving sustained reductions in BP up to 24-h post-intervention, it is unsurprising that BP did not change in the current study as there was no increase in PV 24-h after baseline measures. Therefore, it appears that a single ExHWI session is not enough to reduce morning BP or increase PV in young normotensive individuals.

5.4.4 Flow-mediated dilation

Acute FMD improvements are associated with increased antegrade SR, which is associated with increased NO availability (Carter et al., 2013b). Previous HIIEx (4x4 minutes at 85-95% HR maximum; (Tyldum et al., 2009a)) and HWI (45 minutes of leg immersion at 45°C; (Cheng et al., 2021)) studies have demonstrated increased antegrade SR and FMD after a single session in young, healthy adults. Therefore, combining both HIIEx and HWI was expected to increase antegrade SR and, thus, FMD. The current study did not see an FMD change post-intervention for either ExHWI or ExTWI, although there was a trend for a condition main effect (p=0.070). Indeed, Weston et al. (2022) showed that brachial artery FMD was increased one hour following both moderate (75% $\dot{V}o_{2max}$) and high (90% $\dot{V}o_{2max}$) intensity interval exercise (both 5 x 3 min intervals). The timing of the FMD measure at post-1 for the current study was approximately one hour after HIIEx, a similar time to Weston et al. (2022).

This suggests the changes observed at post-1 for baseline and peak diameter may be driven by the HIIEx in ExTWI and ExHWI.

Antegrade SR for ExHWI was higher than ExTWI at post-1. This increase in antegrade SR would have been driven by increased core and skin temperature via HWI, thus increasing cutaneous and conduit artery blood flow. Increased antegrade SR in long-term interventions is associated with improved endothelial function and arterial restructuring, such as an increased resting baseline diameter (Brunt et al., 2016a). Therefore, despite no increase in FMD, the increase in antegrade SR at post-1 means ExHWI may be a promising long-term intervention to improve endothelial function.

HIIEx is associated with a "bi-phasic" FMD response (Dawson et al., 2013). Specifically, within the first 45 minutes post-HIIEx (first phase), FMD has been shown to decrease due to factors including an increased baseline diameter (Carter et al., 2013a) and inflammatory response (Ohara et al., 1993). In the second phase (approximately one-hour post-intervention), FMD has been shown to increase due to increased NO availability (Cosio-Lima et al., 2006) and a robust anti-inflammatory response (Tyldum et al., 2009b). Therefore, the timing of the FMD following HIIEx will determine the direction of the FMD change. This study included two FMD time points (~30 minutes and ~90 minutes post-intervention, which coincides with ~60 minutes and ~120 minutes post-HIIEx). By including two-time points, it was possible to investigate the difference between the direct effect of elevated T_c and skin temperature (as shown by the increased baseline diameter and antegrade SR at post-1) versus the more prolonged effect of ExHWI once T_c had returned to near baseline at post-2. Data from this

pilot study indicates that ExHWI does follow the typical HIIEx bi-phasic FMD response to an extent. For example, at post-1, there was an increase in baseline and peak diameter, which coincides with approximately one-hour post-HIIEx. However, by post-2 (~90 minutes post-intervention and ~120 minutes post-HIIEx), there was no change in FMD variables for either ExHWI or ExTWI. Therefore, it appears that any acute effects of ExHWI on endothelial function are not present 90 minutes post-intervention (or 120 minutes post-HIIEx).

5.4.5 Mood state

A key strength of this study is that it investigates the effect of ExHWI on mood. Assessing the mood state allows further insight into whether young adults can benefit from reduced stress and increased happiness, which are associated with CVD disease risk (Batty et al., 2014, Rosengren et al., 2004). HIIEx perturbed mood state by reducing calming ratings in both ExHWI and ExTWI. However, mood state ratings were similar to baseline values within 30 minutes of bathing. ExHWI was well tolerated by participants as there were no significant increase in negative mood states, despite increased thermal discomfort and sensation during bathing. Therefore, these findings are promising as they demonstrate that ExHWI is a feasible intervention for young adults with no detrimental effects on mood state.

Some passive heating studies have focussed on examining changes in negative mood states via the Brunel Mood Scale (Terry et al., 1999). For example, in young, healthy males, one hour of seated rest in an environmental chamber (39.6 ± 0.4°C) increased confusion, depression and fatigue scores (though anger, tension, and vigour were unaffected (Malcolm et al., 2018)). In another study, obese and non-obese young females saw an increase in

anger, depression and a reduction in vigour following a water-perfused suit exposure (Caldwell et al., 2018). These studies contrast with Chapter 4, where an increase in negative mood state did not change during HWI30 or post-intervention, despite an increase in peak T_{rec} (1.31±0.36°C) and presumably a rise in skin temperature. Therefore, there are mixed results regarding how passive heating affects negative mood states.

Interestingly, the duration an individual is passively heated, rather than the degree of hyperthermia, may mediate increases in a negative mood state. In the Malcolm et al. (2018) and Caldwell et al. (2018) studies, participants were heated for approximately an hour, which resulted in a ~0.2 and ~1 °C T_{rec} rise, respectively, alongside an increase in negative mood states. Conversely, ExHWI (with 30 minutes of HWI) resulted in a greater T_{rec} rise (~1.6 °C) than HWI30 (Chapter 4) and the Malcolm et al. (2018) and Caldwell et al. (2018) studies, yet saw no change in mood state during bathing. Similarly, there was no reported change in negative mood states in HWI30 in Chapter 4) despite reaching a greater T_{rec} rise (~1.1 °C) than Malcolm et al. (2018). In summary, prolonged heat stress appears to be the driver for increased negative mood states. Secondly, (although this assumption is based upon Chapters 4 and 5 as the Malcolm et al. (2018) and Caldwell et al. (2018) studies did not measure positive mood states)) it seems that passive heating does not increase positive mood state either during passive heating or immediately following ExHWI or a passive heating intervention.

5.4.6 Experimental considerations and perspectives

To our knowledge, this is the first study to investigate the CV response from the combined effect of HIIEx and HWI. The current study has two key strengths that help tease out these CV responses. Firstly, BP was measured during bathing, demonstrating hydrostatic pressure's direct effect on the BP response. Second, the study completed two FMD tests following the intervention to decipher whether ExHWI has a bi-phasic FMD response similar to HIIEx. As this was a pilot study, the sample size was small. Nevertheless, this pilot study has demonstrated that it is feasible for young adults to complete ExHWI and that there is a clear hyperthermic and CV response despite the small sample size. As this was a feasibility study, there was no resting control. Therefore, we cannot elucidate how the CV response would differ if HIIEx were completed apart from water immersion. Finally, there may be variability in the FMD results due to the skill of the ultrasound operator (B.P.). However, during the data collection phase, the operator had already conducted a repeatability study (coefficient of variation from repeatability study one: 6.9%) in Section 3.3.2, and the study from Chapter 4 (>200 scans). Therefore, confidence can be found in the operator's ability to undertake brachial artery FMD scans.

5.4.7 Conclusion

The current study shows that ExHWI created a greater hyperthermic impulse than ExTWI and a greater CV strain, as demonstrated by the higher HR during bathing and post-intervention and a lower DBP and MAP immediately post-intervention. ExHWI appears well tolerated, as shown by no significant increase in negative mood states (e.g. stress) during bathing or post-intervention, despite an increase in thermal comfort and thermal sensation. Additionally,

ExHWI, despite no FMD increase, saw an increased baseline and peak brachial artery diameter and elevation in antegrade SR, which could drive long-term FMD change following multiple bouts of ExHWI. Therefore, ExHWI is a promising intervention to induce a positive, acute BP post-intervention response in young adults.

6. GENERAL DISCUSSION

6.1 Revisiting the problem and objectives

Heat thermotherapy (HT) is a promising method to attenuate CVD risk as it has been demonstrated to lower BP, improve endothelial function, and reduce arterial stiffness (Brunt et al., 2016a). Consequently, there is a need to understand how HT can be optimised to minimise discomfort and heat stress risk (and therefore be feasible for general public use). Also, there is a need to understand how HT can be optimised to result in a positive and meaningful CV response that reduces CVD risk and, thus, advance its use as a preventative therapy for CVD. Therefore, the primary objective of this thesis was to assess the impact of passive HT and HIIEx followed by 30 minutes of HWI (ExHWI) on CV function. A systematic review and meta-analysis (**Chapter 2**) were conducted to evaluate the efficacy of HT in enhancing CV and cardiometabolic health and to identify the gaps in the literature. The experimental chapters examined the effects of different HWI protocols on the acute CV response (**Chapter 4**) and the impact of combined HIIEx and HWI on the acute CV response (**Chapter 5**).

6.2 Summary of main findings

6.2.1 Core body temperature thresholds

As detailed in Chapter 1.5.4., there is a common conception that to improve CV function and cardiometabolic health, T_c needs to rise to 38.5-39°C and be maintained for at least 20-30 minutes (Brunt and Minson, 2021). The rationale originates from Laukkanen et al. (2015) where, after a 20-year follow-up, the greatest reduction in CVD risk was associated with Finnish sauna sessions over 19 minutes long at 80-100°C. Brunt et al. (2016a) attempted to

mimic a similar rise in T_{rec} (38.5-39°C) in a 90-minute HWI study over eight weeks in young, sedentary adults. Due to the improvements in FMD, arterial stiffness and reduced BP, other researchers have used the same method (albeit for 60 minutes rather than 90 minutes) in participants with CVD risk factors, such as increased chronic inflammation (Ely et al., 2019b) and those with type two diabetes (James et al., 2023, Behzadi et al., 2022). Whilst for long-term adaptations, both healthy individuals and those with CVD risk factors have seen improvements in CV function, such as improved FMD and lower BP (Brunt et al., 2016a, Ely et al., 2019b), acute bouts of HWI60 have had mixed results. For example, whilst one session of HWI60 prevented a reduction in FMD following an ischemic reperfusion event, HWI60 did not improve FMD 60 minutes post-intervention for young, healthy adults (Brunt et al., 2016b) or improve FMD in type two diabetics (Behzadi et al., 2022).

Chapter 2 demonstrated that due to variations in HT protocols (e.g., different core temperature measurement methods such as T_{rec} vs. oesophageal) and some studies not recording T_c, subgroup analysis of T_c changes with HT (and associated CV and cardiometabolic responses) was not feasible. Therefore, a meta-regression was completed that investigated whether there was a relationship between cumulative minutes (number of bouts x bout duration) of HT and the CV outcome of interest (e.g., BP). This meta-regression showed no significant effect; therefore, it was unclear the duration of heat stress needed to elicit a beneficial CV adaptation. To investigate the effect of a shorter duration HT protocol, against the more common, longer HT protocol, **Chapter 4** examined the effect of HWI30 vs. HWI60.

Chapter 4 demonstrated that HWI60 resulted in a greater cumulative thermal and CV strain than HWI30, with a prolonged elevation in T_{rec} (Figure 6.1.) and HR (Figure 6.2.), and a prolonged hypotensive response in HWI60 as compared to HWI30. Despite the greater cumulative CV strain, BP was lowered to a similar extent for HWI30 and HWI60. Interestingly, the T_{rec} peak for HWI30 was 38.0±0.4°C. This indicates that T_c did not need to reach >38.5°C or that the elevation in T_{rec} needed to be prolonged to cause a robust CV response. This is advantageous for future HT work because lower T_c rises are more tolerable, and a shorter HT time (30 vs. 60 minutes) is more likely to be adhered to by the public. For example, participants reported in the Bathing Preference Questionnaire that HWI30 was a preferred condition over HWI60 (Appendix Q). For Chapter 5, due to the elevation in T_{rec} from HIIEx, participants entered the bath in ExHWI with a higher rectal temperature, which HWI60 did not match until 35 minutes of bathing was completed (Figure 6.1.). Interestingly, the peak T_{rec} (ExHWI: 38.44±0.18°C vs. HWI60: 38.52±0.27°C) and duration T_{rec} was >38°C was similar for ExHWI (45 minutes) and HWI60 (40 minutes). This similar level of thermal strain indicates that differences in the CV response between ExHWI and HWI60, such as the higher HR response for ExHWI during bathing (Figure 6.2.), was due to other factors unique to exercise (i.e., difference in dilation response (skeletal muscle vs. cutaneous vasculature (Romero et al., 2017b)).

Cardiometabolic health is another key factor associated with CVD (Lotta et al., 2015). **Chapter 2** attempted to investigate the effect of HT on cardiometabolic factors such as fasting glucose and inflammatory markers (interleukin-6). Unfortunately, insufficient studies reported these factors, which meant the analysis was underpowered. From the meta-

analysis, included studies that used an HWI60 protocol (thus increased T_c >38.5°C) showed improvements in fasting glucose, insulin and increased HSP proliferation (Ely et al., 2019a, James et al., 2021). However, increases in eHSP72 have been demonstrated after one session of 45 minutes of lower limb immersion in young adults (45°C) where T_c reached ~37.6°C. Therefore, increasing T_c to >38.5°C might not be necessary to gain meaningful improvements in cardiometabolic health. Cardiometabolic markers were unable to be measured in Chapters 4 and 5. HIIEx intervention studies have shown reductions in inflammatory markers (Ramos et al., 2015) and fasting glucose (Batacan et al., 2017). Thus, how the combination of HIIEx and HT might improve cardiometabolic health remains a promising line for future investigation.

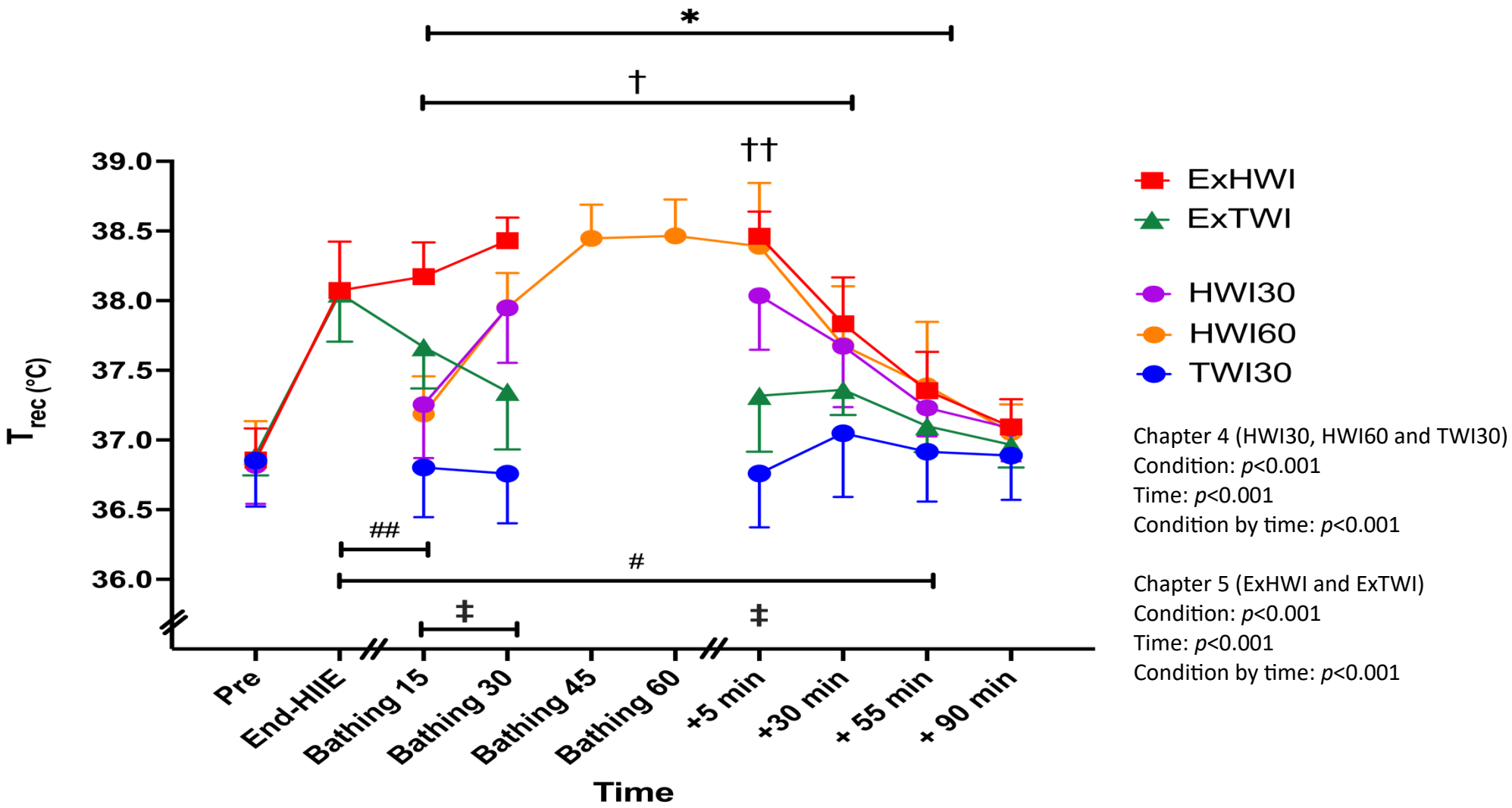

Figure 6.1. Rectal temperature (T_{rec}) responses pre (baseline), during the intervention and post-intervention for Chapters 4 and 5. ExTWI, a 5x1-min HIIEXx cycle bout (100% W_{peak}) followed by 30 minutes of hot water bathing at 41°C; ExHWI, a 5x1-min HIIEXx bout (100% W_{peak}) followed by 30 minutes of hot water bathing at 35°C; HIIEXx, high-intensity interval exercise; W_{peak}, peak power output. TWI30, 30 minutes of thermoneutral water bathing; HWI30, 30 minutes of hot water immersion; HWI60, 60 minutes of hot water immersion. #(p< 0.05), significantly different from pre-bathing value (Pre) in ExHWI alone; ##(p< 0.05), significantly different from pre-bathing value in ExHWI and ExTWI; ‡ p< 0.05), ExHWI significantly different from EXTWI. *(p< 0.05), significantly different from pre-bathing value (Pre) in HWI30 and HWI60; †(p< 0.05), HWI60 and HWI30 significantly different from TWI30; ††HWI60 significantly different from HWI30 (p< 0.05). All data are expressed as mean ± SD.

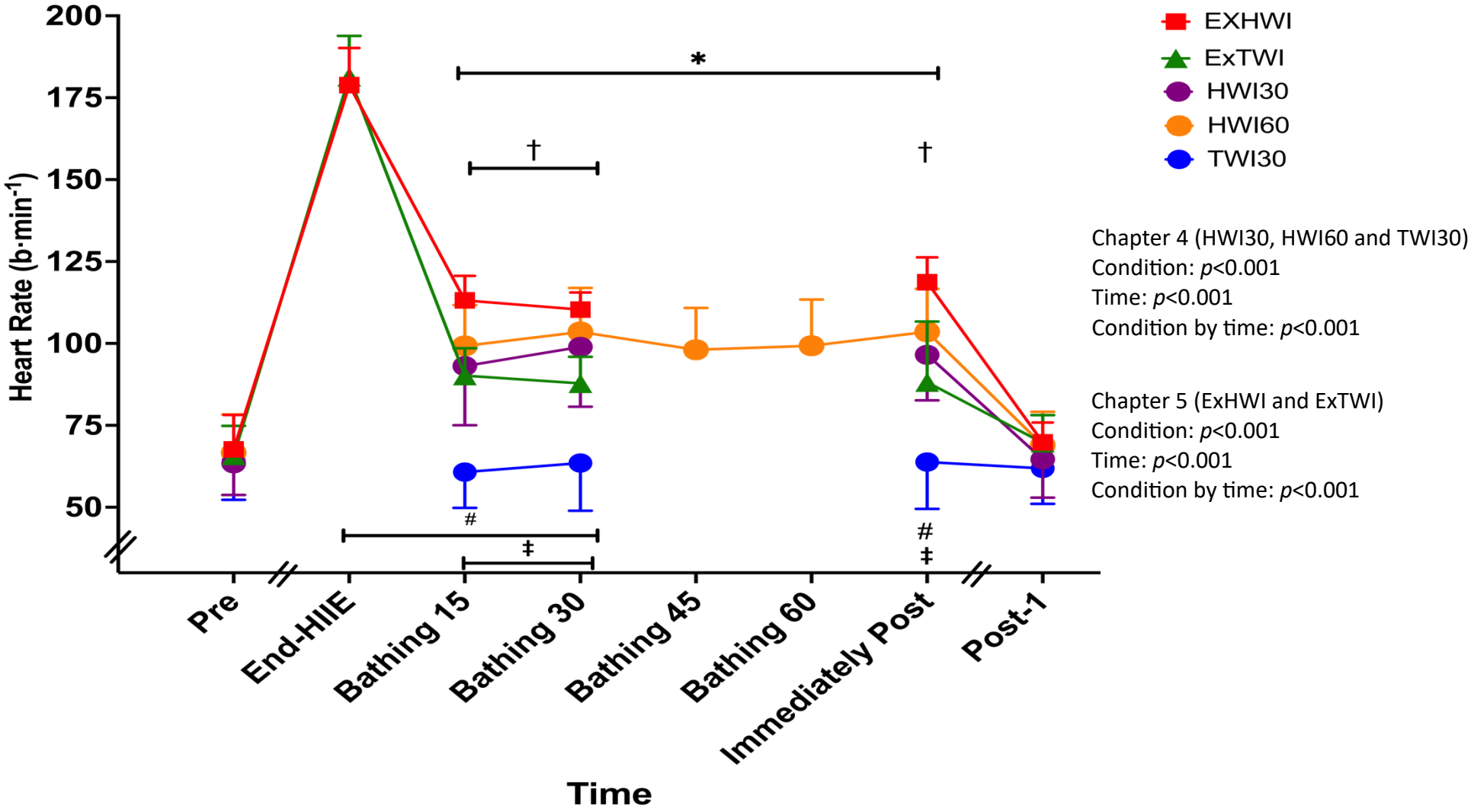

Figure 6.2. Heart rate (HR) responses pre (baseline), during the intervention and post-intervention for Chapters 4 and 5. ExTWI, a 5x1-min HIIEXx cycle bout (100% W_{peak}) followed by 30 minutes of hot water bathing at 41°C; ExHWI, a 5x1-min HIIEXx bout (100% W_{peak}) followed by 30 minutes of hot water bathing at 35°C; HIIEXx, high-intensity interval exercise; W_{peak}, peak power output. TWI30, 30 minutes of thermoneutral water bathing; HWI30, 30 minutes of hot water immersion; HWI60, 60 minutes of hot water immersion. #($p< 0.05$), significantly different from pre-bathing value (Pre) in ExHWI alone; ##($p< 0.05$), significantly different from pre-bathing value in ExHWI and ExTWI; ‡ $p< 0.05$), ExHWI significantly different from EXTWI. *($p< 0.05$), significantly different from pre-bathing value (Pre) in HWI30 and HWI60; †($p< 0.05$), HWI60 and HWI30 significantly different from TWI30. All data are expressed as mean ± SD.

6.2.2 Flow-mediated dilation

Improvements in FMD are associated with a reduced risk of CVD incidence (Green et al., 2011). Therefore, **Chapters 2, 4, and 5** investigated the effect of HT on FMD. Findings from the meta-analysis indicated that whilst there was no long-term (after >1 bout of HT) improvement in FMD, there was an acute improvement in FMD following a single session of HT. Therefore, it was anticipated that in **Chapters 4 and 5** the HWI interventions would improve FMD acutely.

Despite the CV response caused by ExHWI, HWI30 and HWI60, neither condition increased FMD post-intervention at ~30-35 minutes (post-1), ~90-95 minutes (post-2) or 24-h post-baseline measures (Table 6.1.). Interestingly, there was an increase in baseline and peak diameter for ExHWI and HWI30. This may indicate that increasing skin and T_c led to increased brachial artery diameter. This would likely be due to an increase in antegrade SR during bathing, which is associated with an increase in the release of vasodilators such as NO from the endothelial lining of the brachial artery (Cosio-Lima et al., 2006). However, this baseline and peak diameter increase was short-lived, both returning to baseline values by post-2, coincidently when T_{rec} also returned to baseline values for all ExHWI, HWI30 and HWI60.

ExHWI resulted in additional FMD-related benefits compared to HWI30 and HWI60. For example, ExHWI saw an increase in antegrade SR at post-1, with no change for HWI30 and HWI60 demonstrated. As T_{rec} was similar in HWI60 and ExHWI at post-1, it is apparent that adding an HIIEx session before HWI has resulted in the elevated antegrade SR despite a higher baseline diameter. While the cause for the increased antegrade SR from ExHWI is

unclear, it is likely due to the elevated total blood velocity, which is not seen in HWI30 or HWI60. There was no change in 24-h FMD for **Chapters 4 or 5**. These results suggest that neither HWI nor ExHWI is enough of a stimulus to invoke this change in young, healthy adults. However, other studies that have used acute HT interventions have seen improvements in FMD and antegrade SR in young, healthy adults (Cheng et al., 2021, Steward et al., 2024). These studies have completed FMD measurements sooner post-intervention (10 minutes vs. ~30-35 minutes post-intervention) than **Chapters 4 and 5** and could be why there is no observed FMD change in **Chapters 4 and 5.**

Table 6.1. The blood pressure, heart rate and flow-mediated dilation response during experimental laboratory visits for Chapters 4 and 5.

		Chapter 4 (*n*=17)			Chapter 5 (*n*=6)	
Measure	**Time Point**	HWI30	HWI60	TWI30	ExHWI	ExTWI
SBP (mm Hg)	Pre	122±11	123±10	122±9	119±5	124±9
	30-minute Bathing	113±13*	107±12*	114±13	111±5	111±7
	Immediately-post intervention	116±14†	109±9** †	132±13**	110±9	125±6
	Post-1	122±11	120±9	124±10	116±13	122±13
	24-h	123±10	125±9	124±9	112±12	118±11
DBP (mm Hg)	Pre	62±12	62±10	64±8	75±7	72±8
	30-minute Bathing	42±5*** †	40±6*** †	55±6***	39±5##	50±8##
	Immediately-post intervention	47±10*†	47±5* †	69±9	39±6# ‡	62±8
	Post-1	59±8	61±10	64±8	71±4	76±10
	24-h	62±8	64±7	64±9	64±5	61±4
MAP (mm Hg)	Pre	82±11	82±10	83±8	90±6	89±5
	30-minute Bathing	66±6*** †	63±7*** †	75±8***	63±3##	70±6##
	Immediately-post intervention	70±8*†	67±5*†	89±9	63±5# ‡	83±6
	Post-1	80±8	80±9	84±8	91±9	86±3
	24-h	82±8	84±6	83±8	80±6	80±5
HR ($b \cdot min^{-1}$)	Pre	63±10	67±12	64±12	68±11	66±9
	30-minute Bathing	99±18* †	104±13* †	64±15	110±5## ‡	88±8##
	Immediately-post intervention	97±14* †	104±13* †	64±14	111±8## ‡	88±8##
	Post-1	65±12	69±10	62±11	70±8	70±6
Allometrically Scaled FMD (%)	Pre	7.3±2.0	7.2±2.0	7.3±2.0	7.4±2.9	6.9±2.9
	Post-1	7.6±2.1	7.6±2.0	7.4±2.0	8.4±2.9	7.1±3.0
	Post-2	7.9±2.1	6.4±2.0	7.3±2.0	7.5±2.9	6.3±3.1
	24-h	6.8±2.0	7.5±2.0	7.0±2.0	6.7±2.9	6.5±2.9

ExTWI, a 5x1-min HIIEx cycle bout (100% W_{peak}) followed by 30 minutes of hot water bathing at 41°C; ExHWI, a 5x1-min HIIEx bout (100% W_{peak}) followed by 30 minutes of hot water bathing at 35°C; HIIEx, high-intensity interval exercise; W_{peak}, peak power output. TWI30, 30 minutes of thermoneutral water bathing; HWI30, 30 minutes of hot

water immersion; HWI60, 60 minutes of hot water immersion. DBP, diastolic BP; SBP, systolic BP; MAP, mean arterial pressure; HR, heart rate; FMD, flow-mediated dilation. #($p< 0.05$), significantly different from pre-bathing value (Pre) in ExHWI alone; ##($p< 0.05$), significantly different from pre-bathing value in ExHWI and ExTWI; ‡ $p< 0.05$), ExHWI significantly different from EXTWI. *($p< 0.05$), significantly different from pre-bathing value (Pre) in HWI30 and HWI60; **($p< 0.05$), significantly different from pre-bathing value (Pre) in HWI60 and TWI30; ***($p< 0.05$), significantly different from pre-bathing value (Pre) in HWI30, HWI60 and TWI30; †($p< 0.05$), HWI60 and HWI30 significantly different from TWI30. All data are expressed as mean ± SD.

6.2.3 Blood pressure

ExHWI, HWI30, and HWI60 have an apparent hypotensive effect during and immediately after bathing (Table 6.1). Across both studies in **Chapter 4 and 5**, DBP reductions during HWI were similar for all three conditions; however, ExHWI appears to lower DBP to a greater extent than HWI30 and HWI60 immediately post-intervention (DDBP: HWI30 and HWI60 (drop of ~18 mm Hg) vs. ExHWI (~36 mm Hg). This DBP change underscored MAP changes, which appear to be lower immediately post-intervention for ExHWI as compared to HWI30 and HWI60 (DMAP: HWI30 and HWI60 (drop of ~16 mm Hg) vs. ExHWI (drop of ~27 mm Hg). During HWI, ExHWI has a simultaneous demand for blood flow to previously active skeletal muscle beds (Lockwood et al., 2005) and cutaneous vasculature (Rowell et al., 1969), resulting in a reduced venous return, which not be a factor for the HWI30 and HWI60 interventions. Therefore, the venous return reduction may not be the same magnitude as ExHWI (although this was not directly measured). Thus, for ExHWI, a lower venous return could cause a lower CVP and DBP than HWI30 and HWI60.

There was no reduction in SBP during ExHWI compared to HWI30 and HWI60 during bathing. After HIIEx, SBP typically decreases in normotensive individuals (Price et al., 2020b). Hence, HWI immediately post HIIEx appeared to prevent a drop in SBP in ExHWI, despite **Chapter 4** demonstrating that HWI (HWI30 and HWI60) alone resulted in a lower SBP during bathing. However, as it was a pilot study, the SBP results could likely be underpowered. HR was elevated in ExHWI and appears to be higher than HWI30 and HWI60 during bathing (Table 6.1.). This is likely due to the increased demand for cardiac output in ExHWI to compensate for a lower venous return (vasodilated skeletal muscle and cutaneous vascular beds vs.

cutaneous vascular beds only (Lockwood et al., 2005, Kellogg Jr et al., 1995)) than HWI30 or HWI60. In summary, findings from this thesis showed that HWI30 and HWI60 had a similar hypotensive response whilst, there was a greater reduction in DBP and MAP during and immediately post-intervention for ExHWI. Therefore, ExHWI appears to be a more potent CV stimulant to drive a hypotensive effect than HWI30 and HWI60.

6.2.4 Mood state

The use of mood state questionnaires was twofold. Firstly, the HT literature has primarily focused on adherence and thermal perception. While it is useful to know how hot a participant feels, it would be more insightful to know if this makes them have a higher positive or negative mood state response such as increased stress. Secondly, recent literature has shown HT improves depressive symptoms as well as mood in depressed individuals (Hanusch and Janssen, 2019). Currently, no study has investigated if there are any increase in positive mood states in young, healthy individuals without depression following HT. Therefore, **Chapters 4 and 5** investigated if HT increase positive mood states in young and healthy adults within a university population.

Individuals are more likely to engage in an activity if they derive pleasure from it and less likely to engage in an activity if they derive negative emotions (McNeill et al., 2006). In other words, negative emotions are associated with activity disengagement/avoidance (Kahneman et al., 1993). Therefore, if ExHWI, HWI30 and HWI60 increase positive mood states, then participants will be more willing to complete the intervention again.

Whilst there was a slight increase in negative mood states during HWI60 and HIIEx (during ExHWI and ExTWI), there were no other changes in mood states during or post-intervention. Therefore, ExHWI, HWI30, and HWI60 participants had relatively neutral mood state response following the intervention. Interestingly, ExHWI caused no changes in mood states during HWI despite T_{rec} starting and being maintained above 38°C. In contrast, there was an increase in negative mood states during HWI60, despite a similar peak T_{re} (~38.5°C) and duration T_{rec} was >38°C in both trials. Therefore, it may be that completing two 30-minute sections, with different physical demands and environmental conditions within ExHWI prevented the increase in negative mood states as reported with one continuous 60-minute HWI session. This is somewhat supported by the fact that HWI30, a shorter protocol than HWI60, did not see a reduction in happiness or increased stress and frustration, as seen in HWI60. Alternatively, acute exercise bouts are associated with an increased positive mood state (Hansen et al., 2001). This rise in positive mood state could be due to an increase in neurotrophins (e.g., brain-derived neurotrophic factor), which have previously been associated with improved mood and cognitive function (Nay et al., 2021). Potentially, the rise in positive mood state from HIIEx (typically seen 10-30 minutes after exercise, (Hansen et al., 2001)) counteracted the decline in mood caused by heightened thermal discomfort during HWI. However, this was not reflected in Chapter 5, as there was no difference between ExHWI and ExTWI despite T_c returning to near baseline values by the end of ExTWI bathing. In summary, no HT intervention caused an increase in positive mood states. Due to a slight increase in negative mood states during bathing in HWI60, it appears that out of the three conditions (HWI30 vs. HWI60 vs. ExHWI), HWI30 is the most likely, and HWI60 is the least likely to be repeated by participants.

In summary, the main findings of this thesis are:

- HT improves CV related variables as shown by the positive CV response during a single bout (reduced DBP, MAP and increased FMD) and the long-term CV adaptations (reduced resting DBP and MAP) following multiple bouts. These changes were not affected by health status or age, demonstrating that HT can improve CV related variables across multiple population demographics.
- ExHWI, HWI30, and HWI60 caused a robust CV response, including a hypotensive effect during bathing and immediately post-intervention in young, healthy adults. However, this effect is short-lasting, as BP was restored by post-1 and did not change 24 hours post-baseline.
- ExHWI appears to be a more potent CV stressor than HWI30 and HWI60, as shown by a higher elevated HR during bathing a prolonged elevated T_{rec} and a lower DBP and MAP during and immediately post-intervention. Additionally, ExHWI is the only condition to increase antegrade SR at post-1.
- Despite the greater cumulative CV strain in HWI60 than HWI30, DBP and MAP were reduced similarly during and immediately post-bathing. This demonstrates that HWI30 is a more time-efficient response than HWI60 for a hypotensive response immediately post-bathing. Additionally, HWI60 was reported to be less popular than HWI30, as shown in the increase in negative mood states during bathing and the least preferable option selected in the bathing preference questionnaire.
- HWI30 demonstrates that T_{rec} does not need to be >38°C to cause an acute CV response that lowers BP. This contrasts with the popular belief that T_c needs to be

within 38.5 - 39°C and for a prolonged amount of time (≥30 minutes) to result in an acute CV response (Brunt and Minson, 2021).

6.3 Considerations and assumptions/ limitations

The participant recruitment strategy for the experimental chapters was to recruit healthy adults free from CVD, who had not recently visited a hot country or were regular users of a spa facility (see Chapter 3.1). The rationale for this cohort was to select participants within a readily available university population with the incentive of providing research credits. Additionally, the original plan of this thesis was to test the concept on healthy participants before moving on to an at-risk CVD population (i.e. hypertensive individuals) or a short-term intervention study. Unfortunately, due to the COVID pandemic, this was not possible. As highlighted in **Chapters 4 and 5**, participants with CVD risk factors may have demonstrated a more potent CV response, such as lower BP after bathing, than healthy, normotensive individuals. Therefore, as we have shown in **Chapters 4 and 5** HWI and ExHWI can lower BP immediately post-intervention, the next step is to move on to CVD risk factor participants where HT improvements such as reduced BP may be more beneficial.

The recruitment strategy did not restrict the participants' physical activity levels. Therefore, as shown in Appendix O and W, the average physical activity scores are high for both studies, with significant variation (large standard deviations). Physical activity can be classified as Inactive/low (<600 metabolic equivalent of task minutes per week), active (600-1200 metabolic equivalent of task minutes per week) and highly active (>1200 metabolic

equivalent of task minutes per week); (Chandrashekar Nooyi et al., 2019)). From **Chapters 4 and 5**, participants would be classed as (**Chapter 4**: (inactive, *n*=0), (active, *n*=1), (highly active, *n*=16) vs. **Chapter 5**: (inactive, *n*=0), (active, *n*=0), (highly active, *n*=6). Alternatively, another way to consider physical activity is to separate those who do not versus those who do complete the recommended weekly activity guidelines (≥150 minutes of moderate-intensity physical activity or ≥75 minutes of vigorous physical activity or both (Kayal, 2016)). In this respect, from **Chapters 4 and 5**, participants would be classed as (**Chapter 4**: (meets guidelines, *n*=16), (does not meet guidelines; *n*=1), Chapter 5: (meets guidelines, *n*=6), (does not meet guidelines; *n*=0). Therefore, apart from one participant in **Chapter 4**, all participants met the recommended weekly physical activity guidelines. In summary, the International Physical Activity Questionnaire demonstrates that the majority of participants for **Chapters 4 and 5** were physically active.

Despite the majority of participants completing the recommended physical activity guidelines per week (Kayal, 2016), there is still a large variation in the International Physical Activity Questionnaire data (Total average metabolic equivalent of task per week: **Chapter 4** (5721±3550) vs. **Chapter 5** (5014±3156)). This is not surprising as some participants were competing at a national level in university sport competitions. Physical activity has well-established effects on CV factors, including BP (Miller, 2014) and HR (Martinelli et al., 2005). Therefore, there may be some variation in the CV response due to the variation in participant physical activity levels. FMD is lower in elite athletes compared to healthy controls due to the long-term adaptations in brachial artery diameter (Green et al., 2013). However, there are no studies comparing FMD to those who are not elite athletes but are highly physically active

(e.g. university athletes vs. healthy controls). Therefore, it is not certain whether the variation in physical activity scores would have impacted the Chapters 4 and 5 results. However, as both studies were a crossover study design, each participant would have acted as their “own control, " reducing inter-subject variability (Jones and Kenward, 2003) and the risk of physical activity being a confounding variable.

Dietary intake, such as caffeine and alcohol, can influence endothelial function (Thijssen et al., 2019). Therefore, participants were told to restrict their caffeine and alcohol intake before each experimental visit (Chapter 3.2.). Alongside alcohol and caffeine, other natural substances such as polyphenols (e.g. found in tea and wine) when taken in large quantities can acutely increase FMD. For example, in a recent meta-analysis that investigated the effect of polyphenol supplementation to affect FMD (Jalili et al., 2024). From eight randomised control trials, the meta-analysis completed a linear-dose response analysis that suggested that each increase in polyphenols of 200mg/d increased FMD by 1.09%. Increased polyphenol intake, such as orange juice, can prevent FMD attenuation after a high-fat meal in middle-aged adults (which typically reduces FMD (Fewkes et al., 2022)) up to seven hours post-ingestion (Rendeiro et al., 2016). Therefore, it was important that the experimental chapters in this thesis did indeed control for FMD-enhancing substances such as flavonoids to prevent diet from becoming a confounding variable.

Firstly for **Chapters 4 and 5**, participants were told to refrain from eating at least 12 hours before the baseline to prevent any influence of substances such as caffeine, vitamin C and polyphenols (Thijssen et al., 2019). For Chapter 4, participants remained fasted throughout

each experimental visit before being provided with lunch once the final measures had been completed. However, it was considered due to the additional CV strain from HIIEx and from participant feedback in Chapter 4 to provide participants with breakfast for **Chapter 5.** The breakfast provided (food choice and quantity) was kept consistent for both experimental laboratory visits, and participants were given the options of toast with a small amount of spread (e.g. margarine) or cereal such as porridge.

To verify whether participants had completed the dietary restrictions, a 24-hour recall food diary was completed (Appendix J and K). Once the first 24-h food diary was completed, participants were asked to keep a record of the diary entry and to replicate the same diet 24-hours before the next laboratory visit. If participants had admitted on the form to breaking the morning fast (before entering the laboratory) or to have consumed alcohol or caffeine less than 12 hours before the laboratory visit, then their visit would be rescheduled. Fortunately, participants adhered to dietary restrictions for all sessions, as shown by the food diary results. Despite participants being encouraged to be consistent with their meals before an experimental visit, there will be some variation in participants' dietary intake (Appendix J, K, R and S). However, as the main nutritional factors that influence CV function have been controlled (e.g. caffeine intake, alcohol, vitamin C and polyphenols (Thijssen et al., 2019)), we do not believe diet is a confounding variable for **Chapters 4 and 5**.

Improved positive mood states within a young university population, which can experience high levels of anxiety and stress (Griffin, 2010), is beneficial for reducing CVD risk (Krittanawong et al., 2023). Assessing positive and negative mood states via an easy and

unburdensome mood state questionnaire is novel, with only a limited number of studies assessing mood states, (Malcolm et al., 2018, Caldwell et al., 2018) let alone the combination of mood states (positive and negative), thermal perception, and 24-hour sleep quality, all within the same study. However, mood states and sleep quality were considered secondary measures, meaning the inclusion criteria could have been stricter. For example, whilst anxiety, depressive states and sleep quality were assessed at the familiarisation stage, no participants were excluded from the study if they scored highly for insomnia, depressive symptoms or anxiety. Therefore, participants with a high score for the above could have impacted the results. However, we did not see this observation in **Chapter 4 or 5.**

FMD is an informative and non-invasive test to assess endothelial function (Chapter 3.3.1). However, operator variability is a big concern. Operators undergo training and use devices such as model arms to reduce operator variability to acceptable levels (Thijssen et al., 2019). Despite these preventative measures, operator variability still exists and can affect the FMD results. The COVID pandemic did result in considerable disruption of this PhD with a repeatability study completed by March 2020 (coefficient of variation from repeatability study one: 6.9%); section 3.3.2). To compensate for the two-year gap in FMD scanning (resumed March 2022), the operator completed additional training scans before starting data collection for **Chapter 4.** Therefore, by data collection for Chapter 4, the operator had conducted ~250 scans (90 scans post-March 2022). Consequently, we are confident that the FMD operator variability was within the recommended guidelines for research studies (Thijssen et al., 2019).

Two BP devices were used during Chapter 5. Unfortunately, a device connected to the hydrotherapy room's main power supply was not possible. Therefore, the Vicorder® (SMT Medical, Bristol, UK) BP device was used as it could be powered via the laptop. The Tango (M2; SunTechMedical Instruments Inc., United States) was used for all other measures. Therefore, there is the possibility that there could be a slight variation in the BP recordings between devices.

6.4 Perspectives and implications

Chapter 2 has shown that HT results in an acute CV response that increases FMD and lowers BP. Meanwhile, multiple bouts of HT led to lower resting BP across different population demographics and ages, effectively improving CV function. This demonstrates that HT is a strategy to attenuate CVD risk, specifically the main risk factor of increased BP. **Chapter 2** has identified heterogeneity across the HT literature and the need to standardise procedures, such as environmental conditions for post-intervention measures and highlights the need for more studies focusing on cardiometabolic health.

Chapter 4 has demonstrated that shorter-duration HT protocols with a T_{rec} below 38°C can induce a similar CV response to that of a longer, and less comfortable HT protocols such as HWI60. Specifically, HWI30 had a similar HR and BP response to HWI60, with fewer increases in negative mood states while also reported to be a preferable bathing condition. Therefore, HWI30 is a more time-efficient and pleasant HT protocol for participants to complete than the HWI60 protocol, which is the most reported within the literature. For HT to be effective in a public health setting, it must be pragmatic. Reducing the protocol to 30 minutes and lowering T_{rec} is potentially more likely to be adhered to and can provide an answer to the

CVD health crisis. These findings could demonstrate that HWI30 is a feasible intervention to reduce CVD risk in populations who struggle to meet the UK physical activity guidelines, such as those with peripheral arterial disease. However, future research will need to confirm this.

Chapter 5 investigated whether combining two strategies known to induce a CV response (HIIEx and HWI) can complement each other. ExHWI appeared to cause a more substantial hypotensive effect than HWI30 and HWI60 and had smaller increase in negative mood states than HWI60. This is promising as it demonstrates that ExHWI was well tolerated by young, healthy participants and the combination of HIIEx and HWI led to a robust hypotensive response. For those able to complete HIIEx and HWI, this could be an advantageous route to seek long-term improvements in CV function and attenuate CVD risk. However, future research is needed to confirm this.

6.5 Future directions

The thesis findings build upon the current literature by investigating the acute effect of different HWI strategies and combining all the HT literature into one large meta-analysis. The findings open several exciting further research opportunities.

- Based on the findings of **Chapters 4 and 5**, both studies should be repeated in groups at risk of CVD (e.g., hypertensives) and as intervention studies to investigate functional and long-term CV changes.
- Based on the findings of **Chapters 4 and 5**, both studies should be repeated in groups at risk of CVD (e.g., hypertensives) and as intervention studies to investigate

functional and long-term mood states and sleep quality changes. Additionally, whilst this study included males and females, previous research has demonstrated that HWI results in a different response in men compared to women (for example, women have smaller conduit arteries, which results in increased antegrade SR during 60 minutes of HWI (Larson et al., 2021)). Therefore, future studies should consider investigating potential sex differences with long-term HWI30 and ExHWI.

- Based on the findings from **Chapter 2**, more studies should incorporate (when possible) cardiometabolic markers to address the efficacy of whether HT can improve cardiometabolic health. Also, studies need to standardise the post-intervention environmental conditions when assessing measures such as BP and SR to help standardise results across the HT literature.

6.6 Closing comments

In summary, this thesis has demonstrated that HT can induce a robust CV response that leads to long-term reductions in resting BP across different participant health statuses. It has also shown that despite the popularity of HWI60 within the literature, shorter HT protocols such as HWI30 can result in a similar hypotensive response during and immediately after bathing in young, healthy adults. Additionally, HIIEx followed by HWI provides a more robust CV stimulus than HIIEx and TWI, alongside reduced BP immediately post-intervention. Whether this shorter HWI protocol or the combination of HIIEx and HWI are equally effective in different populations, or lead to functional and long-term structural CV function adaptations is yet to be determined.

6.7 Reflections of the researcher

Whilst every PhD thesis and journey is different, this is amplified by completing this journey as a self-funded student and completing a two-year leave of absence during the COVID pandemic. Here are some of the key lessons learned along the way:

- The COVID pandemic was at a critical moment during my PhD! Unfortunately, it meant we did not get to investigate the effects of hypertension and HT or move on to a short-term functional study. Catching up led to 7 days a week of testing for over two months for **Chapter 4** whilst commuting and completing teaching work. However, through perseverance, I have collected data for two human research studies, which is still a considerable achievement.
- The pandemic did lead to new opportunities. During my leave of absence, I attended the research-business seminar which inspired me to contact a local hot tub manufacturer. After a brief meeting (and following discussions with the university business team), I created a helpful industry link to my PhD. Fortunately, the company gave us a loan of a £5000 jacuzzi for my PhD which was a better solution than a bathtub on wheels! Therefore, "How can my PhD studies relate to industry partners?" is always worth asking.
- During the pandemic, I gained professional experience as a Career Coach at Warwick Business School. This was an excellent opportunity to work in a full-time professional services capacity, and it will help me find work post-PhD.
- Self-funding a PhD has been challenging, resulting in wearing many "hats." These have included postgraduate teaching work in the library, sports sciences, and as an

Academic Writing Lecturer for Coventry University. Consequently, I now have a full two-page CV, but with funding, I may have had an opportunity to replace these work experiences with more lab work. Therefore, self-funding has been rewarding but also challenging. However, through graft and determination, I have presented my research across the world, including to over 200 attendees in South Korea!

- Don't be afraid to reach out and network. After meeting Dr Hannah Pallubinsky at a conference in 2019, I reached out and was invited to present my research to her lab group at Maastricht University. It was a great experience to meet the team and to gain a different cardiometabolic research perspective. Additionally, after advertising my desire to undertake a Post Doc opportunity during my International Conference of Environmental Ergonomics presentation in South Korea, I did receive a lot of help and advice from fellow researchers.
- Learn to code! While I learned how to use programs such as Jamovi and Prism and new stats methods such as linear mixed models, R coding would have made my life much easier. By learning code, I will be less reliant on others and improve my co-authorship rate in future research publications.

I am proud to come from a white working-class family from Birmingham and to be the first to attend university. It has been truly special to be a PhD student at my local university and to achieve what others would never consider possible. Whilst it has been hard self-funding, it has been a fantastic experience to learn about an area I am passionate about with world-leading researchers. Thank you for reading this thesis and being a part of my PhD journey at one of the best sports science institutes in the world.

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APPENDIX

Appendix A. Consent forms for Chapters 4 and 5

UNIVERSITY OF BIRMINGHAM

School of Sport, Exercise and Rehabilitation Sciences
Participant Consent Form

Study Title: Effect of Hot Water Immersion on Cardiovascular Health
Principal Investigator: Dr Rebekah Lucas & Mr Ben Price

Participant Name & ID: _____

Date of Birth: _____

Please **INITIAL** each box below to indicate your agreement to the corresponding statement:

1. I have read the attached information sheet (code: ERN_19-1491 V0.5) and have discussed the investigation with the researcher, who has explained the procedures to my satisfaction. ☐
2. I am willing to undergo the investigation and understand that I am free to withdraw at any time without giving an explanation up to two weeks after my final laboratory visit. Doing so will not affect any treatment or care I may receive. ☐
3. I can confirm that I have not been treated for heat stroke, cardiovascular or metabolic conditions in the past. ☐
4. I understand that anonymised data collected during the study may be looked at by responsible individuals on request (i.e. other researchers) from the University of Birmingham. I give permission for these individuals to have access to my data and understand that this information will <u>not</u> include names or contact details and that this information will be kept strictly confidential. ☐
5. I understand that my digital data will be stored for a minimum of 10 years in accordance with University of Birmingham policies on password protected systems accessible only to research personnel associated with this study. I agree to this. ☐
6. I understand that my questionnaire data will be stored for a minimum of 10 years in accordance with University of Birmingham policies in a locked cabinet and only accessible to the research personnel associated with this study. I agree to this. ☐
7. I agree to participate in this study. ☐

Name of Participant (PRINT)	Date	Signature

Name of Researcher (PRINT)	Date	Signature

If you wish to receive the study results, please initial this box: ☐

UNIVERSITY OF BIRMINGHAM

School of Sport, Exercise and Rehabilitation Sciences
Participant Consent Form

Study Title: Effect of Exercise and Water Immersion on Cardiovascular Health
Principal Investigator: Dr Rebekah Lucas, Mr Ben Price & Dr Shane Balthazaar

Participant Name & ID: _____

Date of Birth: _____

Please **INITIAL** each box below to indicate your agreement to the corresponding statement:

1. I have read the attached information sheet (code: ERN_19-1491 V0.6) and have discussed the investigation with the researcher, who has explained the procedures to my satisfaction. ☐
2. I am willing to undergo the investigation and understand that I am free to withdraw at any time without giving an explanation up to two weeks after my final laboratory visit. Doing so will not affect any treatment or care I may receive. ☐
3. I can confirm that I have not been treated for heat stroke, cardiovascular or metabolic conditions in the past. ☐
4. I understand that anonymised data collected during the study may be looked at by responsible individuals on request (i.e. other researchers) from the University of Birmingham. I give permission for these individuals to have access to my data and understand that this information will not include names or contact details and that this information will be kept strictly confidential. ☐
5. I understand that my digital data will be stored for a minimum of 10 years in accordance with University of Birmingham policies on password protected systems accessible only to research personnel associated with this study. I agree to this. ☐
6. I understand that my questionnaire data will be stored for a minimum of 10 years in accordance with University of Birmingham policies in a locked cabinet and only accessible to the research personnel associated with this study. I agree to this. ☐
7. I agree to participate in this study. ☐

_____	_____	_____
Name of Participant (PRINT)	Date	Signature

Name of Researcher (PRINT)	Date	Signature

If you wish to receive the study results, please initial this box: ☐

Appendix B. General Health Questionnaire for Chapters 4 and 5

UNIVERSITY OF BIRMINGHAM

School of Sport, Exercise **Rehabilitation Sciences**

General Health Questionnaire

Name: .....
Address: .....
.....
.....
Phone: .....

Name of the responsible SportExR Staff member for your testing sessions

.....

Please answer the following questions. If you have any doubts or difficulty with the questions, please ask the SportExR Staff member for guidance. These questions are to determine whether the proposed exercise is appropriate for you. Your answers will be kept strictly confidential.

1.	You are.....	Male	Female
2.	What is your exact date of birth? Day..... Month.....Year..... So your age is..... Years		
3.	When did you last see your doctor? In the: Last week..... Last month..... Last six months..... Year..... More than a year.....		
4.	Are you currently taking any medication?	YES	NO
5.	Has your doctor ever said you have “heart trouble”?	YES	NO
6.	Has your doctor ever said you have low or high ?	YES	NO
7.	Have you ever taken medication for blood pressure or your heart?	YES	NO
8.	In the last month have you had pains in your chest when not doing any physical activity?	YES	NO
9.	Has your doctor (or anyone else) said that you have a raised blood cholesterol?	YES	NO
10.	Have you had a cold or feverish illness in the last month?	YES	NO
11.	Do you ever lose balance because of dizziness, or do you ever lose consciousness?	YES	NO
12.	Do you suffer from asthma?	YES	NO

13.	Do you have any joint or bone problems which may be made worse by exercise?	YES	NO
14.	Has your doctor ever said you have diabetes?	YES	NO
15.	Have you ever had heat stroke?	YES	NO
16.	Have you ever had viral hepatitis?	YES	NO
17.	If you are female, to your knowledge, are you pregnant?	YES	NO
18	I am allergic to Bromine (Bromine is a cleaning agent added to water, like chlorine in a public swimming pool).	YES	NO

I have completed the questionnaire to the best of my knowledge and any questions I had have been answered to my full satisfaction.

Signed: .....

Date: .....

Appendix C. Participant information sheets for Chapters 4 and 5

UNIVERSITY OF BIRMINGHAM

Participant Information Sheet

Study Title: Effect of Hot Water Immersion on Cardiovascular Health

An invitation to take part:
Thank you for taking the time to read this leaflet. We would like to invite you to take part in this study. Before you decide if you want to participate or not, it is important that you understand why the research is being done and what it will involve. Please take the time to read the following information carefully and discuss it with friends or relatives, if you wish. Please ask us if there is anything that is not clear or if you would like more information.

1. **What is the purpose of the study?**

Hot water bathing can improve cardiovascular health. For example, hot water bathing has been shown to reduce cardiovascular disease risk factors such as high blood pressure and arterial stiffness. With hot water bathing, blood flow to the limbs increases to help cool the body. This increases the force of blood flowing through arteries, which improves vascular function and can ultimately improve cardiovascular function/health. However, it is unclear how to optimally use hot water bathing to gain the greatest cardiovascular benefit. Therefore, the purpose of this study is to investigate different bathing conditions and their effect on cardiovascular function.

2. **Why have I been chosen?**

You have been chosen because you are:

- Aged between 18-50 years old
- Healthy
- Have no history of heat stroke, cardiovascular or metabolic disease
- Non-smoker and do not use e-cigarettes (vapes or disposable vapes)
- Have not visited a hot climate within the past six weeks or are a regular jacuzzi/sauna/steam room user

3. **Do I have to take part?**

No. Taking part in this study is entirely voluntary. If you would like to participate, you will be given this information sheet to keep and asked to sign a consent form, but you are still free to withdraw at any time and without giving a reason, for up to two weeks after your last

laboratory visit. You should feel under no pressure to participate and if at any time you are asked questions that you are not comfortable with answering (e.g. those asked in the General Health Questionnaire) you are free to not disclose this information. Though, please note that not answering some questions may mean you cannot participate. Please also bear in mind that all information collected will be kept strictly confidential. If you do decide to withdraw, any data collected relating to you will only be retained following your consent at the time of withdrawal.

4. **What will happen to me if I agree to take part?**

You will be invited to the School of Sport, Exercise and Rehabilitation Sciences at the University of Birmingham for an initial screening. An investigator will explain the nature of the procedures to you in detail and you will then be asked to sign a consent form before being asked to fill out a general health questionnaire. If you are female, you will be asked to monitor your menstrual cycle while you are enrolled in the study. You are encouraged to ask questions at any stage if there is anything you do not understand or feel uncomfortable with.

If all the above is fine, then your participation in the study starts and you will be booked in for the data collection trials.

Testing timeline

You will be asked to refrain from vigorous exercise 24 hours before and consuming caffeinated substances and alcohol 12 hours before the experimental trial. You will be asked to eat a similar meal the night before each experimental trial, and you will be expected to arrive fasted for the trial. At the end of the trial, you will be given lunch.

Familiarisation

First, you will be asked to visit the laboratory for a **familiarisation trial.** After arriving at the laboratory, you will rest for 20 minutes. After resting, the researchers will then use the ultrasound machine, and an oscillometer to take cardiovascular measurements at the neck, leg and arm. These vascular function measurements are non-invasive, and they are explained in detail below. After the cardiovascular measurements, will ask you to complete some questions about your mood. Then, we will take you to the hydrotherapy room and show you the hot tub. We will explain how to exit the bath safely and other safety procedures relating to the hot tub. This session will last no longer than 1 hour. Following this session, you will be asked to complete an online questionnaire measuring general wellbeing (e.g., anxiety, depression, fatigue) at home.

Experimental trials

You will be asked to perform **three experimental trials** on three separate occasions. Each experimental trial will be performed under different bathing conditions (duration and temperature).

- 30 minutes of bathing in warm water (approx. 34 °C), immersed to the shoulder.
- 30 minutes of bathing in hot water (approx. 41 °C), immersed to the shoulder.

- 20 – 30 minutes of bathing in hot water (approx. 41 °C), immersed up to the shoulder, followed by 30 – 40 minutes of bathing, immersed to the waist. The total will be 60 minutes of bathing.

These visits will take approximately 2.5-3 hours and are outlined in further detail below.

24-hours after an experimental trial, we will ask you to return to the laboratory to complete some questions about mood and sleep quality and to repeat the cardiovascular measurements. This visit will take approximately 1 hour.

The total time commitment of this study is approximately **12 hours.** There will be 4 trials in total, with at least 48 hours between each trial, but ideally no more than 4 weeks between sessions.

An overview of the protocol timeline is provided below (*Figure 1*).

Activity	Familiarisation	Rest	Experimental trial	Wash out	Experimental trial	Wash out	Experimental trial
Day	0	1-2	3	4-5	6	7-8	9

Figure 1: Study protocol timeline

5. **What do I have to do for the measurements made during the familiarisation and experimental visits?**

<u>Before the experimental visits</u>

Please refrain from vigorous exercise 24 hours prior to testing, alcohol, and caffeinated substances 12 hours prior to testing, as well as have a full night's sleep prior to testing. You will also be asked to record a food diary for up to 24 hours prior to your visit and to consume a similar meal the night before each experimental trial. Please drink plenty of water the day before a session and at least 500ml of water, the morning of the experimental trial. This is to ensure you are fully hydrated before starting the testing session. For the familiarisation session, you do not need to follow any of the restrictions above.

<u>Experimental tests</u>

These tests will take place in laboratory 217 and the hydrotherapy room, located within the SportExR laboratory building. A schematic of the experimental protocol is provided below (*Figure 2*).

When you arrive at the laboratory, we will measure your body weight and ask you to provide a urine sample as a measure of hydration. We will ask you to rest before a blood sample and cardiovascular and body temperature measures are taken. The researcher will ask you to complete some questions about mood and sleep quality. We will then fit you with equipment to measure body core temperature, as well as heart rate.

You will be taken to the hydrotherapy room, where you will complete a bathing condition lasting 30 – 60 minutes. While bathing, you will be asked to rate your feelings of thermal

comfort, sensation, and mood. We will also take your blood pressure at time points during the bathing condition. We will be constantly monitoring your body core temperature and heart rate throughout the trial. You may voluntarily withdraw and exit the hot water bath at any time during the session if you feel too hot or begin to feel faint. Please notify investigators if you feel faint, light-headed, have decreased vision, the sensation of hearing voices distantly, or feel nauseous. If these symptoms are persistent at any point of the study, you will be asked to withdraw from the study.

Immediately after you have finished bathing, we will take your blood pressure, then allow you to get changed. Once changed, we will take you to Laboratory 217 where you will rest and complete the same cardiovascular measures taken before bathing and we will take a second blood sample. One hour after bathing we will take a final cardiovascular measurement and ask you to rate your mood before giving you lunch. Later that evening, at dinner time and before bedtime, we will ask you to rate your mood. In the morning we will ask you to return to the laboratory in a fasted state. We will then conduct cardiovascular measurements and we will ask you to rate your mood and sleep quality.
This visit and the following morning assessments will take approximately 3.5 – 4 hours.

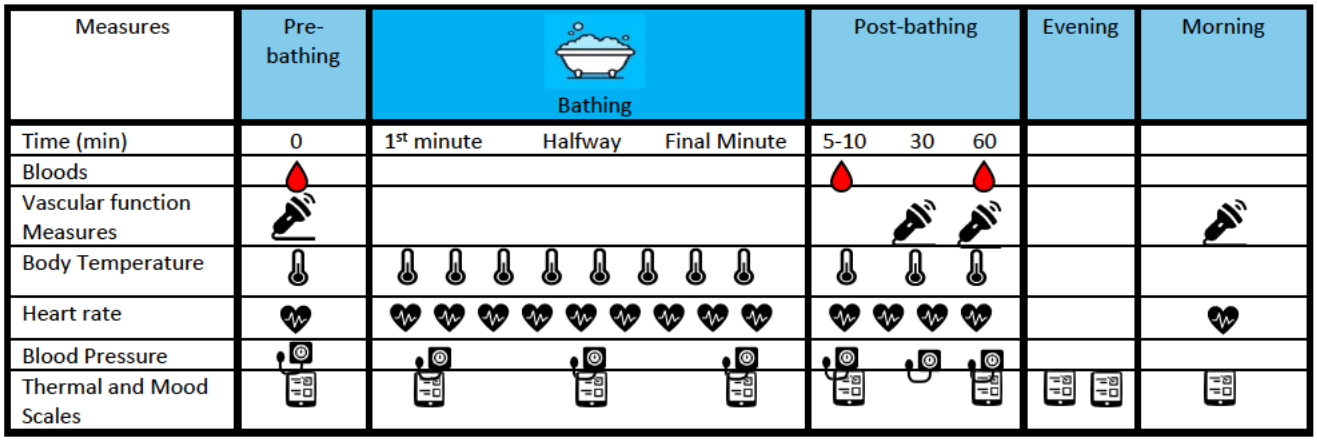


Figure 2: Experimental trial overview

Measures

The following measurements will be made:

Spot Urine Samples: You will be asked to urinate into a container from which we will immediately assess the osmolality and colour of your urine. Your urine sample will be discarded immediately

Venous Blood Samples: Cardiovascular and fluid regulatory markers will be measured from your blood samples. Your blood sample will be spun and stored for later analysis. All samples will be labelled with a key code (i.e., not directly identifiable to your name).

Body Core Temperature: Rectal temperature will be measured using a medical grade, flexible, sterile, and disposable thermistor. You will be instructed on how to insert your own rectal thermistor, to a depth of 10 cm. This procedure may cause slight discomfort initially but should not be painful.

Heart rate: will be measured via a heart rate monitor attached to the chest.

Sweat Volume: By measuring your nude body mass (in a private change room) before and after a session, as well as your fluid intake, we will be able to calculate the volume of sweat that you have produced during the session.

Thermal and Mood scales: Throughout the study, you will be asked to rate your thermal sensation and comfort as well as your mood and sleep quality using visual analogue scales and Likert scales.

Questionnaires: You will be asked to complete an online questionnaire to assess your wellbeing.

Vascular function will be measured by performing an ultrasound scan of the upper arm before and after occluding blood flow to the hand via a cuff placed around the forearm for 5 minutes. The second assessment will use a tonometer attached to your neck, leg and ankle to record the stiffness of your common carotid and ipsilateral common femoral arteries.

Blood pressure will be measured using a cuff wrapped around your upper arm to obtain blood pressure via a standard automated device.

6. **What are the possible disadvantages and risks of taking part?**

There are risks associated with hot water bathing. The General Health Questionnaire is a screening tool to minimize these risks. However, we feel you should be made aware of some of the following risks, as well as some of the things we are doing to minimise these risks:

- Fainting – often related to reduced orthostatic pressure in a supine position and then suddenly moving upright, this will be mitigated by moving slowly into an upright position whilst supervised by researchers. Please notify investigators if you feel faint, light-headed, have decreased vision, the sensation of hearing voices distantly, or feel nauseous. If these symptoms are persistent at any point of the study, you will be asked to withdraw from the study.

- Heat stress – Hot water bathing will increase your internal body temperature. To prevent you overheating to an unsafe level we will monitor your body core temperature closely. If at any stage you feel too uncomfortable with the heat then please let us know and we will stop the session, move you to a cool place and help you cool down. Please let us know in advance if you have had any previous issues with overheating.

- Drawing venous blood can cause some local pain and discomfort, and sometimes bruising and discoloration.

- There may be some initial discomfort associated with measurement of rectal temperature. It should however not be painful, and any initial feeling of discomfort should disappear immediately upon completion of insertion.

The investigators are experienced in performing all the procedures detailed. Investigators will observe you carefully throughout the study and you are encouraged to notify an investigator immediately if you have any worrisome symptoms in addition to those symptoms described above. In addition, if any of the tests show incidental findings related to your health (e.g. hypertension) we will inform you of this observation and ask your permission to inform your GP of this in writing.

7. **What are the possible benefits of taking part?**

You will receive information on your cardiovascular health (i.e. blood pressure). You will also help the researchers better understand the mechanisms underlying cardiovascular health and hot water bathing. If relevant, you can be awarded with **10 research hours** for completing this study.

8. **Will my taking part in this study be kept confidential?**

Yes, your participation in this study will be kept confidential. Data from this study will be managed in accordance with the University's Code of research Practice and the terms and conditions of the Data Protection Act 2018. Specifically, both hard copies and electronic data collected during the study will only be accessible to responsible employees from the University of Birmingham. In accordance with the University of Birmingham's data storage policy, all data will be stored securely for a minimum of 10 years. After this time, it will be permanently destroyed, provided it is of no further use to the academic world (if kept, it will remain stored securely and confidentially).

9. **What will happen to the results of the research study?**

The results of this study will form part of BSc and PhD degree projects and may be published in a scientific journal; however, names of participants will never be published. If you consent to it on the Consent Form, your anonymised data may be made available to other researchers on request.

10. **Who is organising and funding the research?**

The School of Sport, Exercise & Rehabilitation Sciences is funding the research. Dr Rebekah Lucas is organising the research as part of ongoing research studies.

11. **Can I obtain feedback from the study?**

Yes, if you wish to know the results of the study a summary of the results can be provided once the study has concluded. On the Consent Form there is a space to indicate if you would like to receive a study summary.

12. **Do you have any further questions or concerns?**

If you have any further questions about the study, please feel free to contact:

Mr Ben Price- [redacted]

Dr. Rebekah Lucas - [redacted] Tel: [redacted]

If you are at any time unhappy or have cause to complain about any aspect of your treatment during this study, and are uncomfortable approaching the research team, please contact Dr. Gareth Wallis, SportExR Head of Research – [redacted] Tel: +44 [redacted]

UNIVERSITY OF BIRMINGHAM

Participant Information Sheet

Study Title: Effect of Exercise and Water Immersion on Cardiovascular Health

An invitation to take part:

Thank you for taking the time to read this leaflet. We would like to invite you to take part in this study. Before you decide if you want to participate or not, it is important that you understand why the research is being done and what it will involve. Please take the time to read the following information carefully and discuss it with friends or relatives, if you wish. Please ask us if there is anything that is not clear or if you would like more information.

13. **What is the purpose of the study?**

Exercise is one of the most effective means of improving cardiovascular health and reducing/preventing cardiovascular disease. In particular, high-intensity interval training (HIIT) enhances cardiovascular function and cardiorespiratory fitness in less time with greater exercise adherence than continuous, moderate-intensity exercise training. Hot (e.g., 41 °C) water bathing mimics some of the key physiological mechanisms through which HIIT improves cardiovascular function. Subsequently, the use of hot water bathing as an alternative or complimentary treatment/conditioning strategy is of increasing interest. However, it is unclear how to optimally use hot water bathing to gain the greatest cardiovascular benefit. Therefore, the purpose of this study is to investigate cardiovascular responses and functional changes to HIIT, thermoneutral water immersion (TNWI,) and hot water immersion (HWI).

14. **Why have I been chosen?**

You have been chosen because you are:

- Aged between 18-50 years old
- Healthy and exercise regularly
- Have no history of heat stroke, cardiovascular or respiratory disease
- Non-smoker and do not use e-cigarettes (vapes or disposable vapes)
- Have not visited a hot climate within the past six weeks or are a regular jacuzzi/sauna/steam room user

15. **Do I have to take part?**

No. Taking part in this study is entirely voluntary. If you would like to participate, you will be given this information sheet to keep and asked to sign a consent form, but you are still free to withdraw at any time and without giving a reason, for up to two weeks after your last laboratory visit. You should feel under no pressure to participate and if at any time you are asked questions that you are not comfortable with answering (e.g. those asked in the General Health Questionnaire) you are free to not disclose this information. Though, please note that not answering some questions may mean you cannot participate. Please also bear in mind that all information collected will be kept strictly confidential. If you do decide to withdraw, any data collected relating to you will only be retained

following your consent at the time of withdrawal.

16. **What will happen to me if I agree to take part?**

You will be invited to the School of Sport, Exercise and Rehabilitation Sciences at the University of Birmingham for an initial screening. An investigator will explain the nature of the procedures to you in detail and you will then be asked to sign a consent form before being asked to fill out a general health questionnaire. If you are female, you will be asked to monitor your menstrual cycle while you are enrolled in the study. You are encouraged to ask questions at any stage if there is anything you do not understand or feel uncomfortable with.

If all of the above is fine, then your participation in the study starts and you will be booked in for the data collection trials.

Testing timeline

Familiarisation

Firstly, you will be asked to visit the laboratory for a **familiarisation trial.** After arriving at the laboratory, you will rest for 20 minutes. After resting, the researchers will then use ultrasound machines to take cardiovascular measurements at the arm and chest (heart level). These cardiovascular function measurements are non-invasive and are explained in detail below. After these measurements, will ask you to complete some questions about your mood. Then, we will take you to the hydrotherapy room and show you the hot tub. We will explain how to exit the bath safely and other safety procedures relating to the hot tub. This session will last no longer than **1 hour**. Following this session, you will be asked to complete an online questionnaire measuring general wellbeing (e.g., anxiety, depression, fatigue) at home.

VO2max test

You will be asked to visit the laboratory for a **maximal oxygen consumption (VO_{2max}) test.** After a warmup, you will begin your VO_{2max} test by exercising at a steady/easy pace. The exercise intensity will increase every minute, so that you progress from light intensity exercise to maximal effort. The test will finish when you reach volitional exhaustion and/or your exercise cadence can no longer be maintained. The VO_{2max} test will be followed by a 10-minute, self-paced cool down. This visit will take approximately **1 hour**.

Experimental trials

You will be asked to refrain from vigorous exercise 24 hours before and consuming caffeinated substances and alcohol 12 hours before the experimental trial. You will be asked to eat a similar meal the night before each experimental trial, and you will be expected to arrive fasted for the trial. At the end of the trial, you will be given lunch.

You will be asked to perform **two or three experimental trials** on separate occasions. Each experimental trial will involve a different combination of exercise and bathing conditions.

- 30 minutes of HIIT followed by 30 minutes of bathing in warm water (approx. 35 °C), immersed to the waist.
- 30 minutes of HIIT followed by 30 minutes of bathing in hot water (approx. 41 °C), immersed to the waist.
- 30 minutes of bathing in hot water (approx. 41 °C) immersed up to the shoulder, followed by 30 minutes of bathing immersed to the waist. The total will be 60 minutes of bathing.

These visits will take approximately **3.5 hours** and are outlined in further detail below.

The total time commitment of this study is approximately ***9.5-12.5 hours***. There will be 3-to-4 trials in total, with at least 48 hours between each trial, but ideally no more than 4 weeks between sessions.

17. **What do I have to do for the measurements made during the experimental visits (VO_{2max} test, pre- and post-intervention measures)?**

Before the experimental visits

Please refrain from vigorous exercise 24 hours prior to testing, alcohol, and caffeinated substances 12 hours prior to testing, as well as have a full night's sleep prior to testing. You will also be asked to record a food diary for up to 24 hours prior to your visit and to consume a similar meal the night before each experimental trial. Please drink plenty of water the day before a session and at least 500ml of water, the morning of the experimental trial. This is to ensure you are fully hydrated before starting the testing session. For the familiarisation session, you do not need to follow any of the restrictions above.

VO_{2max} Test

When you arrive at the laboratory, we will measure your body weight and then fit you with a chest strap heart rate monitor and a mouthpiece to measure your heart rate and respiratory gases during your VO_{2max} tests. Following a warmup, your VO_{2max} will begin. For the VO_{2max} test the intensity will increase gradually so that you move from light exercise to maximal effort. The test will finish when you reach volitional exhaustion. This test should last ~15-min. After the test (including a 5-min warm-down protocol), you will have the opportunity to ask any questions, be debriefed and be reminded of the protocol for future testing sessions.

Experimental tests

These tests will take place in laboratories located within the SportExR laboratory building.

When you arrive at the laboratory, we will measure your body weight and ask you to provide a urine sample as a measure of hydration. We will ask you to rest before imaging your heart and measuring your vascular function and body temperature. We will then ask you to complete some questions about your mood before taking a blood sample.

You will then undertake an exercise and bathing session (collectively lasting 60 minutes) or a bathing only session (lasting 60 minutes).

- The HIIT exercise session will involve a warmup and then repeated bouts of 1-min high intensity exercise (near or at maximal effort) followed by 4-min active recovery (slow cycling with no resistance). Total exercise time for this session will be 30 minutes.
- The 30-min bathing session will immediately follow the HIIT session and will involve sitting in a hot tub in water up to your waist for 30 minutes. The temperature of the water will be either warm (approx. 35 °C) or hot (approx. 41 °C).

- The 60-min bathing session will involve sitting in a hot tub in water up to your shoulders for 30 minutes and then up to your waist for another 30 minutes. The temperature of the water will be hot (approx. 41 °C).

While exercising or bathing, we will monitor your heart rate and body temperature. Periodically we

will take images of your heart and ask you to rate your feelings of thermal comfort, sensation, and mood. We will also take a capillary blood sample at the end of each session.

You may voluntarily withdraw and stop exercising or exit the hot water bath at any time during the session if you feel too hot or begin to feel faint. Please notify investigators if you feel faint, light-headed, have decreased vision, the sensation of hearing voices distantly, or feel nauseous. If these symptoms are persistent at any point during the study, you will be asked to withdraw from the study.

Immediately after you have finished bathing, we will take your blood pressure and image your heart, then allow you to get changed. Once changed, you will rest and repeat the same heart, vascular function and mood measures taken at the start of the experimental session. We will also take another blood sample. After completing testing, you will be given lunch.

Measures

The following measurements will be made:

- ***Oxygen Consumption (VO_2):*** During the VO_{2max} test, VO_2 and respiratory (***ventilation rate*** and ***volume***) variables will be measured using a breath-by-breath indirect calorimetry system (Vyntus, Carefusion).
- ***Spot Urine Samples***: You will be asked to urinate into a container from which we will immediately assess the osmolality and colour of your urine. Your urine sample will be discarded immediately.
- ***Venous Blood Samples***: Cardiovascular and fluid regulatory markers will be measured from your blood samples. Your blood sample will be spun and stored for later analysis. All samples will be labelled with a key code (i.e., not directly identifiable to your name).
- ***Finger Prick Samples:*** In order to measure changes in your blood and plasma volume, as well as your red blood cell count, we will take a small amount of blood via finger prick. These samples will be analysed immediately and then discarded.
- ***Body Core Temperature:*** Rectal temperature will be measured using a medical grade, flexible, sterile, and disposable thermistor. You will be instructed on how to insert your own rectal thermistor, to a depth of 10 cm. This procedure may cause slight discomfort initially but should not be painful.
- ***Forearm blood flow:*** Forearm blood flow will be measured using a method that measures the small changes of the size of your forearm when the blood leaving your arm is momentarily occluded. For this a cuff will be placed on your upper arm and around your wrist and inflated for less than 1 min before being rapidly deflated.
- ***Vascular function*** will be measured by performing an ultrasound scan of the upper arm before and after occluding blood flow to the hand via a cuff placed around the forearm for 5 minutes.
- ***Cardiac function:*** will be measured by performing an ultrasound scan of the heart before, during, and after your bathing session.
- ***Blood pressure*** will be measured using a cuff wrapped around your upper arm to obtain blood pressure via a standard automated device. In addition, blood pressure may be obtained continuously from a small cuff wrapped around one of your fingers.
- ***Heart rate*** will be measured via a heart rate monitor or a three-lead electrocardiogram (ECG).
- ***Sweat Volume:*** By measuring your nude body mass (in a private change room) before and after a

session, as well as your fluid intake, we will be able to calculate the volume of sweat that you have produced during the session.

Perceived Exertion, Thermal Sensation & Comfort, and Mood: Throughout the study, you will be asked to rate your level of perceived exertion on a scale of 6-20, ranging from very, very light up to maximal exertion. You will also be asked to rate your thermal sensation and comfort as well as your mood using visual analogue scales and Likert scales.

18. **What are the possible disadvantages and risks of taking part?**

There are risks associated with exercise. The General Health Questionnaire is a screening tool to minimize these risks, but exercise will be discontinued immediately if you feel distressed, unduly uncomfortable, wish to stop, or if we become concerned for your welfare. Please let us know if you have had any previous issues with exercise or heat illness.

Drawing venous blood can cause some local pain and discomfort, and sometimes bruising and discoloration. Finger pricking can also cause a small amount of local pain.

There may be some initial discomfort associated with measurement of rectal temperature. It should however not be painful, and any initial feeling of discomfort should disappear immediately upon completion of insertion.

Some of the bathing conditions will be hot and may cause discomfort. To prevent you overheating to an unsafe level we will monitor your body core temperature closely. Please notify investigators if you feel faint, light-headed, have decreased vision, the sensation of hearing voices distantly, or feel nauseous. If these symptoms are persistent at any point of the study, you will be asked to withdraw from the study. There is some risk of fainting as a result of hot temperatures. If you faint at any point of the study, you will be asked to withdraw from the study.

The investigators are experienced in performing all the procedures detailed with many similar sessions completed safely in the recent past. Investigators will observe you carefully throughout the study and you are encouraged to notify an investigator immediately if you have any worrisome symptoms in addition to those symptoms described above. In addition, if any of the tests show incidental findings related to your health (e.g. high resting blood pressure) we will inform you of this observation and ask your permission to inform your GP of this in writing.

19. **What are the possible benefits of taking part?**

You will receive an assessment of your aerobic capacity (i.e., your VO_{2max}). You will also receive information on your cardiovascular health (i.e. blood pressure) and help the researchers better understand the mechanisms underlying cardiovascular health and hot water bathing. If relevant, you can be awarded **10 research hours** for completing this study.

20. **Will my taking part in this study be kept confidential?**

Yes, your participation in this study will be kept confidential. Data from this study will be managed in accordance with the University's Code of research Practice and the terms and conditions of the Data Protection Act 2018. Specifically, both hard copies and electronic data collected during the study will only be accessible to responsible employees from the University of Birmingham. In accordance with the University of Birmingham's data storage policy, all data will be stored securely for a minimum of 10 years. After this time, it will be permanently destroyed, provided it is of no further use to the

academic world (if kept, it will remain stored securely and confidentially).

21. **What will happen to the results of the research study?**

The results of this study will form part of BSc, MSc and PhD degree projects and may be published in a scientific journal; however, names of participants will never be published. If you consent to it on the Consent Form, your anonymised data may be made available to other researchers on request.

22. **Who is organising and funding the research?**

The School of Sport, Exercise & Rehabilitation Sciences is funding the research. Dr Rebekah Lucas is organising the research as part of ongoing research studies.

23. **Can I obtain feedback from the study?**

Yes, if you wish to know the results of the study a summary of the results can be provided once the study has concluded. On the Consent Form, there is a space to indicate if you would like to receive a study summary.

24. **Do you have any further questions or concerns?**

If you have any further questions about the study please feel free to contact:

Mr. Ben Price [redacted]

Dr. Shane Balthazaar [redacted]

Dr. Rebekah Lucas [redacted] Tel: [redacted]

If you are at any time unhappy or have cause to complain about any aspect of your treatment during this study, and are uncomfortable approaching the research team, please contact Dr. Gareth Wallis, SportExR Head of Research – [redacted] Tel: +44 (0)121 414 4129.

Appendix D. Participant information sheet and consent form for the repeatability study

School of Sport, Exercise and Rehabilitation Sciences

Participant Consent Form

Study Title: Repeatability study to assess intra- and inter-day variability in ultrasound based vascular measures

Investigators: Dr Catarina Rendeiro, Dr Samuel Lucas, Dr Rebekah Lucas. Bethany Skinner, Alessio Daniele

Participant Name : _____

Telephone Number: _____ Date of Birth: _____

Initial each box

1. I have read the study information sheet and have discussed the experiment with one of the above named investigators, who have explained the procedures to my satisfaction. ☐

2. I understand that I am volunteering to participate in the experiment by my choice and that I may stop and withdraw from the experiment at any time. I understand that I can choose to participate in either one or both parts of the study. ☐

    a. I am volunteering for the Repeatability study arm (3 sessions) (Initial box to select this study) ☐

    b. I am volunteering for the Repeatability study and the Proof of Concept study arms (4 sessions) (Initial box to select this study) ☐

3. I confirm that I have not been treated for any cardiovascular, metabolic, neurological or respiratory conditions in the past. ☐

4. I understand that the data collected during the study may be looked at by responsible individuals from the University of Birmingham where it is relevant to my taking part in this research. I give permission for these individuals to have access to my data and understand that any information will be kept strictly confidential. ☐

5. I understand that my digital data will be stored for a minimum of 10 years in accordance with University of Birmingham policies on password protected systems accessible only to research personnel associated with this study. I agree to this. ☐

6. I understand that my questionnaire data will be stored for a minimum of 10 years in accordance with University of Birmingham policies in a locked cabinet only accessible to the research personnel associated with this study. I agree to this. ☐

7. I would like to receive a summary of the study findings Yes / No (circle your response)

8. I understand that the principal investigator of this study will inform me of any incidental finding from the tests/measures that I will be completing, and will ask my permission to inform my GP of this. I give permission for this. GP contact details: _____ ☐

9. I consent to be contacted by researchers at the University of Birmingham for future studies ☐

10. I agree to participate in this study. ☐

11. I understand that I can withdraw from the study at any time up to 2 weeks post completing, and I understand that the data collected will be removed unless I give permission for my data to be used. ☐

For cases of withdrawal: I give permission for my data to be used even though I am withdrawing from the study.

Signed: _____

Date:_____

_____	_____	_____
Name of Participant (PRINT)	Date	Signature
_____	_____	_____
Name of Researcher (PRINT)	Date	Signature

UNIVERSITY OF BIRMINGHAM

Participant Information Sheet

Study Title: Repeatability study to assess intra- and inter-day variability in ultrasound based vascular measures

Investigators:
Dr Catarina Rendeiro; *Tel* [redacted], *Email*: [redacted]
Dr. Rebekah Lucas; Tel: [redacted] *Email:* [redacted]
Dr Samuel Lucas; *Tel*: [redacted] *Email:* [redacted]

An invitation to take part:
Thank you for taking the time to read this leaflet. We would like to invite you to take part in this study. Before you decide if you want to participate or not, it is important that you understand why the research is being done and what it will involve. Please take the time to read the following information carefully and discuss it with friends or relatives, if you wish. Please ask us if there is anything that is not clear or if you would like more information.

Technical terminology:

- FMD (flow mediated dilation): a technique that temporarily reduces blood flow to the end of the arm/leg by using an occlusion cuff to allow us to test the elasticity of arteries that feed your arms (brachial) and your legs (femoral or popliteal).

Total Time commitment:
The total time commitment for this study is 6 hours (3 sessions) for the repeatability study arm and 4 hours for proof-of-concept study arm (1 session). You will have the option of only participating in the repeatability study arm if you wish or additionally participate also on the proof of concept study arm.

What is the purpose of the study?
FMD and blood flow measurements are valuable indicators of the vascular health of an individual. However, the accuracy of these measurements is highly dependent on extensive training of the operator. As such, it is fundamental that variability of these measures is established before meaningful vascular measures can be obtained. The aim of this project is to i) firstly determine the variability in the measurements within the same day and between different days in healthy adults (3 sessions, 2 hours each) and ii) test whether we can detect the impact of 1, 2 and 4 hours of sitting time on the elasticity of arm and leg arteries, using these same techniques.

Why have I been chosen?

You have been chosen because you are:

- Aged between 18 and 45
- Healthy
- Have no history of cerebrovascular, cardiovascular or respiratory disease

1. Do I have to take part?

No. Taking part in this study is entirely voluntary. If you would like to participate, you will be given this information sheet to keep and be asked to sign a consent form, but you are still free to withdraw at any time and without giving a reason for up to two weeks after your last laboratory visit. You should feel under no pressure to participate. Please also bear in mind that all information collected will be kept strictly confidential.

2. What will happen to me if I agree to take part?

You will be invited to the School of Sport, Exercise and Rehabilitation Sciences at the University of Birmingham for 3 or 4 occasions. An investigator will explain the nature of the procedures to you in detail. You are encouraged

to ask questions prior to and throughout the study protocol if there is anything you do not understand or feel comfortable with. You will then be asked to sign a consent form. You will also have your height and weight measured. Following provision of informed consent, you will be familiarised with experimental methods.

What do I have to do for the measurements made during the experimental visit?

Before the experimental visit

You will be asked to refrain from exercising and alcohol for 24 h prior to the visit and avoid caffeine for at least 6 hours prior to the session. All the sessions will be performed at the same time of the day.

During Experimental Visits

Repeatability study Arm: During each study visit, you will lay down in supine position 20 min before ultrasound measurements are initiated. Whilst in supine position, FMD and/or Carotid/Internal Carotid Blood Flow will be measured at baseline (0 h) and exactly 1 hour later. The same procedures will be repeated in Sessions 2 and 3 to assess variability of the measurement within the same day and across different days.

Proof-of-concept Arm: In Session 4, you will undergo similar procedures at Baseline (0 h) and 1, 2 and 4 h. During this time you will remain seated, unless vascular measurements are taking place, in which case you will be in supine position. If during this 4 hour period you need to use the toilet, you will have to do so whilst seated. The researcher will take you to the toilet in a wheel chair so you do not have to stand up at any point.

During all the tests, the following measurements will be made:

- **Brachial/femoral/popliteal blood flow and Flow-mediated dilatation (FMD) using Duplex Doppler:** Blood flow in the brachial artery (upper arm) and the femoral or popliteal artery (upper leg, only one of the leg arteries will be assessed, not both) will be assessed. This consists of placing one ultrasound probe on your right, upper, inner arm or upper leg. Ultrasound gel will be placed between the probe and your skin to obtain the highest quality images. A mechanical arm will be used to hold the probe in place during the testing to keep it in the same place throughout. A cuff will be placed on the lower part of your right arm/ right leg during FMD to occlude blood flow for a total of 5 minutes. The total time of the measurement lasts 11 minutes. In order to access the brachial artery (in your arm) and femoral artery (in your leg) we will advise you to bring with you a t-shirt and shorts that you can wear whilst in the laboratory.
- **Carotid/Internal Carotid Blood Flow using Duplex Doppler:** This consists of placing one ultrasound probe on the neck area to locate both the carotid and internal carotid. A short 4 min measurement will take place for to record blood velocity and diameter of the arteries.

3. What are the possible disadvantages and risks of taking part?

You might feel a small discomfort in your arm and/or leg when the blood pressure cuff is occluded during the Flow mediated Dilatation (FMD) measurements. This is a temporary sensation that will go away after a few seconds of the cuff release.

The investigators are experienced in performing all the procedures detailed with hundreds of similar sessions completed safely in the recent past. Investigators will observe you carefully throughout the study and you are encouraged to notify an investigator immediately if you have any worrisome symptoms in addition to those symptoms described above.

4. What are the possible benefits of taking part?

SportExR undergraduates who participate in this study will also receive hours credit towards their research hour requirement for each data collection session they attend.

At your request, you will find out about your blood pressure and peripheral and extra-cranial blood flow responses.
In the event of any incidental findings we will advise you to visit your GP and will contact your GP directly to provide any necessary information with your consent.

5. Will my taking part in this study be kept confidential?

Yes, your participation in this study will be kept confidential. In accordance with the Data Protection Act 2018 (DPA 2018/ GDPR) all physical data (questionnaires, consent forms, manual entry forms) will be stored in a locked cabinet in a locked room that only researchers will have access to. Electronic data will be stored on password protected computers, both of which will only be accessible by researchers named on this information sheet. In accordance with the University of Birmingham's data protection guidelines all data will be stored securely for a minimum of 10 years after which it will be permanently destroyed.

6. What will happen to the results of the research study?

The results of this study are expected to be published anonymously in a scientific journal and within an MSc/PhD thesis; however, names of participants will never be published.

7. Who is organising and funding the research?

The School of Sport, Exercise & Rehabilitation Sciences is funding the research. Dr Catarina Rendeiro, Dr Rebekah Lucas and Dr Samuel Lucas are organising the research as part of ongoing research studies.

8. What will happen if I wish to withdraw from the study?

You are free to withdraw from the study at any time, including following data collection, without giving a reason. If the data collected until the time of withdrawal could be used, you will specifically be asked to give your consent to having the data included in any analysis. Additionally, you can withdraw your data from the study for up to two weeks following completion of the data collection, by notifying us via email or telephone. If you withdraw, the data collected to date cannot be erased but it will not be used in any data analysis or publications. Finally, if you took part in this study for research hours, you will receive the amount of hours you completed.

9. Can I obtain feedback from the study?

Yes, if you wish to know the results of the study a summary of the results can be provided once the study has concluded. On the Consent Form, there is a space to indicate if you would like to receive a study summary.

10. Do you have any further questions or concerns?

If you have any further questions about the study please feel free to contact:

Dr. Catarina Rendeiro: [redacted] Tel: [redacted] -
Dr. Rebekah Lucas: [redacted] Tel: [redacted]
Dr. Samuel Lucas: [redacted] Tel: [redacted]

Appendix E. Mood State Questionnaire from Liao et al. (2017b). Please note that this questionnaire was completed using an online cloud based questionnaire application (Smart Survey, Smartline International Ltd, UK (https://www.smartsurvey.co.uk/company/about-us)

How happy are you feeling right now?

1. Not at all
2. A little
3. Moderately
4. Quite a bit
5. Extremely

How cheerful are you feeling right now?

1. Not at all
2. A little
3. Moderately
4. Quite a bit
5. Extremely

How calm or relaxed are you feeling right now?

1. Not at all
2. A little
3. Moderately
4. Quite a bit
5. Extremely

How stressed are you feeling right now?

1. Not at all
2. A little
3. Moderately
4. Quite a bit
5. Extremely

How frustrated are you feeling right now?

1. Not at all
2. A little
3. Moderately
4. Quite a bit
5. Extremely

How tense or anxious are you feeling right now?

1. Not at all
2. A little
3. Moderately
4. Quite a bit

5. Extremely

How sad or depressed are you feeling right now?

1. Not at all
2. A little
3. Moderately
4. Quite a bit
5. Extremely

How energetic or full of pep are you feeling right now?

1. Not at all
2. A little
3. Moderately
4. Quite a bit
5. Extremely

Appendix F. Groningen Sleep Quality Scale from Meijman et al. (1990). Please note that this questionnaire was completed using an online cloud based questionnaire application (Smart Survey, Smartline International Ltd, UK (https://www.smartsurvey.co.uk/company/about-us)

I had a deep sleep last night
1. True
2. False

I feel like I slept poorly last night
1. True
2. False

It took me more than an hour to fall asleep
1. True
2. False

I felt tired after waking this morning
1. True
2. False

I woke up several times last night
1. True
2. False

I feel like I didn't get enough sleep last night
1. True
2. False

I got up in the middle of the night
1. True
2. False

I felt rested after waking up this morning
1. True
2. False

I feel like I only had a couple of hours of sleep
1. True
2. False

I feel I slept well last night
1. True
2. False

I didn't sleep a wink last night

1. True
2. False

I didn’t have any trouble falling asleep last night

1. True
2. False

After I woke up last night, I had trouble falling asleep

1. True
2. False

I tossed and turned all night last night

1. True
2. False

I didn’t get more than 5 hours sleep last night

1. True
2. False

Appendix G. Familiarisation questionnaires for Chapters 4 and 5. Please note that these questionnaires were completed using an online cloud based questionnaire application (Smart Survey, Smartline International Ltd, UK (https://www.smartsurvey.co.uk/company/about-us)

Hollands Sleep Disorder Questionnaire from Mulder-Hajonides Van Der Meulen (1981)

Do you have trouble falling asleep?

1. Never
2. Rarely
3. Occasionally
4. Most nights/days
5. Always

Do you have trouble staying asleep?

1. Never
2. Rarely
3. Occasionally
4. Most nights/days
5. Always

Do you take anything to help you sleep?

1. Never
2. Rarely
3. Occasionally
4. Most nights/days
5. Always

Do you use alcohol to help you sleep?

1. Never
2. Rarely
3. Occasionally
4. Most nights/days
5. Always

Do you have any medical conditions that disrupt your sleep?

1. Never
2. Rarely
3. Occasionally
4. Most nights/days
5. Always

Have you lost interest in hobbies or activities?

1. Never
2. Rarely
3. Occasionally

4. Most nights/days
5. Always

Do you feel sad, irritable or hopeless?

1. Never
2. Rarely
3. Occasionally
4. Most nights/days
5. Always

Do you have trouble falling asleep?

1. Never
2. Rarely
3. Occasionally
4. Most nights/days
5. Always

Do you feel nervous or worried?

1. Never
2. Rarely
3. Occasionally
4. Most nights/days
5. Always

Do you think something is wrong with your body?

1. Never
2. Rarely
3. Occasionally
4. Most nights/days
5. Always

Are you a shift worker or is your regular sleep schedule irregular?

1. Never
2. Rarely
3. Occasionally
4. Most nights/days
5. Always

Are you legs restless and/or uncomfortable before bed?

1. Never
2. Rarely
3. Occasionally
4. Most nights/days
5. Always

Have you been told that you are restless or that you kick your legs in your sleep?

1. Never
2. Rarely
3. Occasionally
4. Most nights/days
5. Always

Do you have any unusual behaviours or movements during sleep?

1. Never
2. Rarely
3. Occasionally
4. Most nights/days
5. Always

Do you snore?

1. Never
2. Rarely
3. Occasionally
4. Most nights/days
5. Always

Has anyone said that you stop breathing, gasp, snort, or choke in your sleep?

1. Never
2. Rarely
3. Occasionally
4. Most nights/days
5. Always

Do you have difficulty staying awake during the day?

1. Never
2. Rarely
3. Occasionally
4. Most nights/days
5. Always

Pittsburgh Sleep Quality Index from Buysse et al. (1989)

Instructions: The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.
1. During the past month, what time have you usually gone to bed at night?
2. During the past month, how long (in minutes) has it usually taken you to fall asleep each night?
3. During the past month, what time have you usually gotten up in the morning?
4. During the past month, how many hours of actual sleep did you get at night? (This may be different than the number of hours you spent in bed.)

During the past month, how often have you had trouble sleeping because you…

	1. Not during the past month	2. Less than once a week	3. Once or twice a week	4. Three or more times a week
a. Cannot get to sleep within 30 minutes	◯	◯	◯	◯
b. Wake up in the middle of the night or early morning	◯	◯	◯	◯
c. Have to get up to use the bathroom	◯	◯	◯	◯
d. Cannot breathe comfortably	◯	◯	◯	◯
e. Cough or snore loudly	◯	◯	◯	◯
f. Feel too cold	◯	◯	◯	◯
g. Feel too hot	◯	◯	◯	◯
h. Have bad dreams	◯	◯	◯	◯
i. Have pain	◯	◯	◯	◯
j. Other reason(s), please describe:	◯	◯	◯	◯

j. Other reason(s) - state here

During the past month, how often have you taken medicine to help you sleep (prescribed or “over the counter”)?
1. Not during the past month
2. Less than once a week
3. Once or twice a week
4. Three or more times a week

During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?
1. Not during the past month
2. Less than once a week
3. Once or twice a week
4. Three or more times a week

During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?
1. Not during the past month
2. Less than once a week
3. Once or twice a week
4. Three or more times a week

During the past month, how would you rate your sleep quality overall?
1. Not during the past month
2. Less than once a week
3. Once or twice a week
4. Three or more times a week

Do you have a bed partner or roommate?
1. Not during the past month
2. Less than once a week
3. Once or twice a week
4. Three or more times a week

During the past month, how often have you taken medicine to help you sleep (prescribed or "over the counter")?
1. Not during the past month
2. Less than once a week
3. Once or twice a week
4. Three or more times a week

During the past month, how often have you taken medicine to help you sleep (prescribed or "over the counter")?
1. Not during the past month
2. Less than once a week
3. Once or twice a week
4. Three or more times a week

During the past month, how often have you taken medicine to help you sleep (prescribed or "over the counter")?
1. Not during the past month
2. Less than once a week
3. Once or twice a week
4. Three or more times a week

During the past month, how often have you taken medicine to help you sleep (prescribed or "over the counter")?
1. Not during the past month
2. Less than once a week
3. Once or twice a week
4. Three or more times a week

If you have a room mate or bed partner, ask him/her how often in the past month you have had:

	1. Not during the past month	2. Less than once a week	3. Once or twice a week	4. Three or more times a week
a. Loud Snoring	◯	◯	◯	◯
b. Long pauses between breaths while sleeping	◯	◯	◯	◯
c. Legs twitching or jerking while you sleep	◯	◯	◯	◯
d. Episodes of disorientation or confusion during sleep	◯	◯	◯	◯
e. Other restlessness while you sleep, please describe:	◯	◯	◯	◯

Perceived Stress Scale from Cohen et al. (1994)

How often have you been upset because of something that happened unexpectedly?

0. Never
1. Almost Never
2. Sometimes
3. Fairly Often
4. Very Often

How often have you felt that you were unable to control the important things in your life

0. Never
1. Almost Never
2. Sometimes
3. Fairly Often
4. Very Often

How often have you felt nervous and “stressed”

0. Never
1. Almost Never
2. Sometimes
3. Fairly Often
4. Very Often

How often have you felt confident about you ability to handle your personal problems

0. Never
1. Almost Never
2. Sometimes
3. Fairly Often
4. Very Often

How often have you felt that things were going your way

0. Never
1. Almost Never
2. Sometimes
3. Fairly Often
4. Very Often

How often have you found out that you could not cope with all the things that you had to do

0. Never
1. Almost Never
2. Sometimes
3. Fairly Often
4. Very Often

How often have you been able to control irritations in your life

0. Never
1. Almost Never

2. Sometimes
3. Fairly Often
4. Very Often

How often have you felt that you were on top of things

0. Never
1. Almost Never
2. Sometimes
3. Fairly Often
4. Very Often

How often have you been angered because of things that were outside of your control

0. Never
1. Almost Never
2. Sometimes
3. Fairly Often
4. Very Often

How often have you felt difficulties were pilling up so high that you could not overcome them

0. Never
1. Almost Never
2. Sometimes
3. Fairly Often
4. Very Often

Hospital Anxiety and Disorder Scale from Zigmond and Snaith (1983)

I feel tense or wound up

3. Most of the time
2. A lot of the time
1. From time to time, occasionally
0. Not at all

I still enjoy the things I used to enjoy

0. Definitely not as much
1. Not quite so much
2. Only a little
3. Hardly at all

I get a sort of frightened feeling as if something awful is going to happen

3. Very definitively and quite badly
2. Yes, but not too badly
1. A little, but it doesn't worry me
0. Not at all

I can laugh and see the funny side of things

0. As much as I always could
1. Not quite so much now
2. Definitely not so much now
3. Not at all

Worrying thoughts go through my head

3. A great deal of the time
2. A lot of the time
1. From time to time, but not too often
0. Only occasionally

I can sit at ease and feel relaxed

0. Definitely
1. Usually
2. Not often
3. Not at all

I feel as if I am slowed down

3. Nearly all of the time
2. Very often
1. Sometimes
0. Not at all

I get a sort of frightened feeling like “butterflies” in the stomach

0. Not at all
1. Occasionally
2. Quite often
3. Very often

I have lost interest in my appearance

3. Definitely
2. I don’t take as much care as I should
1. I may not take quite as much care
0. I take just as much care as ever

I feel restless as I have to be on the move

3. Very much indeed
2. Quite a lot
1. Not very much
0. Not at all

I look forward with enjoyment to things

0. As much as I ever did
1. Rather less than I used to
2. Definitely less than I used to
3. Hardly at all

I get sudden feelings of panic

3. Very often indeed
2. Quite often
1. Not very often
0. Not at all

I can enjoy a good book or radio or TV program

0. Often
1. Sometimes
2. Not often
3. Very seldom

International Physical Activity Questionnaire from Booth (2000)

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE
We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the last 7 days. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport. Think about all the vigorous and moderate activities that you did in the last 7 days. Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY
The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?
Yes
No Skip to PART 2: TRANSPORTATION

The next questions are about all the physical activity you did in the last 7 days as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging, heavy construction, or climbing up stairs as part of your work? Think about only those physical activities that you did for at least 10 minutes at a time.
_____ days per week

No vigorous job-related physical activity Skip to question 4

3. How much time did you usually spend on one of those days doing vigorous physical activities as part of your work?
_____ hours per day
_____ minutes per day

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate physical activities like carrying light loads as part of your work? Please do not include walking.
_____ days per week

No moderate job-related physical activity Skip to question 6

5. How much time did you usually spend on one of those days doing moderate physical activities as part of your work?

_____ hours per day
_____ minutes per day

6. During the last 7 days, on how many days did you walk for at least 10 minutes at a time as part of your work? Please do not count any walking you did to travel to or from work.
_____ days per week

No job-related walking Skip to PART 2: TRANSPORTATION

7. How much time did you usually spend on one of those days walking as part of your work?
_____ hours per day
_____ minutes per day

PART 2: TRANSPORTATION PHYSICAL ACTIVITY
These questions are about how you travelled from place to place, including to places like work, stores, movies, and so on.

8. During the last 7 days, on how many days did you travel in a motor vehicle like a train, bus, car, or tram?
_____ days per week

No traveling in a motor vehicle Skip to question 10
9. How much time did you usually spend on one of those days traveling in a train, bus, car, tram, or other kind of motor vehicle?
_____ hours per day
_____ minutes per day

Now think only about the bicycling and walking you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the last 7 days, on how many days did you bicycle for at least 10 minutes at a time to go from place to place?
_____ days per week

No bicycling from place to place Skip to question 12

11. How much time did you usually spend on one of those days to bicycle from place to place?
_____ hours per day
_____ minutes per day

12. During the last 7 days, on how many days did you walk for at least 10 minutes at a time to go from place to place?
_____ days per week

No walking from place to place Skip to PART 3: HOUSEWORK,

HOUSE MAINTENANCE, ANDCARING FOR FAMILY
13. How much time did you usually spend on one of those days walking from place to place?
_____ hours per day
_____ minutes per day

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY
This section is about some of the physical activities you might have done in the last 7 days in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, chopping wood, shoveling snow, or digging in the garden or yard?
_____ days per week

No vigorous activity in garden or yard Skip to question 16

15. How much time did you usually spend on one of those days doing vigorous physical activities in the garden or yard?
_____ hours per day
_____ minutes per day

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate activities like carrying light loads, sweeping, washing windows, and raking in the garden or yard?
_____ days per week

No moderate activity in garden or yard Skip to question 18

17. How much time did you usually spend on one of those days doing moderate physical activities in the garden or yard?
_____ hours per day
_____ minutes per day

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate activities like carrying light loads, washing windows, scrubbing floors and sweeping inside your home?
_____ days per week

No moderate activity inside home Skip to PART 4: RECREATION,

SPORT AND LEISURE-TIME PHYSICAL ACTIVITY
19. How much time did you usually spend on one of those days doing moderate physical activities inside your home?
_____ hours per day
_____ minutes per day

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY
This section is about all the physical activities that you did in the last 7 days solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the last 7 days, on how many days did you walk for at least 10 minutes at a time in your leisure time?
_____ days per week

No walking in leisure time Skip to question 22

21. How much time did you usually spend on one of those days walking in your leisure time?
_____ hours per day
_____ minutes per day

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do vigorous physical activities like aerobics, running, fast bicycling, or fast swimming in your leisure time?
_____ days per week

No vigorous activity in leisure time Skip to question 24

23. How much time did you usually spend on one of those days doing vigorous physical activities in your leisure time?
_____ hours per day
_____ minutes per day

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis in your leisure time?
_____ days per week

No moderate activity in leisure time Skip to PART 5: TIME SPENT SITTING

25. How much time did you usually spend on one of those days doing moderate physical activities in your leisure time?
_____ hours per day
_____ minutes per day

PART 5: TIME SPENT SITTING
The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the last 7 days, how much time did you usually spend sitting on a weekday?
_____ hours per day
_____ minutes per day

27. During the last 7 days, how much time did you usually spend sitting on a weekend day?
_____ hours per day
_____ minutes per day

This is the end of the questionnaire; thank you for participating.

Appendix H. Bathing Preference Questionnaire for Chapter 4. Please note that this questionnaire was completed using an online cloud based questionnaire application (Smart Survey, Smartline International Ltd, UK (https://www.smartsurvey.co.uk/company/about-us)

This questionnaire is designed to assess your enjoyment of your recent bathing exposures. Please select your answer and add comments when prompted.

Bathing conditions were as follows
Condition 1 – 30 minutes bathing at 34°C
Condition 2 – 30 minutes bathing at 41°C
Condition 3 – 60 minutes bathing at 41°C

Which condition was your favourite bathing experience and why?
Condition 1 – 30 minutes bathing at 34°C
Condition 2 – 30 minutes bathing at 41°C
Condition 3 – 60 minutes bathing at 41°C

Comments:

Which condition was your least favourite bathing experience and why?
Condition 1 – 30 minutes bathing at 34°C
Condition 2 – 30 minutes bathing at 41°C
Condition 3 – 60 minutes bathing at 41°C

Comments:

Out of the three conditions, which would you undertake again? Choose all that are applicable
Condition 1 – 30 minutes bathing at 34°C
Condition 2 – 30 minutes bathing at 41°C
Condition 3 – 60 minutes bathing at 41°C

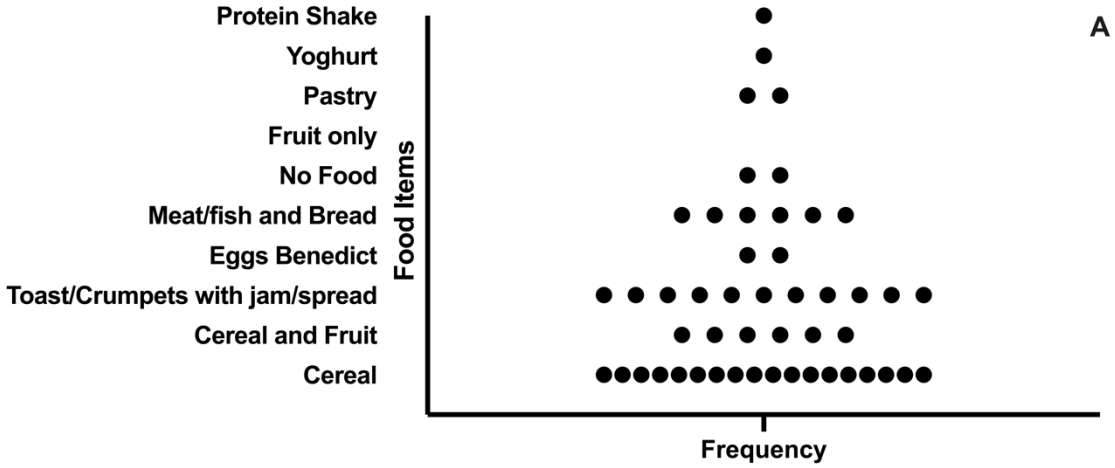
Would you repeat any of these bathing conditions in your own time? If not, then why?

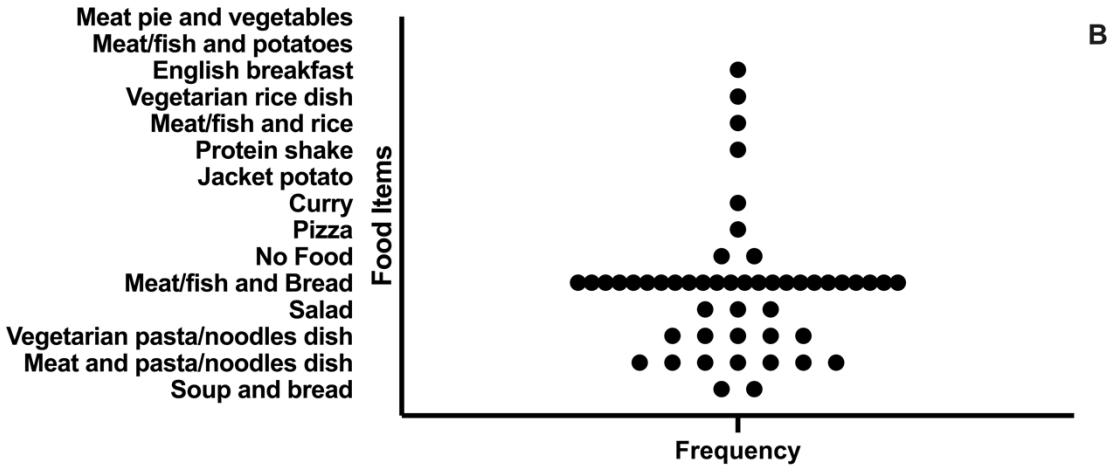
Appendix I. Thermal Sensation and Thermal Comfort from Gagge et al. (1967). Please note that this questionnaire was completed using an online cloud based questionnaire application (Smart Survey, Smartline International Ltd, UK (https://www.smartsurvey.co.uk/company/about-us)

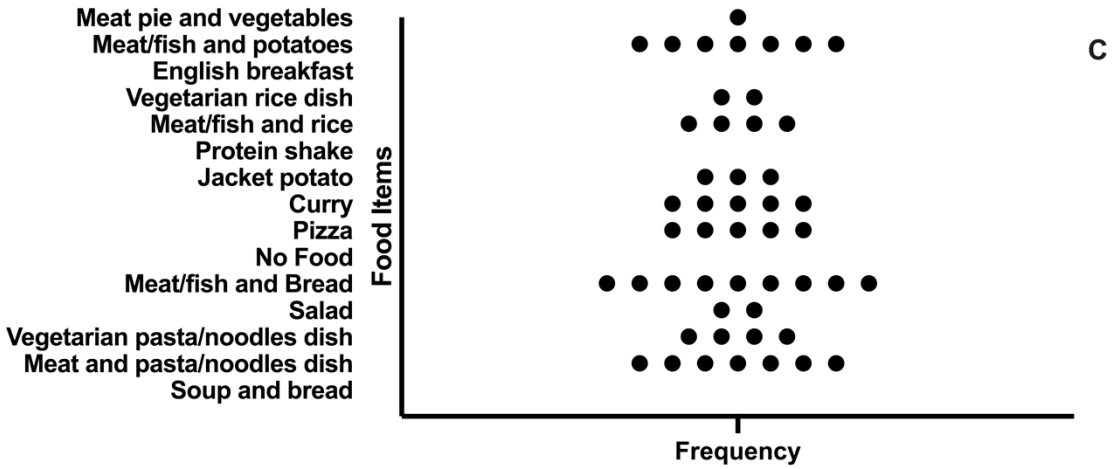
How does the temperature of your body feel?
1. Unbearably Cold
2. Extremely Cold
3. Very Cold
4. Cold
5. Cool
6. Slightly Cool
7. Neutral
8. Slightly Warm
9. Warm
10. Hot
11. Very Hot
12. Extremely Hot
13. Unbearably Hot

How comfortable do you feel at that temperature?
1. Comfortable
2. Slightly Uncomfortable
3. Uncomfortable
4. Very Uncomfortable
5. Extremely Uncomfortable

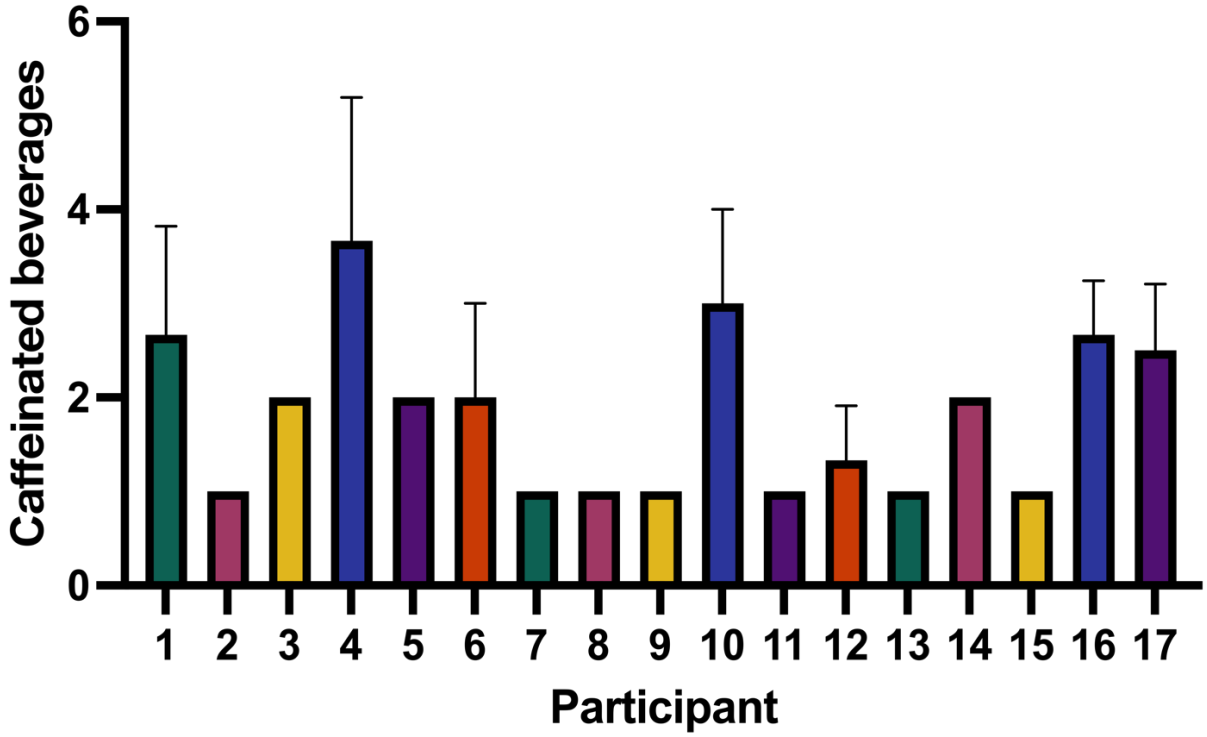
Appendix J. 24-hour recall of diet before a lab visit in Chapter 4. A) breakfast, B) Lunch & C) Dinner.



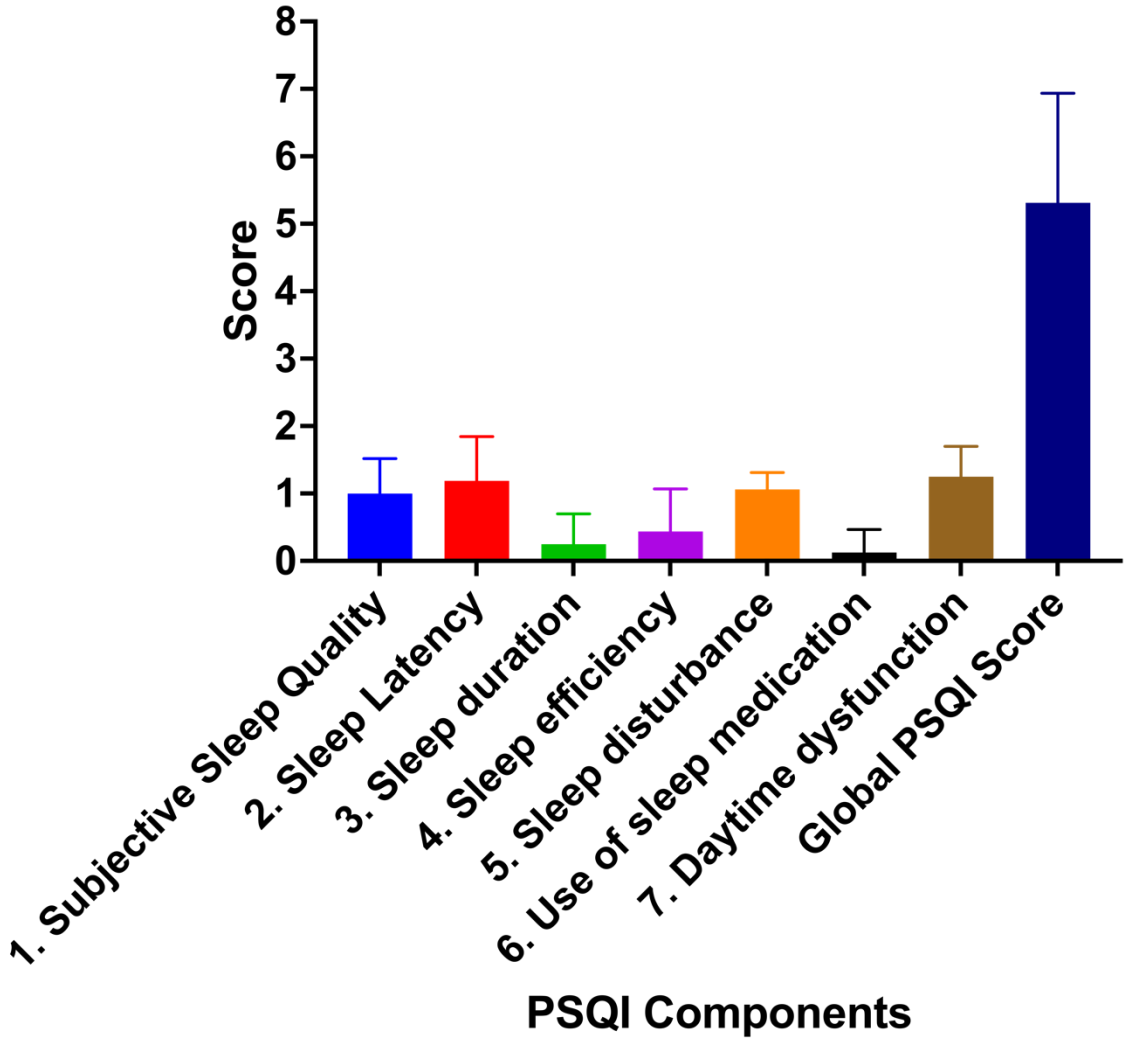




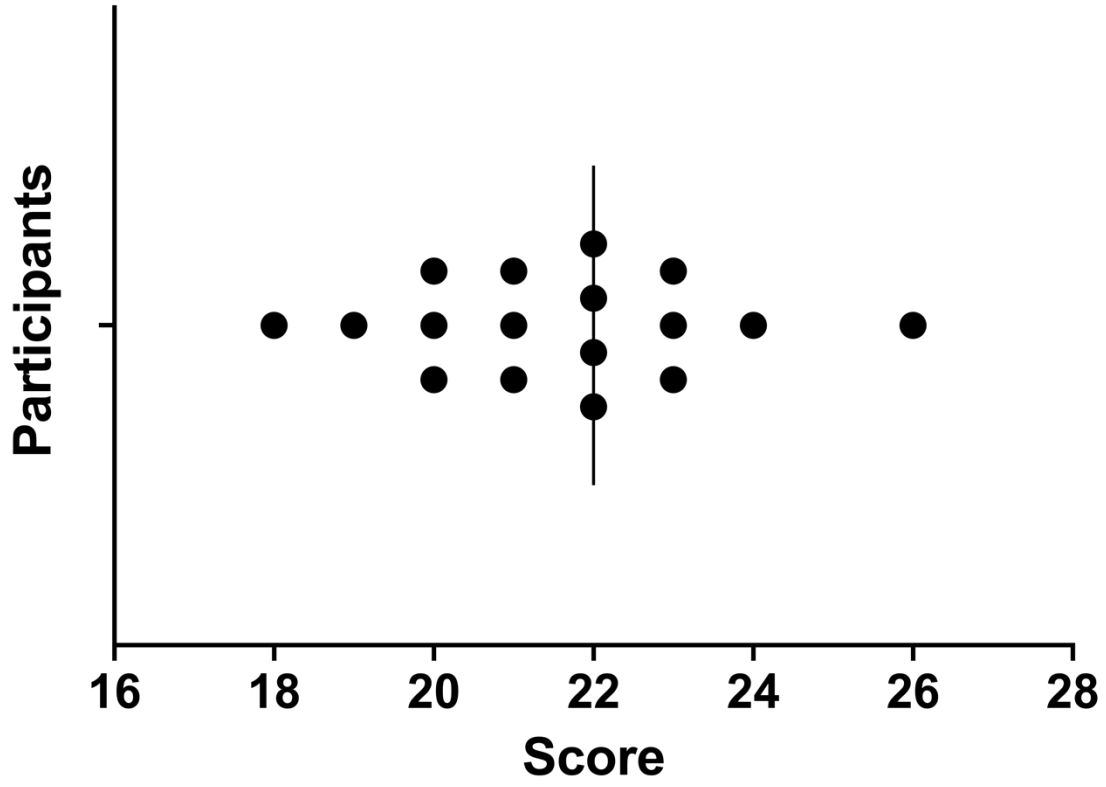
Appendix K. 24-hour recall of the number of caffeinated beverages consumed 24 hours before a lab visit in Chapter 4. Note, that participants in the same questionnaire confirmed that they did not take any of these beverages 12 hours before the lab visit commenced. Data presented as means ± SD.



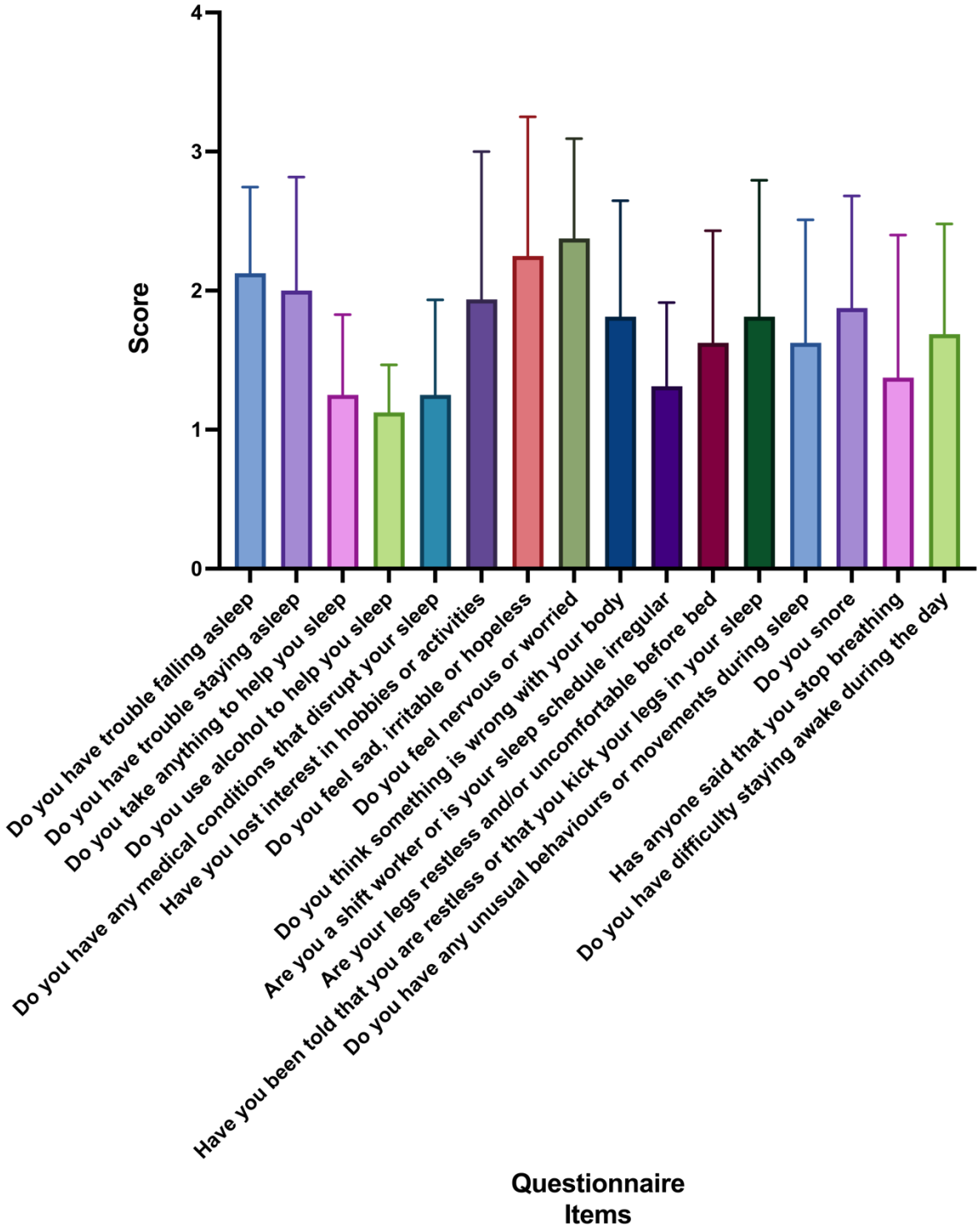
Appendix L. Pittsburgh Sleep Quality Index (PSQI; (Buysse et al., 1989)) results for Chapter 4. Note, that the global score is a combination of components 1-7. Each component is scored from 0-3, whilst the global score can range from 0-21. A higher global score indicates a poorer sleep quality. Data presented as means ± SD.



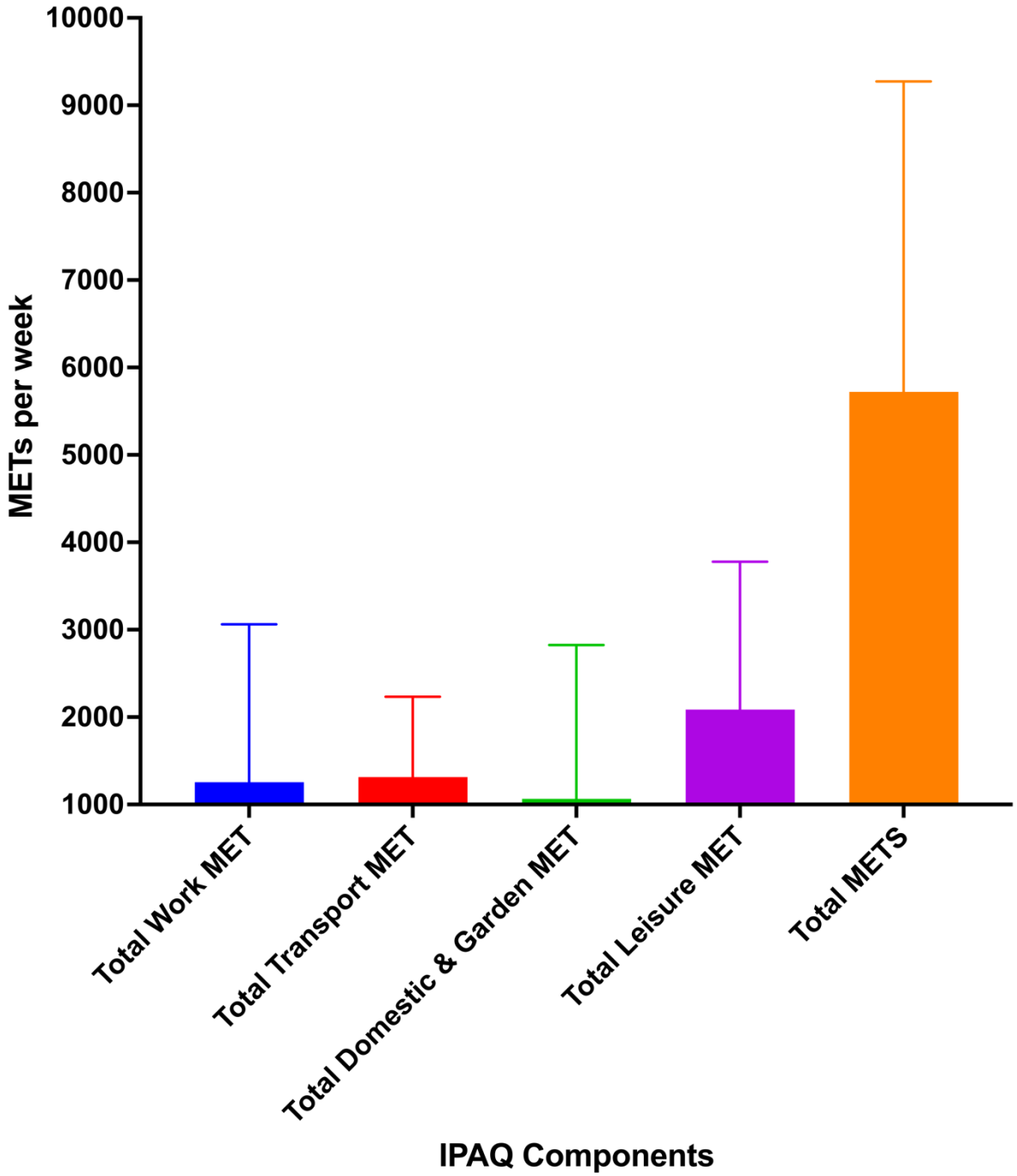
Appendix M. Perceived Stress Questionnaire results (Cohen et al., 1994) from Chapter 4. Scores ranging from 0-13, 14-26 and 27-40 indicate low, moderate and high perceived stress.



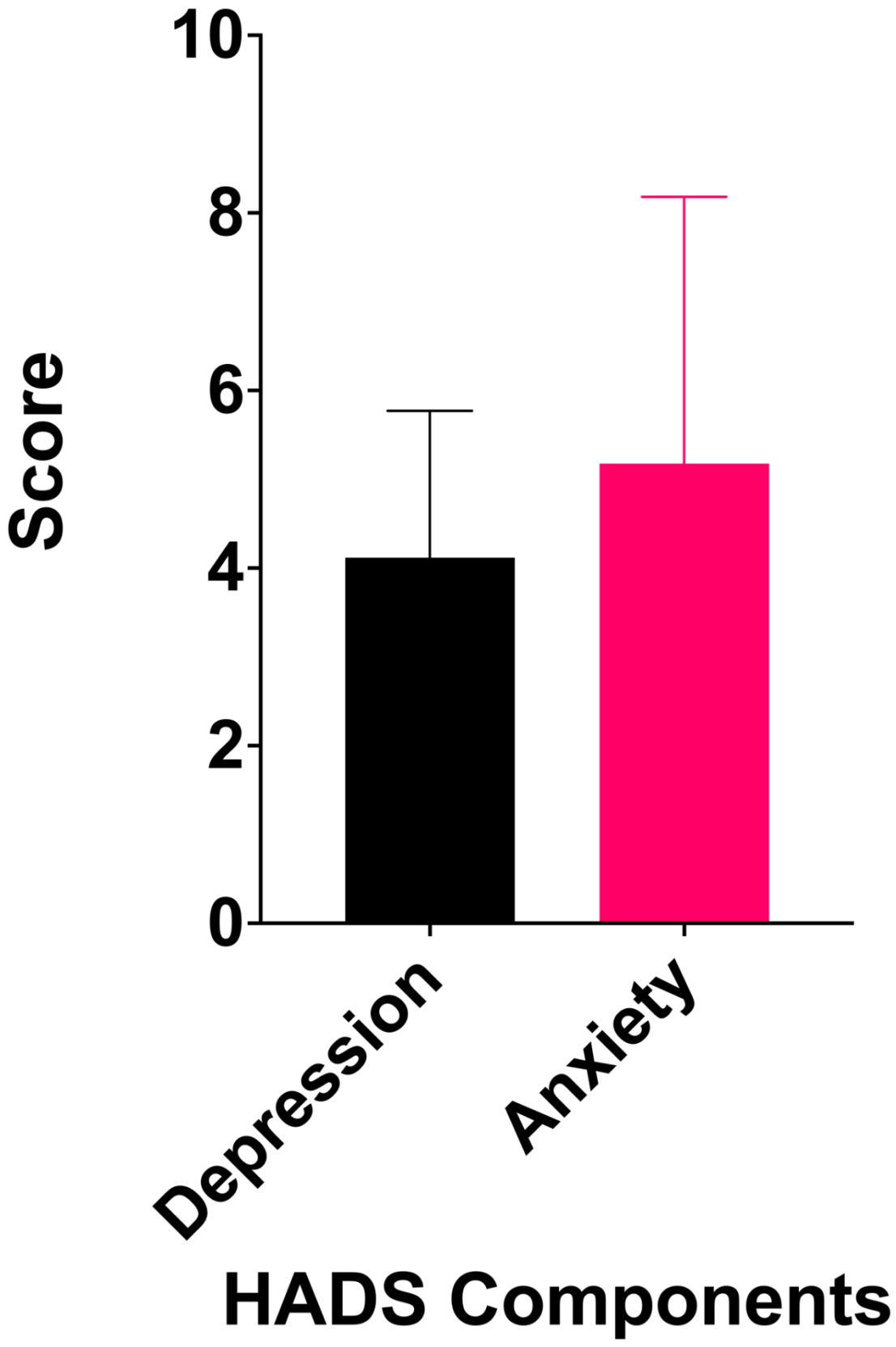
Appendix N. Holland Sleep Disorder Questionnaire (Mulder-Hajonides Van Der Meulen, 1981) scores from Chapter 4. Scores are scaled from 0 to 5 with 5 being the highest number of concern. Data presented as means ± SD.



Appendix O. International Physical Activity Questionnaire (long format; IPAQ) scores from the familiarisation session in Chapter 4. A higher METs score indicates increased physical activity. MET, Metabolic equivalents. Data presented as means ± SD.



Appendix P. Hospital and Anxiety Depression Scale (HADS; (Zigmond and Snaith, 1983)) scores from the familiarisation session in Chapter 4. A score of 0-7 indicates normal functioning, whilst a score of 8-10 indicates abnormalities. Data presented as means ± SD.



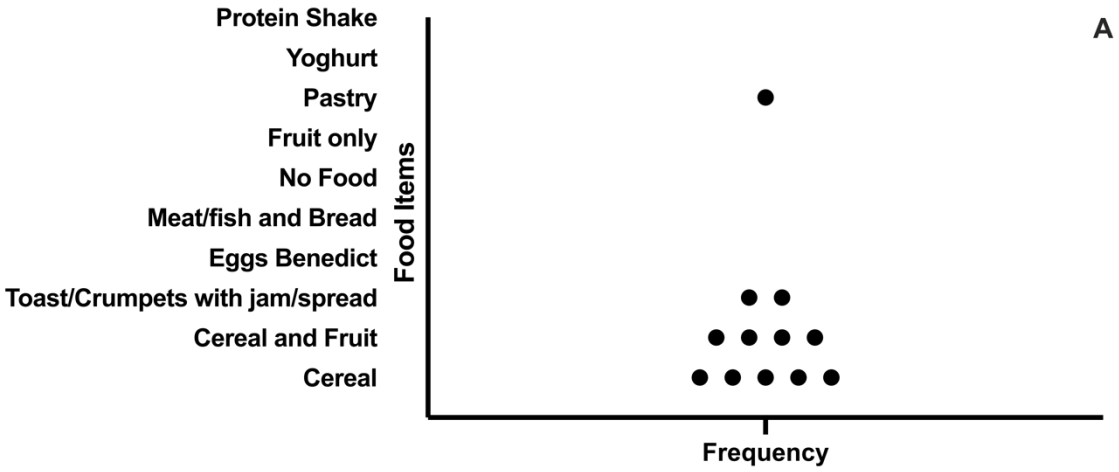
Appendix Q. Bathing Preference Questionnaire responses after the last bathing visit in Chapter 4.

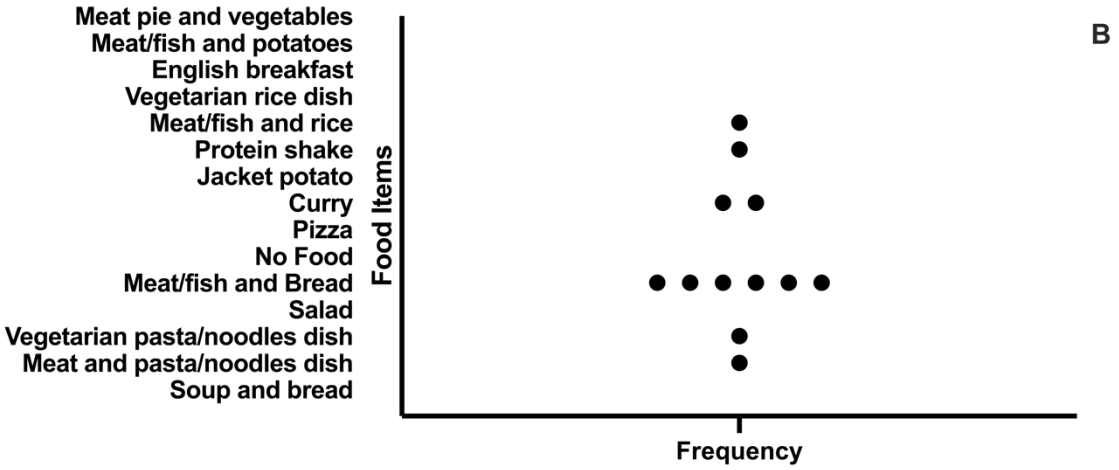
Participant	Favourite bathing experience	Reason for favourite bathing experience	Least favourite bathing experience	Reason for least favourite bathing experience	Would you undertake TWI30 again?	Would you undertake HWI30 again?	Would you undertake HWI60 again?	Would you repeat any of these bathing conditions in your own time, if not then why?
1	TWI30	Shortest and easiest temperature to manage	HWI60	The heat and extent of time to which I was submerged was difficult	Yes	Yes	No	Probably not because I've done them here and know I'm a healthy, fit athlete so I don't believe there would be any requirement to do it in my own time.
2	HWI30	Nice temperature and for a good time so didn't get overwhelmingly boiling	TWI30	Felt a bit cold after a while so less enjoyable	No	Yes	Yes	30 mins at 41
3	HWI30	Shorter and more manageable than 60 mins to get the CV benefits	HWI60	Long duration and got uncomfortable after a while	Yes	Yes	No	I'd repeat the 30 minutes at 41 as I felt like it would be something I could fit into my busy schedule but still reap the benefits of hot bathing
4	TWI30	Cool and relaxed, not too hot	HWI30	Felt uncomfortable, too hot	Yes	No	No	I wouldn't, 41 degrees was too hot and I felt discomfort and the 34 degrees Bath was cold however it felt nicer than the hot Bath
5	HWI60	The 60 minute was my favourite because the contrast of full to half immersion allowed me to not feel as hot and therefore weary when in the water. It allowed the	HWI30	The 30 mins at 41 was my least favourite because of the physical fatigue it left on my body after. I feel like without a fast, this condition would have been pleasant to give energy	Yes	No	Yes	I would repeat condition 1 and 3. I would repeat condition 2 but at a reduced time, like 20 mins?

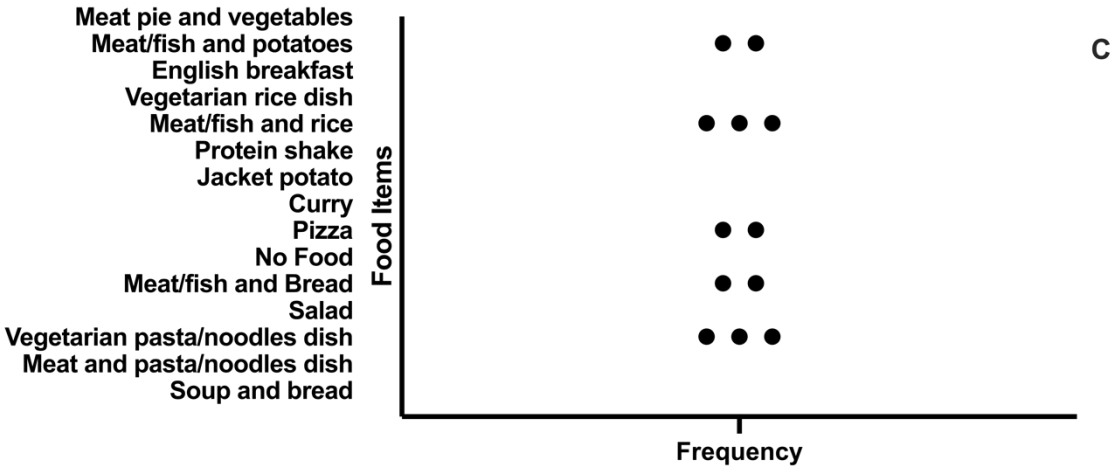
		contrast of feeling hot but not boiling like the 30 minute felt at the same temperature		however the last 10 minutes of the study were not an enjoyable experience				
6	TWI30	Water was a comfortable temperature and felt nice to sit in	HWI60	It was ok for about 10 minutes, but after that the water was too hot and felt uncomfortable fully submerged	Yes	No	No	Maybe somewhere in between the two temperatures, like 37 degrees as it's nice to sit in the water if it's not too hot. I may do the hotter temperature again but for less time and with shoulders above the water
7	TWI30	I did not get overly hot or cold, it wasn't uncomfortable	HWI60	It was very hot and it felt very uncomfortable	Yes	No	No	Yes
8	TWI30	Not too hot and not too cold	HWI60	Unbearably hot and felt dizzy when getting out	Yes	Yes	No	I felt as though the hot baths helped my knee injury. But the hot baths were slightly too hot for me to be comfortable
9	HWI30	It was the most relaxing and the shorter time frame was more comfortable	TWI30	I was quite cold during this session	No	Yes	No	I would repeat the 30 minute session as it made me feel refreshed
10	HWI30	Just enough time in the heat to enjoy it without feeling sick	TWI30	It was cold!! And I couldn't relax enough	No	Yes	Yes	No comment
11	TWI30	Perfect temp	HWI60	Too hot for too long	Yes	No	No	Yes
12	HWI30	No comment	HWI60	Felt too hot for too long. Felt slightly faint during.	Yes	Yes	Yes	Yes
13	HWI30	Warm enough to keep warm afterwards Felt warm afterwards	HWI60	Hot and sweaty for too long Was very tired afterwards	Yes	Yes	No	Yes

14	TWI30	Not difficult., more relaxed. Heated conditions were a bit more uncomfortable	HWI30	Felt like I was going to pass out after leaving bath	Yes	Yes	Yes	Not in my own time. If prescribed for health complications or scientific reasons, yes I would do so But as a healthily individual, I wouldn't put the time in to do it
15	HWI30	Enjoyed the temperature and wasn't too long	TWI30	Felt pretty cold during the bathing and wasn't too comfortable.	No	Yes	Yes	Condition 2
16	TWI30	This was the most comfortable	HWI60	This was too long	Yes	Yes	No	No. I prefer a hot tub whirlpool or shower
17	HWI30	Wasn't enjoyable to begin with but became more bearable towards the end. 60 min was too long	HWI60	Too long too hot	No	Yes	No	If I had an access to a hot tub I would

Appendix R. 24-hour recall of diet before a lab visit in Chapter 5. A) breakfast, B) Lunch & C) Dinner.







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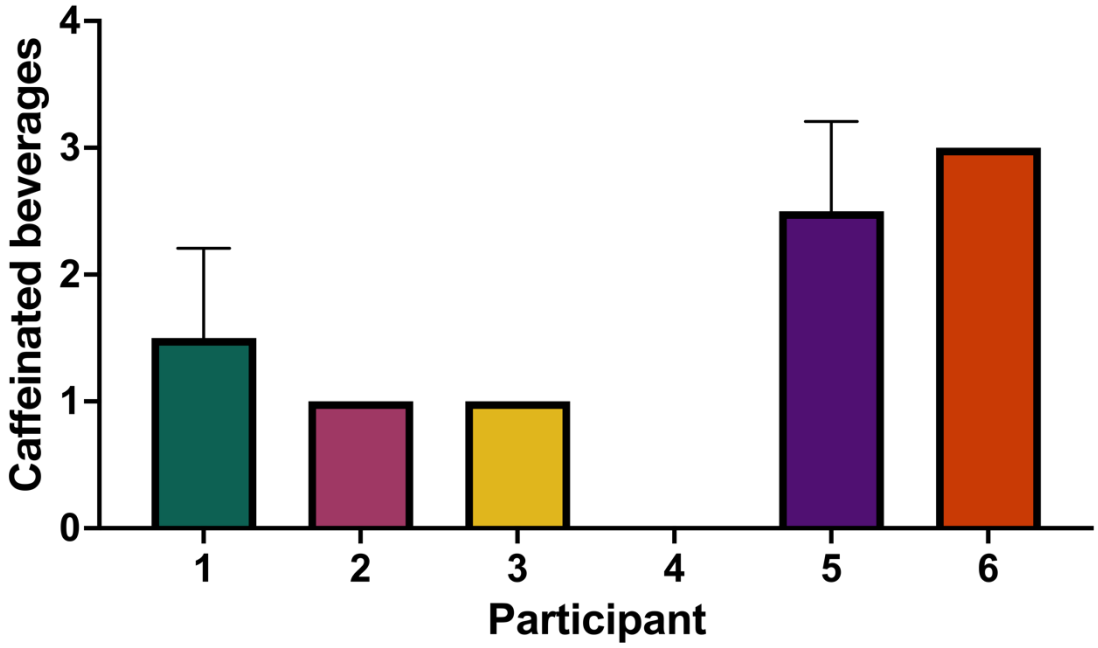

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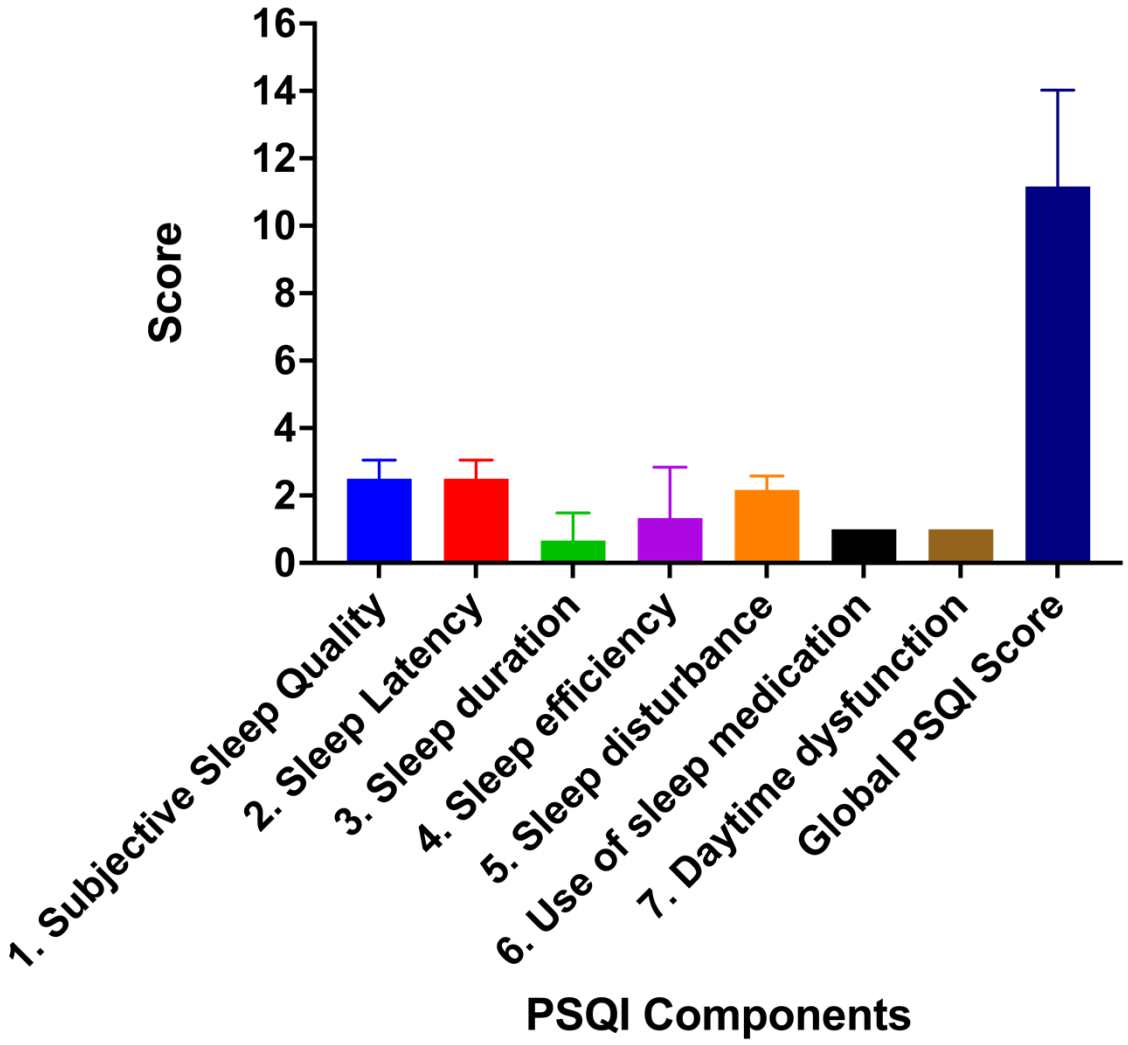

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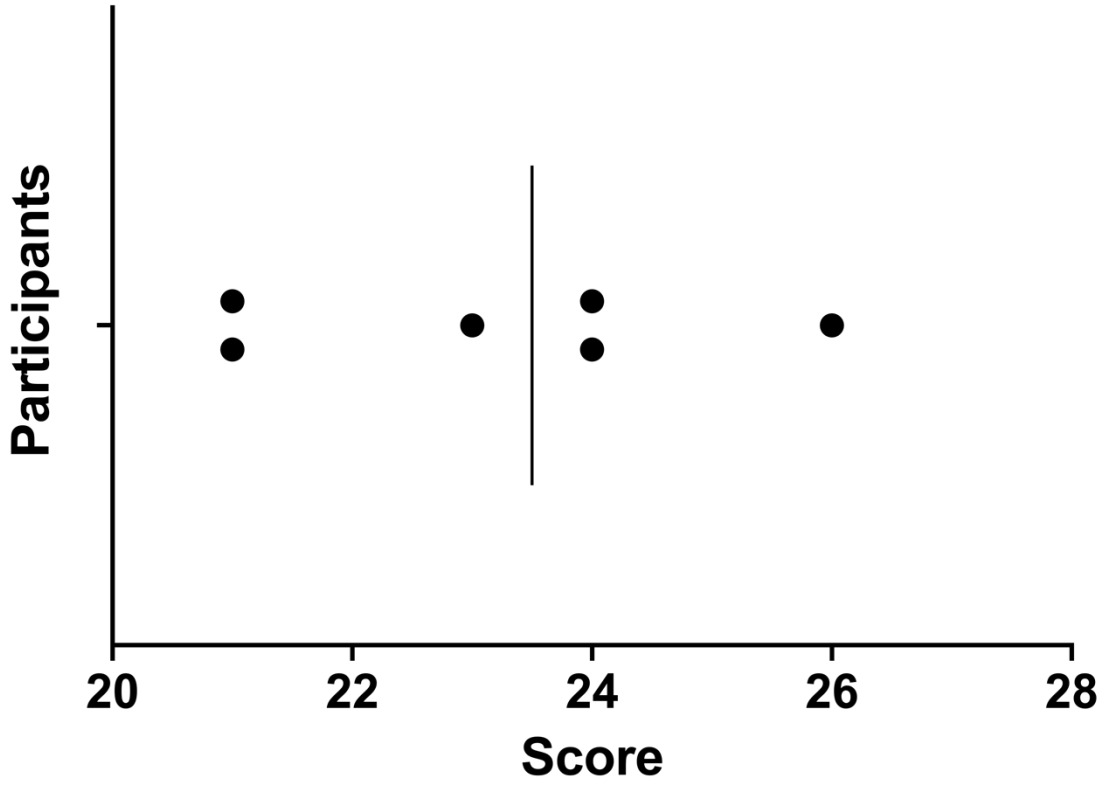

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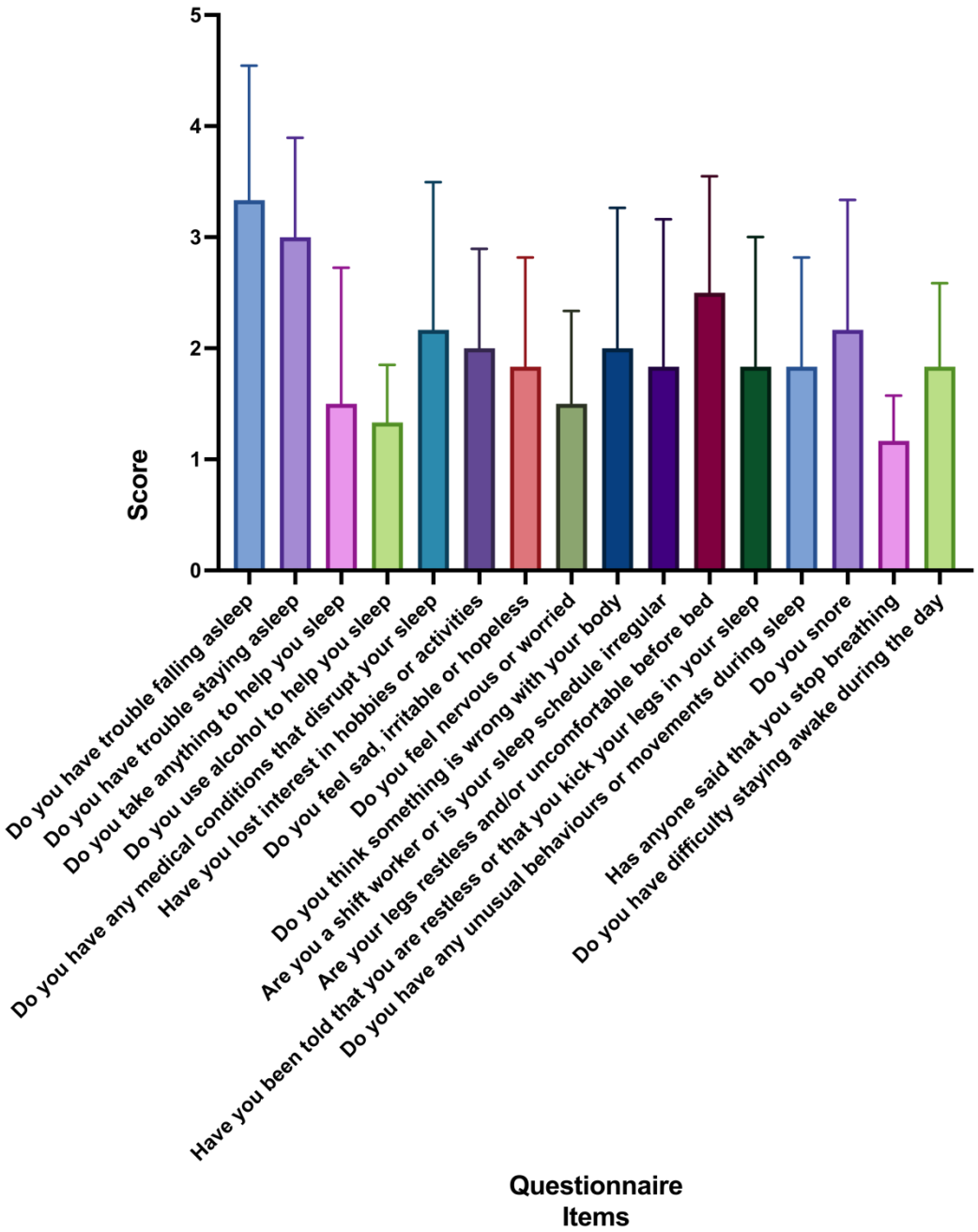

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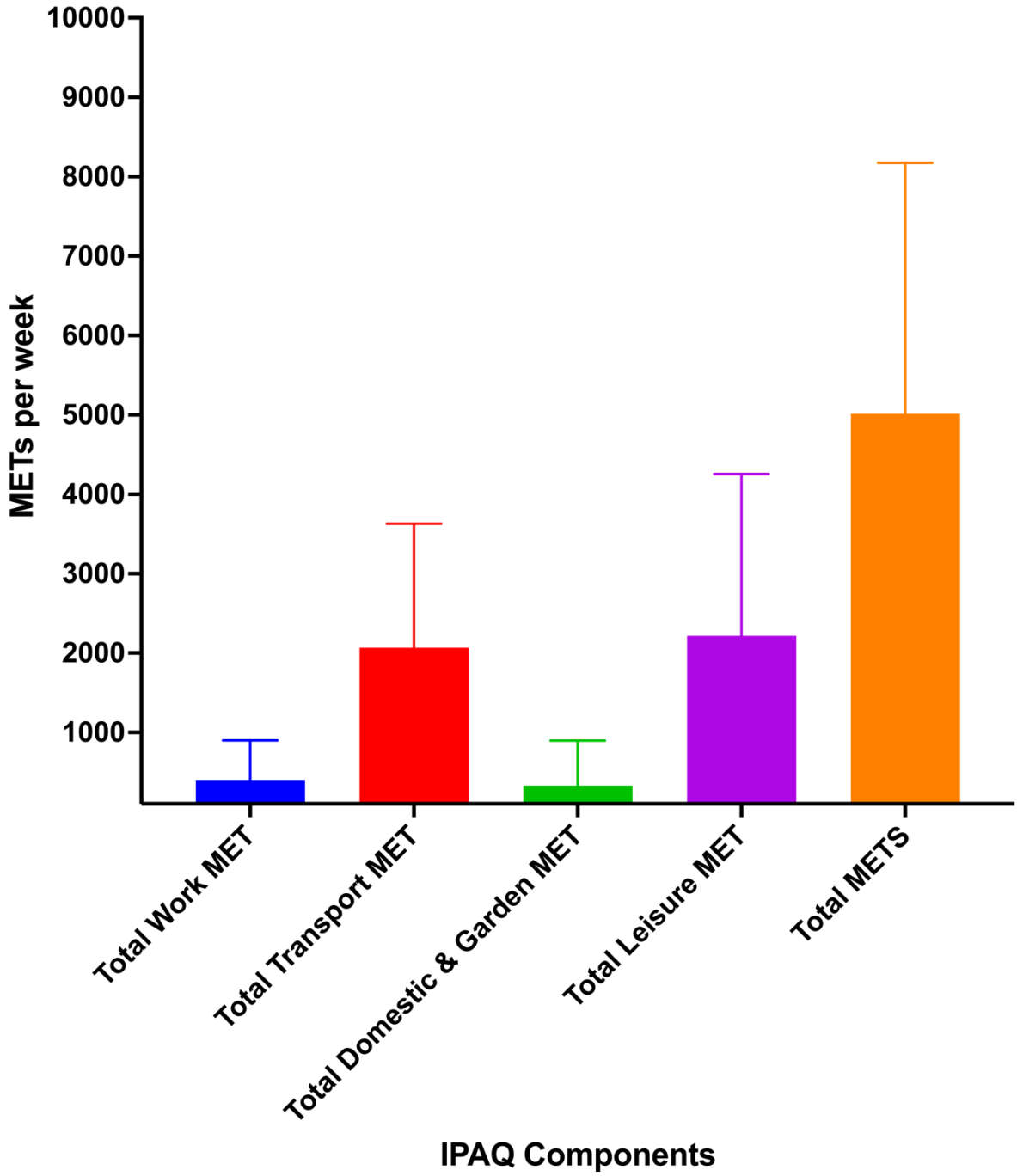

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