THE IMPACT OF EXERCISE AND THERMAL TRAINING INTERVENTIONS ON THERMOREGULATORY AND CARDIOVASCULAR FUNCTION IN YOUNG AND POST-MENOPAUSAL FEMALES

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Abstract

The female reproductive hormone oestrogen influences cardiovascular and thermoregulatory control. A dramatic decline in oestrogen during the menopause causes cardiovascular and thermoregulatory dysfunction resulting in hot flushes (HFs). HFs consist of feelings of intense heat with rapid increases in cutaneous vasodilation and sweating that severely affect quality of life and increase cardiovascular disease risk. Treatment using hormone replacement therapy for HFs is contraindicated in some symptomatic females meaning an alternative strategy is warranted. Exercise training may reduce HFs; however no study to date has examined the physiological mechanisms that cause changes in the frequency and severity of HFs following a period of exercise training. Exercise training is known to enhance thermoregulatory efficiency via an earlier core temperature onset for cutaneous vasodilation and sweating in pre-menopausal females. Exercise training is also known to positively impact vascular function in the conduit, cutaneous and cerebral vessels and thus can also decrease cardiovascular risk in symptomatic post-menopausal females. Heat acclimation interventions target the same thermoregulatory and cardiovascular physiological mechanisms, and may also be beneficial. The primary aim of this thesis was to (i) examine whether exercise training reduces objectively measured HFs via improving cardiovascular and thermoregulatory dysfunction in symptomatic post-menopausal females, and to (ii) assess the efficacy of an exercise-independent stimulus in improving thermoregulatory and cardiovascular function in pre-menopausal females.

Twenty-one symptomatic post-menopausal females completed a 16-week exercise training intervention (*n*=14, 52±4y, 29±6 kg/m^2) or a no-exercise control intervention (*n*=7, 52±6y, 30±7 kg/m^2). Cardiorespiratory fitness (VO_{2peak}) and brachial artery endothelial function was assessed using flow-mediated dilation (FMD). Participants underwent a passive heat stress in a water-perfused suit (~48^{o}C) to obtain core temperature thresholds and sensitivities for cutaneous vascular conductance (CVC) and sweat rate at two sites (chest and forearm). Middle cerebral artery velocity (MCAv) was measured at rest and throughout the heat stress. All measurements were repeated following the intervention period. HFs reduced by 39 HF·wk (95% CI= 31, 47) following exercise training compared to no change in control. HF-severity reduced by 101 (AU) (95% CI= 80, 121) following exercise training compared to no change in control. VO_{2peak} and FMD improved (*P*<0.05), along with a lower core temperature following exercise training [0.14^{o}C (95% CI=0.03, 0.20; *P*=0.04)]. Sweat rate and CVC body/core temperature thresholds occurred ~0.22^{o}C earlier alongside an increase in sweating sensitivity, at both sites, following exercise training. Resting MCAv was 3.12 cm/s (95% CI, 1.20, 5.01; *P*=0.03) higher, with decreases in MCAv attenuated during heat stress, following exercise training.

HFs recorded in symptomatic females during the passive heat stress prior to and following the exercise training (*n*=9) or no-exercise control (*n*=6) intervention were used to assess changes in thermoregulatory and (cerebro)vascular responses during an acute HF. HFs were objectively identified and divided into eight equal segments, with each segment representing 12.5% of HF duration, for analysis. Exercise training decreased HF duration by 63s (95% CI, 14, 113; *P*=0.08) compared to a negligible decrease of 17s (95% CI, -43, 66) following control. Chest sweat rate decreased by 0.04 mg·cm^2·min^{-1} (95% CI, 0.02, 0.06; *P*=0.01) during HFs after exercise training compared to no change in control (*P*>0.05). This was accompanied by a reduction in chest skin blood flow of 26 AU (95% CI,

21, 30; *P*=0.01) during HFs following exercise training compared to no change in control (*P*=0.10). MCAv was attenuated by 3.4 cm/s (95% CI, 0.7, 5.1; *P*<0.001) during a HF following exercise training compared to control [0.6 cm/s (95% CI, -0.7, 1.8; *P*=0.93)].

Eighteen pre-menopausal females (25±8y) were assigned to 3x30-min of cycling exercise (70% HR_{max}) or warm water immersion (42°C) to the level of the sternum for 8-weeks. FMD (*P*=0.003) and VO_{2peak} (*P*<0.001) improved following both interventions. Core body temperature reduced by 0.14°C (95% CI, 0.04, 0.23; *P*=0.004) following both interventions. Sweat rate mean body temperature thresholds at the chest and forearm occurred 0.10°C (95% CI=-0.14, 0.33, *P*<0.001) and 0.19°C (0.12, 0.23°C, *P*<0.001) earlier following the interventions, alongside an increase in sweat rate sensitivity of 1.18 $mg \cdot cm^2 \cdot min^{-1}$ (95% CI= 0.68, 1.67; *P*<0.001) following water immersion compared to 0.28 $mg \cdot cm^2 \cdot min^{-1}$ (95% CI= 0.23, 0.78) following exercise training. CVC core temperature thresholds occurred ~0.20°C earlier at the chest and forearm (*P*<0.001). Resting MCAv was 2.30 cm/s (95% CI=1.20, 3.34; *P*<0.001) higher, with decreases in MCAv attenuated during heat stress, following both interventions.

The findings from this thesis suggest that reductions in the frequency and severity of HFs with exercise training are mediated by improvements in thermoregulatory function, alongside cerebral, conduit and cutaneous adaptation. This coincided with objective reductions in HF severity following exercise training, with attenuation in the physiological perturbations observed during an acute HF. Consequently, interventions that target thermoregulatory function may be useful in reducing post-menopausal HFs. In keeping with the exercise mediated physiological changes, warm water immersion training also elicits similar favourable thermoregulatory, conduit- and cerebrovascular adaptations to a period of moderate intensity exercise training in pre-menopausal females. Immersion therapy may therefore be applicable to HF-symptomatic post-menopausal females.

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Declaration

I declare that the work contained within this thesis is entirely my own.

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List of abbreviations

Abbreviation	Title
ACh	Acetylcholine
ANOVA	Analysis of Variance
AVAs	Arteriovenous Anastomoses
BMI	Body Mass Index
BP	Blood Pressure
CBF	Cerebral Blood Flow
CBVC	Cerebro-Vascular Conductance
CI	Confidence Interval
CVC	Cutaneous Vascular Conductance
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure
DICOM	Digital Imaging and Communications in Medicine
EDHF	Endothelial Derived Hyperpolarising Factor
eNOS	Endothelial Nitric Oxide Synthase
FMD	Flow-Mediated Dilation
FSH	Follicle Stimulating Hormone
GnRH	Gonadotropin Releasing Hormone
HR	Heart Rate
HRR	Heart Rate Reserve
HDL	High Density Lipoprotein
HF(s)	Hot Flush(es)
ICA	Internal Carotid Artery
KNDy	Kisspeptin Neurokinin-B Dynorphin
LDF	Laser Doppler Flux
LDL	Low Density Lipoprotein
LH	Luteinizing Hormone
L-NAME	*N*-nitro-L-arginine Methyl Ester
L-NMMA	N^G-monomethyl-L-arginine
MAP	Mean Arterial Pressure
MCA	Middle Cerebral Artery
MCAv	Middle Cerebral Artery Velocity
MSNA	Muscle Sympathetic Nerve Activity
NPY	Neuropeptide-Y
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
P_aCO_2	Partial Pressure of Arterial Carbon Dioxide
PCA	Posterior Cerebral Artery
$P_{ET}CO_2$	End-tidal of Carbon Dioxide
PO/AH	Preoptic Anterior Hypothalamus
RCT	Randomised Control Trial
RPE	Rating of Perceived Exertion
ROI	Region of Interest

ROS	Reactive Oxygen Species
SBP	Systolic Blood Pressure
SD	Standard Deviation
sGC	Soluble Guanylyl Cyclase
SKBF	Skin Blood Flow
SR	Sweat Rate
SRAUC	Shear Rate Area Under the Curve
SSNA	Skin Sympathetic Nerve Activity
TCD	Transcranial Doppler
TRVP-1	Transient Receptor Vanilloid
VEGF	Vascular Endothelial Growth Factor
VO_{2peak}	Maximal Oxygen Consumption
WHI	Women's Health Initiative
WHO	World Health Organisation

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CHAPTER 1: INTRODUCTION

Female reproductive hormones have a profound influence on nonreproductive organs and systems. The primary ovarian hormone oestrogen exerts a significant influence on the integrative mechanisms that regulate cardiovascular and thermoregulatory function (Charkoudian and Stachenfeld, 2014). Endogenous oestrogen is secreted cyclically during the female menstrual cycle and reduces core body temperature (Brooks et al., 1997, Charkoudian, 2003), enhances conduit artery and cutaneous vascular function (Hashimoto et al., 1995, Kawano et al., 1996, Williams et al., 2001, Charkoudian, 2001, Charkoudian and Johnson, 1997b) and brain blood flow (Krejza et al., 2004).

The onset of the female menopause occurs between the age of ~47-55 years and is characterised by a dramatic reduction in oestrogen causing dysfunction in the cardiovascular and thermoregulatory control systems (Freedman, 2001, Freedman, 2014b). The menopause is a time of life associated with increased cardiovascular disease risk e.g. atherosclerosis, thought to be largely due to the decline in oestrogen (Zaydun et al., 2006, Rexrode et al., 2003, Weinberg et al., 2006). Nevertheless, the major pathophysiological symptom of the menopause in ~70% of females is the occurrence of hot flushes (HFs) (Kronenberg, 1990). A HF is defined as the sudden and intense sensation of heat causing skin reddening, flushing and profuse sweating (Freedman, 2014a). The occurrence of HFs are likely linked to disruptions to core body temperature and dysfunctional thermoregulatory control that are evident during the menopausal transition (Kronenberg, 2010, Freedman, 2001, Freedman, 2014a, Freedman and Krell, 1999). Importantly, there is also evidence of higher cardiovascular risk in post-menopausal females who suffer from HFs compared to asymptomatic females (Bechlioulis et al., 2010, Bechlioulis et al., 2012, Thurston et al., 2008b).

Pharmacological treatment for HFs is usually hormone replacement therapy (HRT). Whilst effective in alleviating HFs (Maclennan et al., 2004, Utian et al., 2004), uptake of this treatment modality is poor due to concerns about increased risk of cardiovascular disease (Rossouw et al., 2002, Manson et al., 2003), breast cancer (Beral, 2003, Manson, 2014, Tworoger et al., 2005) and other contraindications including age, time since menopause and absence of ovaries (Rossouw et al., 2007, NICE, 2009, Ossewaarde et al., 2005). Clearly, alternative treatments are required but other pharmacological and non-pharmacological options are limited due to lack of evidence. Exercise training has recently been suggested as a lifestyle modification that reduces HFs in post-menopausal females (Daley et al., 2009). For example, a recent combined randomised control trial found a reduction in self-reported HFs following 6-months of unsupervised exercise training, alongside improvements in symptoms of depression and sleep quality (Luoto et al., 2012, Moilanen et al., 2012). Not all studies have reported reduced HFs following a period of exercise training (Sternfeld et al., 2014, Wilbur et al., 2005), which may be due to differences in exercise mode and prescription (i.e. walking, duration, intensity), and programme supervision. Improvement in the frequency and intensity of HFs with exercise training is difficult to elucidate, since no studies to date have reported the physiological adaptations that occur following exercise training interventions in post-menopausal females. Furthermore, published studies generally rely on subjective markers i.e. a self-reported HF diary, as the outcome measure. In addition, no previous research has investigated the potential physiological mechanisms by which exercise training can improve menopausal HFs or, in other words, the effect of an exercise training intervention on the thermoregulatory and cardiovascular dysfunction associated with menopausal HFs. Therefore ***aim 1*** of this thesis was to determine whether improving

thermoregulatory control and vascular function with exercise training would alleviate post-menopausal HFs.

The acute physiological changes that occur during a HF are not only rapid in onset but can be extreme (e.g. ~80% change in cutaneous blood flow) and can last ~10 mins in severe cases (Kronenberg, 1990). Research investigating the acute thermoregulatory, cardiovascular and cerebrovascular changes that occur during a HF suggests a rapid onset of cutaneous vasodilation and sweating that is predominantly neurally mediated (Hubing et al., 2010, Low et al., 2010) and is followed by a reduction in core body temperature. Additionally, acute reductions (~5-30%) in brain blood flow and blood pressure are evident whilst heart rate increases (Lucas et al., 2013, Low et al., 2008a). No study to date has examined if these acute physiological changes are linked to objective HF severity or are responsive to interventions. Therefore ***aim 2*** of this thesis was to compare the acute thermoregulatory and (cerebro)vascular changes that occur during HFs following a period of exercise training in symptomatic post-menopausal females.

The impact of exercise training on thermoregulatory and cardiovascular control in pre-menopausal females is well reported (Ichinose et al., 2009, Kuwahara et al., 2005a, Kuwahara et al., 2005b, Armstrong et al., 2005). Cardiovascular and thermoregulatory adaptations may also be possible using other interventions that target the same physiological mechanisms. For example, repeated exposure to external passive heat stress over a short time-frame i.e. 10 days (e.g. resting in a hot and humid environment) induces thermoregulatory (Eichna et al., 1950, Fox et al., 1963a, Fox et al., 1963b, Shido et al., 1999, Armstrong and Kenney, 1993) and cardiovascular (Imamura et al., 2001, Kihara et al., 2002) adaptations. However a strong criticism of this technique is to induce

the necessary changes in core body temperature is time consuming. More recently local warm water immersion training has shown improvements in cutaneous and conduit artery endothelial-dependent function (Carter et al., 2014a, Naylor et al., 2011). The effect of a whole-body, repeated, warm water immersion training programme on thermoregulatory and (cerebro)vascular function is, to date, unknown. This may be important as targeting HFs with interventions (exercise or passive heat training) that improve thermoregulatory and (cerebro)vascular control may constitute a viable alternative to HRT in post-menopausal females. However, the physiological impact of exercise training and heat interventions, e.g. warm water immersion has not been directly compared. Therefore ***aim 3*** of this thesis was to compare the effects of an exercise training intervention with whole-body warm water immersion training in pre-menopausal females on thermoregulatory and (cerebro)vascular function.

In summary, there is evidence that cardiovascular and thermoregulatory control is under direct influence from female reproductive hormones. Oestrogen has a cardio-protective effect on the female vasculature i.e. endothelial dysfunction evident at the onset of the menopause, whilst temperature control becomes dysfunctional. This reduction in oestrogen leads to the occurrence of HFs in post-menopausal females. Exercise training is a potential non-pharmacological treatment. Yet, the underlying physiological mechanisms that lead to reductions in HFs with exercise training, or other interventions that can improve thermoregulatory and (cerebro)vascular function, are not yet understood. Therefore the overarching aim of this thesis is to explore the impact of exercise and thermal training interventions on thermoregulatory and (cerebro)vascular function in young (pre) and post-menopausal females.

1.1 Aims

The aims of this thesis are to:

1. Determine whether improving thermoregulatory control and (cerebro)vascular function with exercise training alleviate HFs in post-menopausal females compared to a no-exercise control group.
2. Compare the acute physiological thermoregulatory and (cerebro)vascular changes that occur during HFs prior to and following a 16-week exercise intervention compared to a no-exercise control group.
3. Compare the effects of an exercise training intervention with warm water immersion training in pre-menopausal females on thermoregulatory and (cerebro)vascular function.

1.2 Objectives

The aims outlined above will be achieved through the following objectives:

In line with ***Aim 1***:

1. Engage symptomatic post-menopausal females in either a 16-week supervised moderate intensity aerobic exercise programme or a 16-week no-exercise control period.
2. Compare the effect of exercise training and no-exercise control on cardio-respiratory fitness and conduit artery endothelial function.
3. Compare the effects of exercise training and no-exercise control on subjective frequency and severity reports of HFs using a validated 7-day questionnaire.
4. Use a passive heat stress to directly assess the vascular and thermoregulatory adaptations in symptomatic post-menopausal females following a 16-week exercise programme vs. a no-exercise control.

In line with ***Aim 2***:

5. Directly measure the physiological, vascular and thermoregulatory responses during a menopausal HF
6. Compare the effects of exercise training or no-exercise control interventions on the physiological perturbations that occur during a HF.

In line with ***Aim 3***:

7. Engage pre-menopausal females in an 8-week moderate intensity aerobic cycling training or warm-water immersion training programme and compare thermoregulatory and (cerebro)vascular adaptations using a passive heat stress.
8. Compare the effect of exercise training and warm water immersion training on cardio-respiratory fitness and conduit artery endothelial function following the 8-week intervention period in pre-menopausal females.

CHAPTER 2: LITERATURE REVIEW

2.1 Menstrual cycle

The female menstrual cycle comprises of the hypothalamus, the pituitary gland, the ovaries and the uterus that interact synergistically to cause cyclical hormonal changes that are essential for human reproduction. The cyclical variation in reproductive hormones begins at the age of menarche and ceases at the menopause. Each cycle typically lasts ~28 days, but ranges from 21-35 days. The menstrual cycle consists of three distinct phases (follicular, ovulatory and luteal) and the hormones follicle-stimulating hormone (FSH), luteinising hormone (LH), progesterone and oestrogens are stimulated and inhibited during the different phases (Figure 2.1).

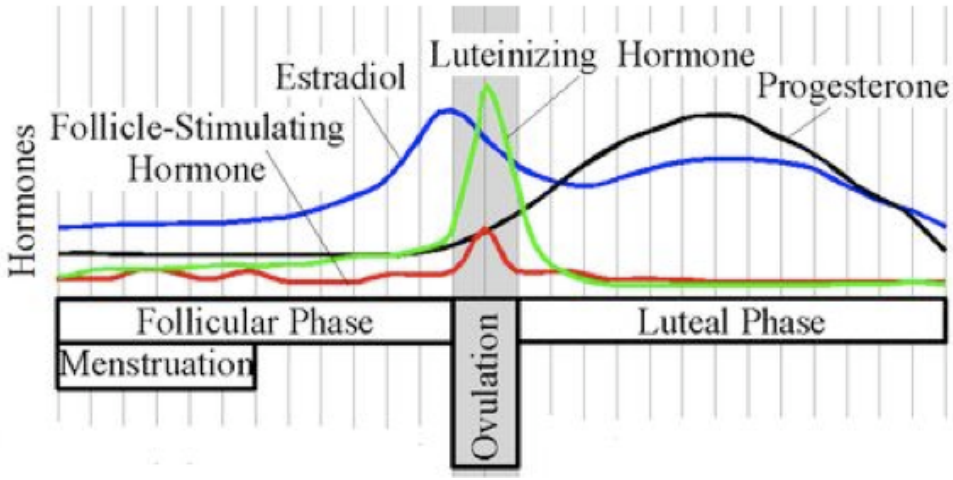


Figure 2.1 Schematic representing the changes in anterior pituitary and ovarian hormones throughout the female menstrual cycle.

Gonadotropin-releasing hormone (GnRH) secreted by the hypothalamus controls the female menstrual cycle, and stimulates the release of FSH and LH from the anterior pituitary. Both FSH and LH stimulate the ovarian follicles to secrete progesterone and importantly oestrogens, with β-estradiol the most abundant. During the follicular phase, oestrogens secreted by ovarian follicles suppress the production of LH until a threshold is reached and a subsequent surge in LH is released to prepare the body for ovulation (Figure 2.1). During the luteal phase, FSH and LH cause the dominant follicle to transform

into the corpus luteum, which secretes progesterone. It is the surge in progesterone that induces the production of oestrogens in luteal phase. These hormones suppress the release of FSH and LH, resulting in atrophy of the corpus luteum and subsequently progesterone declines triggering menstruation.

The hormones involved in the menstrual cycle have also been shown to impact on other physiological mechanisms besides reproductive function. The cyclical changes in oestrogen and progesterone affect thermoregulatory control and cardiovascular function in females (Charkoudian and Stachenfeld, 2014). The focus of this literature review is to discuss the impact of female reproductive hormones, with a specific emphasis on oestrogen, on thermoregulatory, conduit and cerebrovascular function at rest and in response to exercise training.

2.2 Thermoregulation

The human body maintains core body temperature around a set point of ~37°C, orchestrated by changes in the generation and conservation of heat (metabolism, shivering and cutaneous vasoconstriction) and heat loss (cutaneous vasodilation and sweating) in response to a variety of internal and external stimuli. Early in the 20th century, the existence of a thermoregulatory centre within the hypothalamic region of the brain was identified (Kahn, 1904). It is now well established that the central control of thermoregulation is located in the preoptic/anterior hypothalamus (PO/AH) of the brain (Boulant, 2006, Boulant, 2000, Boulant, 1996), which is the primary site that contains both temperature-sensitive and temperature-insensitive neurons that interact to control core body temperature (Charkoudian and Stachenfeld, 2014, Boulant, 2006) Figure 2.2). Afferent information from the skin surface, spinal and visceral thermal receptors are

important for optimal core body temperature regulation in response to alterations to both internal and external environments. Thus, skin temperature afferent information will augment heat dissipation responses under heat stress and will also increase heat generation responses in cooler environments (Charkoudian and Stachenfeld, 2014). When the PO/AH neurons receive afferent signals above a threshold temperature there are proportional increases in the efferent responses of cutaneous vasodilation and sweating, alongside behavioural changes that promote heat loss and peripheral cooling (Boulant, 2006). Each of these responses is unique and is evoked at slightly different hypothalamic temperatures, known as the set-point and/or temperature threshold. With this in mind, the ultimate effectors of thermoregulatory responses to heat stress are the cutaneous blood vessels and sweat glands of the skin.

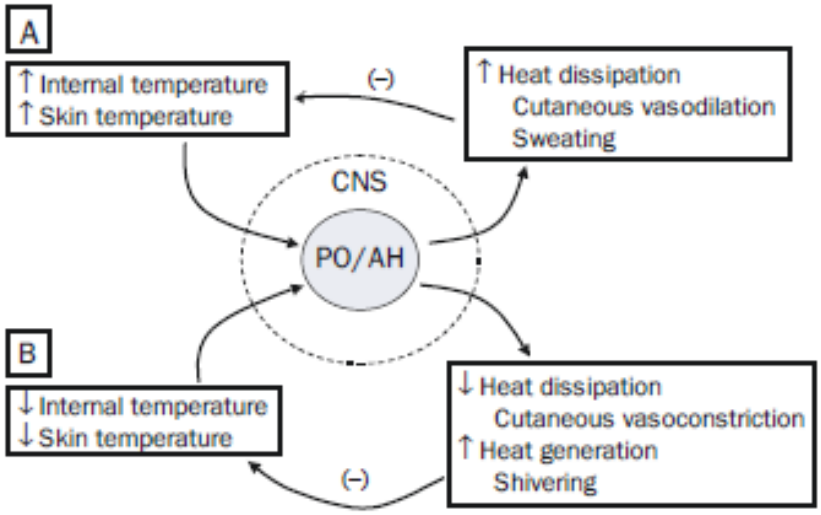


Figure 2.2 Human thermoregulatory control occurs via negative feedback. (A) Increases in internal and/or skin temperature are sensed by the Preoptic-anterior hypothalamus (PO/AH) that results in increased heat dissipation, via cutaneous vasodilation and sweating responses. This corrects the increase in temperature. (B) Decreased internal and/or skin temperature results in reflex decreases in heat dissipation (cutaneous vasoconstriction) and increased heat generation (shivering). This corrects the decreases in internal temperature. CNS = Central Nervous System. Minus signs (-) refer to the correction of the error signal by the appropriate efferent response. Adapted from Charkoudian (2003).

2.2.1 The skin

The skin is a vital component of human thermoregulation. The arterioles and venules of the cutaneous microcirculation represent two vital physiologically important areas in the skin, located in the dermis plexus in both superficial and deep skin layers (Kellogg, 2006). Most of the microvasculature is contained within the superficial papillary dermis 1-2mm below the epidermal surface (Figure 2.3), and consist of high-resistance terminal arterioles, papillary loops (true capillaries) and post-capillary venules.

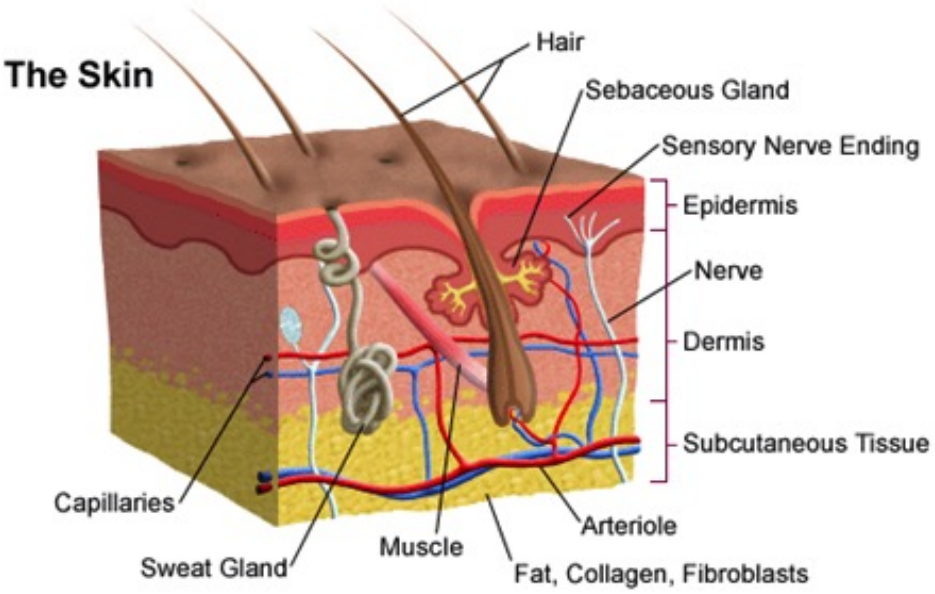


Figure 2.3 Sectional of the skin (cutaneous membrane) and subcutaneous layer; consisting of a superficial, thin epidermis and a deep, thicker dermis. Deep to the skin is the subcutaneous layer, which attaches the dermis to the underlying organs and tissues. Smooth muscle, nerves and sweat glands are located in the dermis layer.

The papillary loops are located close to the dermal-epidermal junction, with this site maintaining a high thermal gradient due to its large surface area and high blood flow, acting as a major determinant for heat exchange (Rowell, 1974a, Charkoudian and Stachenfeld, 2014). Blood flow through the papillary loops is controlled by highly

innervated arterioles that consist of a lining of endothelial cells that are encompassed by two layers of vascular smooth muscle cells. The upper and lower plexuses are located in both glabrous, or non-hairy, (plantar feet, palms and lips) and nonglabrous skin (most of the body surface including the limbs, torso and head), with arteriovenous anastomoses (AVAs) primarily found in glabrous skin (Johnson et al., 1986, Roddie, 2003). AVAs are extensively innervated by sympathetic adrenergic fibres (vasoconstrictor nerves) which control blood flow through the AVAs. An increase in sympathetic activity to the AVAs during mild-to-moderate cold stress causes vasoconstriction, whereas a reduction in sympathetic activity during heat stress causes a drop in vasoconstrictor tone that elevates skin blood flow. A transient reduction in sympathetic activity to the AVAs also mediates cold-induced vasodilation during a prolonged severe cold-stress (Roddie, 2003). Resting skin blood flow in normothermia, when cutaneous arterioles are under minimal neural tone, is approximately 250 mL·min (30 to 40 $mL \cdot min^{-1} \cdot 100g^{-1}$ of skin) which results in a heat dissipation of approximately 80-90 Kcal·hour which is equal to metabolic heat production (Johnson, 1996).

A reduction in skin/core temperature, or if skin temperature is lower than core temperature, triggers a thermoregulatory reflex-mediated reduction in skin blood flow to conserve body heat, through enhanced arteriolar vasoconstrictor tone that enables an arteriolar vasoconstriction, and hence reduced skin blood flow. Equally, during heat exposure (when skin temperature rises above core temperature) or exercise-induced increases in body temperature, vasoconstrictor activity is reduced and active vasodilator tone to the cutaneous arterioles is engaged to trigger cutaneous vasodilation and subsequently sweat rate to promote evaporative heat loss and skin cooling (Johnson et al., 2014, Rowell, 1974b).

During whole-body heat stress, there is a steady increase in skin blood flow that correlates most closely with core body temperature (Taylor and Bishop, 1993), whilst the clearest role for skin temperature is that it affects the threshold for internal temperature at which skin blood flow begins to rise exponentially (see *2.2.4 core temperature threshold and sensitivity*) (Brengelmann et al., 1981, Farrell and Bishop, 1995). Clearly, at higher skin temperatures the internal threshold temperature for increasing skin blood flow (alongside sweating) is lowered such that thermoregulatory effector responses are initiated to stimulate heat loss earlier. Both local and reflex (neural) effectors contribute to changes in skin blood flow during environmental stress.

2.2.1.1 Neural control of skin blood flow.

Skin blood flow is controlled by both neurogenic (reflex) and local thermal mechanisms. In nonglabrous skin, neurogenic changes in the cutaneous circulation are unique in that they are mediated by dual sympathetic neural mechanisms (Kellogg et al., 2002), that are the primary effectors of thermoregulatory control. These consist of non-adrenergic vasoconstrictor nerves (as also found in glabrous skin) and the sympathetic active vasodilator system (Charkoudian, 2003, Johnson et al., 2014, Lewis and Pickering, 1931) (Figure 2.4). In contrast, the vasoconstrictor response with cooling is dependent on intact noradrenergic cutaneous active vasoconstrictor nerves. The mechanisms of (cold-induced) vasoconstriction have been reviewed extensively (Johnson et al., 2014, Johnson and Kellogg, 2010a) and are only included in this literature review in relation to their role in enhancing skin blood flow during heat stress.

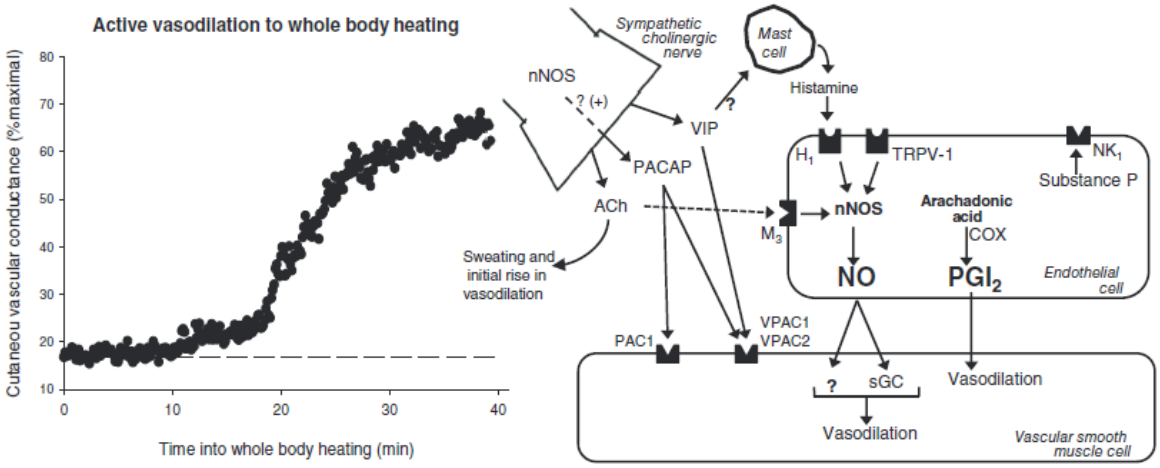


Figure 2.4 Summary of the mechanisms that contribute to reflex cutaneous active vasodilation. The left panel displays a typical trace of the skin blood flow response to whole-body heating. The initial rise in skin blood flow is from the release of vasoconstrictor tone, with the subsequent rise attributed to active cutaneous vasodilation. The right panel displays a schematic of the current theory of active cutaneous vasodilation (Johnson *et al,* 2014).

Sympathetic vasoconstrictor nerves are tonically active in normothermic environments (Brengelmann et al., 1981). Subtle changes in the activity of this system during daily activities or ambient temperature are responsible for the maintenance of body temperature, with decreases in vasoconstrictor nerve activity during exposure to slightly warmer environments causing a passive vasodilation with relatively small changes in skin blood flow responsible for larger changes in heat dissipation (Charkoudian and Stachenfeld, 2014). This allows for a zone of thermoregulation that is controlled solely by changes in vasomotor tone. During heat stress the withdrawal of vasoconstrictor nerve activity is responsible for between 10-20% of cutaneous vasodilation (Brengelmann et al., 1981). In contrast, the sympathetic active vasodilator system is unique to human skin and does not demonstrate tonic activity, and is predominantly responsible (80-90%) for large increases in skin blood flow during hyperthermia, via active vasodilator nerve activation in the skin (Rowell, 1974b).

The mechanisms responsible for cutaneous active vasodilation are complex. Nevertheless, the initiation of active cutaneous vasodilation is by cholinergic nerves and co-transmitters (Johnson et al., 2014). The action of acetylcholine (ACh) on the mechanisms of cutaneous vasodilation are mostly apparent in effecting the initial vasodilator responses to whole body heating, but not in maintaining those responses during prolonged heat stress (Johnson et al., 2014, Kellogg, 2006, Kellogg et al., 1995). Kellogg *et al.* (1995) used Botulinum A, a toxin which prevents the release of ACh and its associated cotransmitters from cholinergic nerves (Dolly and Aoki, 2006), and established that cutaneous active vasodilation is abolished in the treated area during heat stress, yet the reflex noradrenergic vasoconstrictor responses to cooling effects were unchanged. In line with previous studies (Fox and Hilton, 1958, Roddie, 2003, Shastry et al., 2000) they also found that atropine treatment (ACh inhibitor) prior to whole-body heat stress delayed, and slightly attenuated the cutaneous active vasodilator response to heat stress (Kellogg et al., 1995). It thus appears that cutaneous active vasodilation is only partly mediated by ACh during the initial stages of heat stress, and ~80% of the vasodilatory response is mediated by cholinergic nerve co-transmitters (Johnson et al., 2014).

Shibasaki *et al.* (2002) postulated that ACh released from cholinergic nerves during heat stress causes vasodilation via muscarinic receptor mediated NO production. Kellogg *et al.* (1998) established that the increase in skin blood flow mediated by active cutaneous vasodilation during heat stress was significantly reduced by infusing the NOS inhibitor N^G-monomethyl-$_L$-arginine ($_L$-NMMA). The infusion of $_L$-NMMA beyond cutaneous vasodilation during heat stress reduced forearm skin blood flow by ~30% (Shastry et al., 1998). It is widely accepted that 30-45% of the increases in skin blood flow during heat stress are mediated by nitric oxide synthase (NOS) and the generation of NO, as the

inhibition of NO and of soluble guanylyl cyclase (sGC) (a secondary messenger in mediating the effects of NO) attenuates cutaneous active vasodilation during thermal stress (Kellogg et al., 2011). The remainder of the cutaneous active vasodilator response to heat stress is presumed to be due to pathways that are independent of NO and secondary NO-dependent mechanisms (Johnson et al., 2014, Kellogg, 2006), such as vasoactive intestinal peptide, substance P, histamine and prostanoids (Bennett et al., 2003, Kellogg et al., 1995). These are summarised in Figure 2.4.

2.2.1.2 Local control of skin blood flow

In response to alterations in local skin temperature, cutaneous blood flow changes via local temperature-dependent mechanisms. The vasoconstrictor response to local cooling have been reviewed extensively (Hodges and Johnson, 2009), with neuropeptide-Y (NPY) and noradrenaline playing significant roles in the reflex vasoconstrictor response. Vasodilator mechanisms that underlie the cutaneous hyperaemic response to local heating of human skin are different than those involved in reflex cutaneous active vasodilation. The local control of skin blood flow does not require reflex innervation but includes locally mediated vasodilation dependent on the degree and speed of local skin warming (Johnson and Kellogg, 2010a), with a local skin temperature of ~42°C leading to maximal skin vasodilation (Black et al., 2008b, Johnson et al., 1986). The current understanding of the skin blood flow response to local heating includes complex interactions between neural components and locally produced chemical messengers (Johnson and Kellogg, 2010b). Local heating of nonglabrous skin results in a bi-phasic response, characterised by an early transient peak predominantly mediated by neural factors, followed by a brief nadir and a more prolonged secondary increase in skin blood flow to a plateau that is predominantly a result of locally produced chemical factors

(Minson et al., 2001). Sustained heating at the plateau phase eventually results in a return of skin blood flow to resting levels despite the continuation of local heating highlighting that bio-availability of the local chemical factors involved in vasodilation are limited.

Minson *et al*. (2001) reported a 50% reduction in the initial peak response to local heating following the blockade of local nerve function using a topical anaesthetic cream. Interestingly, the blockade of all sensory afferent and sympathetic efferent activity proximal to the site of local heating had no effect on increases in skin blood flow, suggesting that the activation of local sensory nerves acting via an axon-reflex, and not a central perception of heat by the CNS, was required for cutaneous vasodilation. NO is known to play a role in the sensory nerve contribution to the axon reflex component of local heating, with $_{L}$-NAME infusion blocking NOS and subsequently removing (Hodges et al., 2008) and/or delaying (Houghton et al., 2006) the axon reflex response. It was recently suggested that the NO component of the axon reflex is due to activation of transient receptor vanilloid (TRVP-1) channels that are located on free nerve endings of sensory skin fibres (Wong and Fieger, 2010), as TRVP-1 is known to be activated by, or increase the production of, NO (Lin et al., 2007, Vriens et al., 2009).

The prolonged plateau phase of local temperature-dependent vasodilation has been shown to be mediated by the local generation of NO, and is significantly attenuated by ~50% by pre-treating the warmed skin with the NOS-inhibitor $_{L}$-NAME (Kellogg et al., 1999, Minson et al., 2001, Gooding et al., 2006). NO is thought to contribute ~60% to the longer plateau phase in local cutaneous vasodilation, with the remaining ~40% attributed to endothelial-derived hyperpolarising factor (EDHF) that causes vasodilation via hyperpolarisation of the vascular smooth muscle (Brunt and Minson, 2012).

2.2.2 Sweating

Sweating increases the dissipation of heat from the body when internal and/or skin temperature rises, with increases in skin blood flow necessary to initiate and increase sweating (Smith et al., 2013b). The evaporation of sweat from the eccrine sweat glands cools the skin such that heat dissipation can occur more effectively and efficiently at the skin surface. Eccrine sweat glands are distributed over nearly the entire body surface, with the number varying from 1.6 to 4 million, with sweat gland density associated with thermoregulation being greatest on the forehead, upper limbs, the trunk and lower limbs (Kondo et al., 1998, Sato and Dobson, 1970).

The structure of the eccrine sweat gland consists of a bulbous secretory coil leading to a duct, with the secretory coil located in the lower dermis with the duct extending through the dermal layer that opens directly onto the surface of the skin (Figure 2.3). The uncoiled dimension of the secretory portion of the gland is between 2-5mm in length and 30-50 µm in diameter, whilst the secretory coil ranges from 1-8 $x10^{-3}$ mm^3 (Sato and Sato, 1983). The size of the sweat gland has been found to be important, with Sato and Sato (1983) establishing a positive correlation between the individual sweat gland size and maximal sweat gland sweat rate production. The identification of neural tracks in humans is somewhat challenging, meaning the exact neurological pathways that are responsible for sweating are not entirely understood.

2.2.2.1 Neural control of sweat rate

Efferent sympathetic cholinergic nerves innervate eccrine sweat glands to induce sweating, releasing ACh. ACh then binds to cholinergic muscarinic receptors at the sweat gland (Randall and Kimura, 1955, Torres et al., 1991). Increases in sweat rate occur

through a combination of increasing the number of sweat glands that are activated and increasing the amount of sweat released per gland (Randall, 1946, Kondo et al., 2001). The initial increase in sweat rate relates to a greater number of activated glands, with further elevations in the sweat rate response occurring through increases in gland secretion (Kondo et al., 2001), with average maximum sweat rate outputs ~1.4 L/hour in healthy individuals (Shibasaki et al., 2006).

The local and systemic administration of atropine (muscarinic-receptor agonist) abolishes sweating during heat stress, and also in response to the exogenous administration of ACh or its associated analogues. This strongly suggests that sweating primarily occurs via muscarinic receptor stimulation (Foster and Weiner, 1970, Kellogg et al., 1995, Kolka and Stephenson, 1987, MacIntyre et al., 1968). A number of possible co-transmitters e.g. vasoactive intestinal polypeptide, have been identified in and adjacent to cholinergic sudomotor nerve terminals (Spreca et al., 2000, Tainio et al., 1987, Yamashita et al., 1987), however the role of these peptides in modulating sweat rate remains unclear.

Lee and Mack (2006) reported that muscarinic-receptor mediated (pharmacologically-induced) sweating is reduced by NOS inhibition, supporting the suggestion that NO acts to augment local sweat gland activity. However, the authors concluded that they did not know whether NOS inhibitors acted by blocking an intrinsic NOS system within the eccrine sweat gland cells, or reduced NO production in adjacent tissues i.e. the vascular endothelium, that were dependent on skin blood flow. It is proposed that the impact of NOS inhibition on local sweat rate may depend on the existence of adequate sudomotor activity mediated by muscarinic-receptors during thermal stress (Welch et al., 2009). Consequently, NO may augment local sweat rate by directly influencing ACh release from

sympathetic sudomotor nerves and augment sweat gland production, although this is yet to be established.

2.2.2.2 Local control of sweat rate

Sweating responses, can be influenced by changes in skin and local temperature (Vanbeaumont and Bullard, 1965, Wingo et al., 2010, Nielsen and Nielsen, 1965). For example, changes in skin temperature can modify sweat rate, as evidenced by accentuated sweating with local skin heating (MacIntyre et al., 1968, Nadel et al., 1971, Ogawa, 1970), and attenuation with cooling (Bullard et al., 1967, Crawshaw et al., 1975, Nadel et al., 1971), despite increases in core body temperature and/or a stable whole body skin temperature. Additionally, reduced sweat rates are reported during limb ischemia where blood flow is severely reduced (MacIntyre et al., 1968, Collins et al., 1959).

Wingo *et al.,* (2010) recently established that decreases in skin blood flow and local skin temperature independently attenuate sweat rate relative to mean body temperature during whole-body heat stress. The precise mechanisms for this modulation have not been elucidated, but may relate to reductions in shear stress-mediated NO release inducing reduced sweating (Lee and Mack, 2006, Welch et al., 2009) secondary to skin blood flow. There may also be direct effects of decreased local temperature on sweat gland receptors or neurotransmitter release, or an indirect temperature lowering effect of reduced blood flow (Wingo et al., 2010).

2.2.4 Core body temperature threshold and sensitivity

Reflex control of skin blood flow and sweating (effectors) can be studied by evaluating the response as a function of body temperature, with factors that influence threshold and sensitivity of the effector responses providing insight into altered central and peripheral control mechanisms (Charkoudian and Stachenfeld, 2014, Shibasaki et al., 2006). A core body temperature threshold can be identified at which the onset of the effector response occurs, with the slope of the post-threshold relationship analysed to provide an index of sensitivity to increments in core body temperature (Figure 2.5).

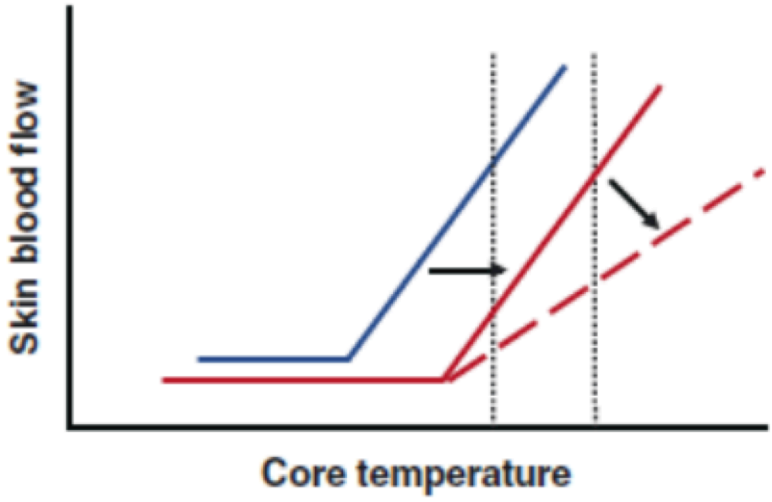


Figure 2.5 Schematic example of cutaneous vasodilation (and/or sweating) as a function of core body temperature. As core temperature increases, a threshold is reached at which the heat dissipation mechanism begins to increase. The slope of the relationship beyond this threshold is referred to as the sensitivity. A rightward shift in the threshold and/or a decrease in the sensitivity will decrease the heat dissipation for a given core temperature. Vertical lines show the change in the amount of a given effector response caused by a shift in threshold or sensitivity (Charkoudian and Stachenfeld, 2014).

A rightward shift in the core temperature threshold and/or a decrease in sensitivity will decrease the amount of heat dissipation for a given core temperature resulting in a less efficient heat loss. Conversely, a shift to the left or increase in sensitivity will result in a

more efficient heat loss. Examples of factors that can modify the heat loss mechanisms of cutaneous vasodilation and sweating are exercise training (Ichinose et al., 2009, Ichinose-Kuwahara et al., 2010, Okazaki et al., 2002), heat acclimation (Armstrong et al., 2005, Lorenzo et al., 2010) ageing (Smith et al., 2013a, Holowatz and Kenney, 2010) and reproductive hormone status (Kuwahara et al., 2005a, Charkoudian and Stachenfeld, 2014, Charkoudian et al., 1999).

2.2.5 Reproductive hormones and temperature control

The cyclical changes in oestrogen and progesterone during the menstrual cycle modify resting core body temperature with an increase of ~0.5°C, in the mid-luteal phase when progesterone and oestrogen are elevated compared with early-follicular when both hormones are low (Charkoudian and Johnson, 2000, Inoue et al., 2005). These alterations also cause a rightward shift in the core temperature threshold for both active cutaneous vasodilation and sweating in response to heat stress during the mid-luteal phase of the menstrual cycle (Charkoudian, 2010, Charkoudian and Johnson, 1997a, Inoue et al., 2005), but no differences in cutaneous vasodilation sensitivity or total sweating output (Inoue et al., 2005). This shift also occurs with exogenous progestins and oestrogens found in oral contraceptives (Stachenfeld et al., 2000, Charkoudian and Johnson, 1997b, Grucza et al., 1993, Houghton et al., 2005), and is consistent with a shift in the onset of reflex cutaneous vasodilation in response to an elevated core temperature during passive heating (Charkoudian and Johnson, 1997a) and acute exercise (Rogers and Baker, 1997). This shift also persists in the presence of local noradrenergic inhibition using bretylium highlighting a shift in the central (neural) control of the cutaneous active vasodilator system to higher internal temperatures with these hormones (Charkoudian and Johnson, 1997b, Charkoudian and Stachenfeld, 2014). The sensitivity of cutaneous active

vasodilation beyond the threshold appears to be unaltered by oestrogen with increasing internal body temperature during heat stress (Stephenson and Kolka, 1993). Conversely, rising oestrogen (but low progesterone) during the pre-ovulatory phase is characterised by a lower resting core body temperature and cutaneous and sweating thresholds at rest and during exercise (Stephenson and Kolka, 1999).

With regard to the specific effects of each hormone, oestrogen appears to have a decreasing effect on core body temperature and thermoregulatory thresholds whereas progesterone stimulates the opposite effect and promotes increases in core body temperature during the menstrual cycle (Charkoudian and Johnson, 2000), with the effects of progesterone appearing to dominate the effects of oestrogen when both are elevated (Stephenson and Kolka, 1985, Stephenson and Kolka, 1993, Kolka and Stephenson, 1989). In summary, female reproductive hormones have widespread influences on the control of thermoregulatory mechanisms, specifically affecting the cutaneous circulation, sweating responses and temperature control.

2.2.6 Ageing and temperature control

With human ageing, even in the absence of pathology, the vascular circulations as well as their control mechanisms are attenuated (Holowatz and Kenney, 2010, Holowatz et al., 2007, Tew et al., 2012b), rendering aged individuals more vulnerable to heat-related cardiovascular complications and hyperthermia (Willie et al., 2014). It is difficult to elucidate differences in male and female temperature control with ageing alone as primarily studies use either older males or males and females combined (~65 years old). For the purpose of this section, ageing is reviewed without specific emphasis on

menopausal status (see section 2.8.1 for temperature control in post-menopausal females).

Control in reflex neural and local mechanisms of the cutaneous circulation change with advancing age (Holowatz et al., 2007). The combined effect is the attenuation of cutaneous vasodilator thresholds and sensitivities to whole-body and/or local heat stress (Holowatz and Kenney, 2010). Ageing is known to reduce the cardiac output (due to a lower stroke volume) and blood flow response to whole-body heat stress, whilst ~30% less blood flow is redistributed to the skin during thermal stress in older populations (Minson et al., 1998).

Reductions in skin blood flow with ageing are also a consequence of vasomotor control in the skin itself (Johnson et al., 2014). A reduced magnitude of cutaneous vasodilation during heat stress has been reported (Pierzga et al., 2003), and attributed to attenuation in NO-dependent mechanisms in older compared to younger males and females (Holowatz et al., 2003, Minson et al., 2002). Furthermore, the substrate L-arginine (a substrate required for NO production by NOS) is reduced with age due to upregulation of arginase (which competes with NOS to convert L-arginine) and is associated with endothelial dysfunction with advancing age (Holowatz et al., 2006b). This age-related decline in NOS has also been attributed to an age-associated increase in oxidative stress that limits NO-bioavailability due to NO 'quenching' (Holowatz et al., 2006a, Stanhewicz et al., 2012).

An age-related decline in thermoregulatory sweating is observed due to decreased thermal sensitivity to passive heat stress or exercise (Natsume et al., 1992, Armstrong and

Kenney, 1993), and sweat gland atrophy (Kondo et al., 2001, Kondo et al., 1998) that produces a lower sweat output during thermal stress. However this has only been reported in older males. Recently, Smith *et al.* (2013) reported greater male and female (~65 vs ~23 years old) age-related decrements in regional sweating compared with cutaneous vasodilation during whole-body heat stress that were not attributable to decreased post-synaptic cholinergic sensitivity. This suggests regional age-related decrements in heat-activated sweat gland function but not cholinergic sensitivity that exhibit greater age-related decrements compared to the attenuation in skin blood flow (Smith et al., 2013a).

2.2.7 Exercise training and thermoregulatory adaptations in females

Exercise training has direct effects on thermoregulatory adaptation (Ichinose-Kuwahara et al., 2010, Inoue et al., 2005). Gender differences exist in the control of sweat rate (see 2.2.5 reproductive hormones and temperature control), independent of training status, with smaller increases in the sweat gland responses to physical training in females than males that is suggested to be due to the inhibitory action of oestrogen on peripheral sweat gland activation with exercise training (Ichinose-Kuwahara et al., 2010).

The function of the thermoregulatory control system (i.e. sweating and cutaneous vasodilation) during heat stress can be enhanced with exercise training in pre-menopausal females (Armstrong et al., 2005, Ichinose et al., 2009, Araki et al., 1981). For example, 60 days of endurance cycle exercise improved heat loss via reduced time to the onset of sweating and increased peak sweat output in the luteal phase during exercise in an a hot environment (~30^{o}C) (Araki et al., 1981), Furthermore, 3 months of moderate intensity endurance training can enhance the efficiency of the thermoregulatory control

system by decreasing the core temperature threshold for the onset, and sensitivity of sweating and cutaneous vasodilation independent of menstrual phase (Ichinose et al., 2009). Importantly, exercise training status has also been shown to lower basal core body temperature and reduce physiological strain during both acute exercise and passive heating in young trained females compared to untrained counterparts (Kuwahara et al., 2005a, Kuwahara et al., 2005b).

Enhanced thermoregulatory function in trained pre-menopausal females is evident irrespective of menstrual phase compared to untrained females (Kuwahara et al., 2005a), whilst an 8-week period of exercise training (60-85% VO_{2max}) and passive heat acclimation (using a heat chamber ~36.5^{o}C) with or without exogenous oestrogen administration in females decreased basal core body temperature with no change in sweating sensitivity (Armstrong et al., 2005). However, the study design made it difficult to establish whether the effects of exercise training and passive heating acclimation were exclusively independent of each other. Advances in sweating sensitivity with exercise training have been suggested to be dependent on subsequent improvements in aerobic capacity (~15-20%) (Ichinose et al., 2009, Pandolf, 1979), with such improvements unattained following exercise training in the Armstrong *et al*. (2005) study.

Heat acclimation, involving repeated exposure to heat stress (i.e. during exercise in a hot environment) has been shown to improve thermoregulatory control by decreasing the internal/body temperature threshold for the onset of sweating and cutaneous vasodilation (Armstrong et al., 2005, Kuwahara et al., 2005a, Kuwahara et al., 2005b, Lorenzo and Minson, 2010) and/or the slope of the sweating and cutaneous vasodilation response (Inoue et al., 2005, Lorenzo and Minson, 2010, Takeno et al., 2001, Sawka et al.,

1989, Sawka et al., 2000). Repeated exposure to external heat stress independent of exercise training (e.g. resting in a hot and humid environment) has only been employed in a small number of studies using a short time-frame i.e. 2 weeks of heat acclimation to induce thermoregulatory adaptations, including reduced basal core body temperature and the earlier onset of sweating in both males and females (Eichna et al., 1950, Fox et al., 1963a, Fox et al., 1963b, Shido et al., 1999, Armstrong and Kenney, 1993). Whether thermoregulatory adaptations can be enhanced to a similar degree with exercise training or passive heat acclimation over a longer time-frame i.e. 8 weeks, in females is yet to be considered.

In summary, temperature control is important to consider in females as it is regulated by reproductive hormone status that can delay the temperature thresholds for cutaneous vasodilation and sweating. Additionally, temperature control is affected in older females via a reduced capacity of the cutaneous vasculature and function of the sweat glands. However, in younger females, differences in temperature control are offset, and improved, by regular physical exercise training over a number of weeks. It is also possible that targeting the thermoregulatory control system with a specific exercise-independent heat stress intervention i.e. passive heating, may induce similar thermoregulatory improvements in the efficiency of sweating and cutaneous vasodilation to that of exercise training in pre-menopausal females.

2.3 Conduit artery function

Conduit arteries are characterised by a thick tunica-media that contain a larger amount of smooth muscle cells compared to other branches in the arterial tree (e.g. resistance

arteries and cutaneous vessels; Figure 2.6). It is the contractility of these vessels that allows them to actively vasodilate either by sympathetic control or circulating vasoactive substances. The endothelium produces numerous paracrine substances, including nitric oxide (NO) which helps regulate vasomotor function and the health of the vessel wall. NO is synthesised in endothelial cells from the amino acid L-arginine through the action of eNOS (Palmer et al., 1988). NO rapidly diffuses into the vascular smooth muscle cell where it binds to the enzyme guanylate cyclase (Ignarro et al., 1986), resulting in an increase in cyclic guanosine monophosphate that induces smooth muscle relaxation and subsequent vasodilation (Green et al., 2004b, Furchgott and Jothianandan, 1991). The function of the endothelium is vital to maintain arterial health, and in addition is important in regulating blood flow to skeletal and cardiac muscle at rest and during elevated metabolic demand.

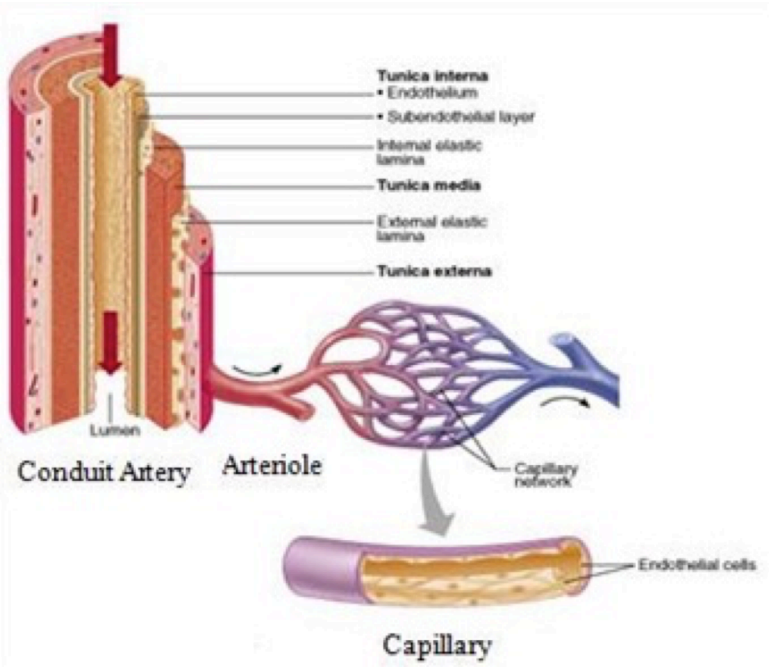


Figure 2.6 The arterial tree from conduit vessels through to capillaries

NO is tonically secreted by the endothelium and contributes ~50% to basal vascular tone, while NO production can be upregulated via increases in blood flow and shear stress. The signalling cascade leading to the secretion of NO, whilst not entirely understood, has been characterised. Increases in arterial shear stress have been reported to induce endothelial potassium channel activation (Oleson and Johnson, 1988, Dull and Davies, 1991), calcium influx in endothelial cells (Dull and Davies, 1991) and release of bradykinin and/or phosphorylation of serine residue (Groves et al., 1995) that enhance NO bioavailability. Furthermore, prostacyclin and EDHF are also released by the endothelium in response to shear stress and are known to be vasoactive (Grabowski et al., 1985, Spilk et al., 2013). NO bioavailability is commonly used as a surrogate marker of endothelial (dys)function (Wilk et al., 2013) and can be assessed using the flow-mediated dilation (FMD) technique. FMD is regarded as a surrogate marker of cardiovascular disease (Green et al., 2011, Vita and Keaney, 2002, Mutlu et al., 2011), with its impairment significantly associated with future cardiovascular events in asymptomatic individuals (Shechter et al., 2009, Inaba et al., 2010).

2.3.1 Reproductive hormones and conduit artery function

Oestrogen promotes the formation of the vasodilator substance prostacyclin (Novella et al., 2012), modulates numerous molecular pathways that improve vascular function (Tostes et al., 2003), may confer direct protection on the vessel wall (Williams et al., 2001, Hashimoto et al., 1995, Tostes et al., 2003), and is suggested to improve endothelial function (Arora et al., 1998). A change in reproductive hormone levels affects the circulating factors, eNOS and vascular endothelial growth factor (VEGF) demonstrating cyclic variation in line with the menstrual cycle (Taguchi et al., 2000, Agrawal et al., 1999). This variation in endothelial-vasodilators has led to the idea that changes can occur in

conduit artery function during the menstrual cycle. Changes in endothelial-dependent conduit function during the menstrual cycle using FMD has been numerously demonstrated (Kawano et al., 1996, Williams et al., 2001, Hashimoto et al., 1995), reporting a higher brachial artery endothelial-dependent vasodilation in the follicular phase compared to the luteal phase in pre-menopausal females. One study reported no influence of reproductive hormone status on carotid and femoral conduit artery function across the menstrual cycle (Willekes et al., 1997), however the differences may relate to methodological discrepancies with Willekes *et al.* (1997) using peripheral brachial pressures to investigate changes in systemic responses i.e. carotid and femoral function. It is suggested that the rise and fall in oestrogen during the follicular and luteal phases, respectively, is responsible for these changes in vascular function primarily via its ability to upregulate eNOS activity in the vasculature (Gonzales et al., 2001, Hayashi et al., 1995).

2.3.2 Ageing and conduit function

Ageing is associated with a reduction in NO bioavailability (Nyberg et al., 2012) and endothelial dysfunction (Holowatz et al., 2010, Parker et al., 2006, Celermajer et al., 1994) that is accompanied by increased levels of several cardiovascular risk factors and incidence of cardiovascular disease (Seals et al., 2006). Brachial artery FMD is impaired, independent of traditional cardiovascular risk factors, in late peri- to early post-menopausal females compared to pre-menopause (Figure 2.7) (Moreau et al., 2012). To what extent the decline in endothelial-dependent vascular function in post-menopausal females is a function of ageing and/or a consequence of reduced oestrogen is difficult to elucidate (Bittner, 2009, Moreau et al., 2012).

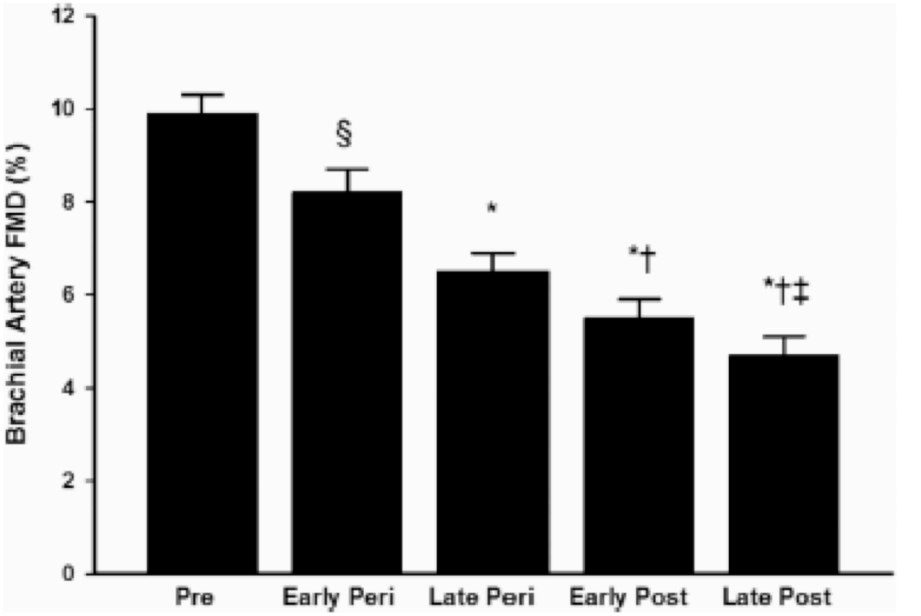


Figure 2.7. Brachial artery FMD in premenopausal females (Pre) and across the menopausal transition into late post-menopause (late post) (Moreau et al., 2012)

2.3.4 Exercise training and conduit function

Exercise training improves endothelial function in populations at risk of cardiovascular disease (Thijssen et al., 2010), with the benefits predominantly focussed on the vascular endothelium (Green et al., 2004b). The benefits in asymptomatic populations with healthy function are less consistent (Green et al., 2004b, Maiorana et al., 2003, Goto et al., 2003). It is proposed that these differences occur due to the level of endothelial dysfunction induced by exercise (Goto et al., 2003). Furthermore, Tinken and colleagues recently examined the time course of change in conduit artery endothelial function and subsequently established that vascular function increases initially, but then declines to pre-training levels in healthy young individuals suggesting modification in arterial structure i.e. a larger vessel following initial improvements in endothelial function (Tinken et al., 2008, Tinken et al., 2010). Importantly, the decline in NO bioavailability with ageing is offset with lifelong regular physical activity (Black et al., 2009, Nyberg et al., 2012), with trained older participants demonstrating a higher eNOS response following arterial ACh

infusion and increased endothelial-dependent vasodilation, compared to their untrained counterparts (Nyberg et al., 2012).

In summary, conduit artery function is altered by reproductive hormone status throughout the menstrual cycle primarily through the actions of oestrogen on endothelial-dependent vasodilation. With ageing, pre-menopausal females have a maintained vascular function compared to males of a similar age, whilst reductions in NO bioavailability with age reduce vascular function in both genders. Exercise training enhances conduit artery function in the presence of previous endothelial dysfunction i.e. cardiovascular disease, alongside attenuating the reduction in vascular function observed in older individuals who have a history of exercise training.

2.4 Cerebral Blood Flow

The regulation of cerebral blood flow (CBF) is critical for the maintenance of oxygen and nutrient supply to the brain. Despite the brain only occupying 2-3% of total human body mass, the metabolic demand of the cerebral tissue is ~20% of total body oxygen consumption (Bain et al., 2014). Consequently, substantial reductions in CBF rapidly leads to unconsciousness (Van Lieshout et al., 2003) and if sustained, brain damage and death ensues (Smith et al., 2011). The control of CBF is a multifactorial integrated process, influenced largely by the partial pressure of arterial carbon dioxide (P_aCO_2), mean arterial pressure (MAP), cerebral metabolism and the autonomic nervous system (Figure 2.8) (Ainslie and Bailey, 2013).

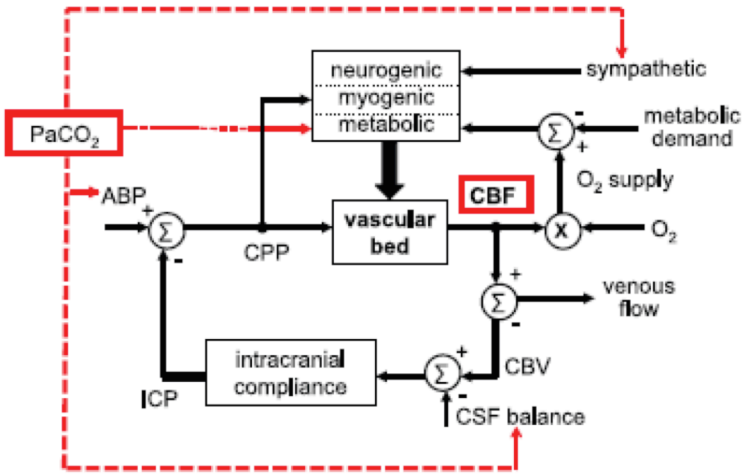


Figure 2.8. Diagram of the main mechanisms responsible for control of cerebral blood flow (CBF) (Ainslie and Duffin, 2009).

Steady-state CBF is reported to remain relatively stable across a wide range of blood pressures i.e. ~60-160 mmHg (Paulson et al., 1990), which requires reflex adjustment in cerebrovascular resistance in response to changes in blood pressure. This physiological response is termed cerebral autoregulation (Franco Folino, 2007, ter Laan et al., 2013, van Beek et al., 2008, Willie et al., 2014). Current studies illustrate that the CBF-MAP relationship is not stable through a broad range of pressures and is dependent on the severity and direction of change in perfusion pressure above or below baseline (Tan and Taylor, 2014, Willie et al., 2014). Blood pressure is a critical component of cerebral autoregulation and CBF (Lucas et al., 2010), with both hypoxia and elevations in P_aCO_2 impairing the capability of the brain, unable to defend against blood pressure changes (Tzeng et al., 2012).

2.4.1. Cerebral blood flow and heat stress

Heat stress results in marked reductions in CBF and reduced orthostatic tolerance (Bain et al., 2014, Brothers et al., 2009, Keller et al., 2009, Low et al., 2008b), with reductions in central blood volume and end-tidal carbon dioxide contributing to the observed

reductions in brain blood flow (Brothers et al., 2009, Nelson et al., 2011). As CBF is highly sensitive to arterial hypocapnia (hyperventilation-induced reduction in CO_2) (Fan et al., 2008), hyperthermia-induced hyperventilation and consequent hypocapnia severely reduces CBF (Crandall et al., 2008), and is proposed to account for ~50% to the reductions in cerebrovascular conductance (Wilson et al., 2006). This is supported by Brothers *et al.* (2009) who recently found that restoration of end-tidal $PaCO_2$ during passive heat stress partially restored CBF to normothermic levels. Additionally, the neurogenic response during hyperthermia, resulting in cerebral vasoconstriction via increased sympathetic nerve activity, is also suggested to account towards the observed CBF reduction (Brothers et al., 2009).

Steady state blood pressure, and hence perfusion pressure to the brain, during mild passive supine hyperthermia is only moderately decreased, with cerebral autoregulation unaltered (Crandall and González-Alonso, 2010, Low et al., 2009), but decreases significantly under dynamic blood pressure challenges that can severely compromise CBF i.e. orthostatic tolerance or haemorrhage (Brothers et al., 2011). It is suggested that the redistribution of cardiac output to the cutaneous circulation during heat stress may be responsible for reductions in CBF perfusion (Ogoh and Ainslie, 2009), with passive hyperthermia-induced elevations in cardiac output relating to decreased middle cerebral artery velocity (MCAv) (Fan et al., 2008). Nevertheless, cardiac output was unrelated to reductions in CBF during passive heat stress in one study, that instead suggested stroke volume and end-diastolic volume were responsible for the observed reductions in CBF during heat stress (Nelson et al., 2011). The level of heat stress in inducing reductions in CBF, and as such the ranges of cardiac output, may be responsible for this discrepancy.

Stroke volume shares a direct linear relationship with central blood volume (Levine et al., 2002), which decreases during hyperthermia (Kempton et al., 2009) and may result in decreases in CBF. However, acute blood volume expansion does not reverse the hyperthermia-induced reductions in CBF at rest (Schlader et al., 2013), but did improve maintenance of CBF during a simulated haemorrhage that was likely a function of augmented stroke volume and the preservation of arterial blood pressure prior to, and during the simulation. This suggests a possible role for carotid and/or pulmonary baroreceptor control over CBF during hyperthermia (Charkoudian et al., 2005, Nelson et al., 2011, Levine et al., 2002).

2.4.2 Reproductive hormones and cerebral blood flow

The interactions between reproductive hormones, namely oestrogen, and the cerebrovascular system are complex and not fully understood (Diomedi et al., 2001). A variety of evidence suggests oestrogen protects against cerebrovascular disease in pre-menopausal females (Sarrel, 1999, Krejza et al., 2004), who are at a lower risk of stroke at pre-menopause compared to males of a similar age (Yoo et al., 1998, Caplan et al., 1986), while risk dramatically increases following the menopause when oestrogen is severely reduced (van der Schouw et al., 1996). Furthermore, treatment with oestrogen prior to induced cerebral ischemia in an animal model of both sexes decreases brain injury, which is suggested to be due to an oestrogen receptor-mediated vasodilation (Alkayed et al., 2000, Sawada et al., 2000).

Diomedi *et al.* (2001) established peak levels of oestrogen during the pre-ovulatory phase are associated with an increased cerebrovascular CO_2 reactivity using the breath-holding index compared to the menstrual phase. Despite potential influences of oestrogen on

cerebral autoregulation and thus vasodilatory capacity, the authors found no differences on basal MCAv in the two menstrual phases. Furthermore, along with increases in oestrogen during the late follicular phase and pre-ovulation, a decrease in cerebral vascular resistance (indexed by internal carotid pulsatility index) was observed in young females (Krejza et al., 2004), that may promote the notion of an oestrogen-related elevation in CBF caused primarily via reductions in cerebral vascular impedance during the menstrual cycle. This is further supported by an elevated reactivity to CO_2 in pre but not post-menopausal females which is attributed to differences in oestrogen concentrations on the cerebrovasculature (Kastrup et al., 1998, Belfort et al., 1995).

2.4.3 Ageing and cerebral blood flow

CBF declines gradually with age in adults between 20-80 years of age, by ~5% per decade (Ainslie et al., 2008, Scheel et al., 2000, Stoquart-ElSankari et al., 2007, Fisher et al., 2013), with ageing associated with global cerebral atrophy that reflects a global decrease in cerebral perfusion without disturbance of regional perfusion or oxygen consumption (Ainslie and Bailey, 2013, Ainslie et al., 2008). The effect of healthy ageing on cerebrovascular reactivity is less clear, with no or little changes to both hypercapnic-induced elevations and hypocapnic-induced reductions in older populations (Kastrup et al., 1998, Galvin et al., 2010, Ito et al., 2002, Tsuda and Hartmann, 1989).

2.4.4 Exercise training and cerebral blood flow

CBF is elevated during submaximal exercise in both young and older individuals by ~5-15% (Ainslie et al., 2008, Ogoh et al., 2005, Fisher et al., 2013, Marsden et al., 2012), and is intensity-dependent up to ~60-70% $\dot{V}O_{2max}$ (Hellstrom et al., 1996, Murrell et al., 2013) and then declines due to hyperventilation-induced hypocapnia that reduces cerebral perfusion (Moraine et al., 1993). Furthermore, MCAv increases to a lesser extent in older

compared to younger individuals during acute submaximal exercise (Murrell et al., 2013, Marsden et al., 2012). Exercise training exerts positive effects on CBF, as age-induced cerebral atrophy appears to be less marked in those with a higher aerobic fitness, increases brain volume in sedentary adults (Colcombe et al., 2003, Colcombe et al., 2006) and improves cerebrovascular reactivity in stroke patients (Ivey et al., 2011), but not basal blood flow, following 6 months of exercise training. Animal studies provided preliminary evidence that the benefits of exercise training may extend to the cerebrovasculature with improvements in CBF and reductions in stoke-induced infarct size following a period of exercise training via improved angiogenesis and elevated CBF (Gertz et al., 2006, Endres et al., 2004). The corollary is that MCAv is increased by ~10-25% with a higher aerobic fitness across the human adult lifespan (Ainslie et al., 2008), resulting in enhanced grey matter volume (Colcombe et al., 2006), improved NO bioavailability (Green et al., 2004a) and cerebrovascular angiogenesis (Rhyu et al., 2010, Swain et al., 2003).

Murrell *et al.* (2013) recently established that a 12-week aerobic exercise training intervention resulted in an elevation in cerebrovascular reactivity to hypercapnia at rest and during sub-maximal exercise in young and old individuals. Importantly, this fitness-induced elevation in cerebrovascular reactivity was independent of age. This observation may relate to improved resting MCAv, but when normalised for changes in blood pressure, cerebrovascular conductance was unchanged (Murrell et al., 2013). Furthermore, there was no effect of gender on the cerebrovascular reactivity response at baseline or following exercise training across the adult lifespan (Murrell et al., 2013).

In summary, there may be an oestrogen-related increase in CBF during the menstrual cycle, however despite increases in cerebrovascular reactivity and decreases to

cerebrovascular resistance in the presence of elevated oestrogen no study to date has reported oestrogen-related increases in basal CBF *per se*. CBF declines with age but this can be offset by both lifelong and short-term (~12 weeks) endurance exercise training in individuals with or without cardiovascular disease i.e. stroke rehabilitation.

2.5 The Menopause

As females approach the menopause, menstruation becomes irregular and less frequent. This portion of menstrual life is termed peri-menopause, and is known as the menopausal transition (Figure 2.9). The peri-menopause is associated with a gradual increase in FSH that leads to irregular ovulation, lasting ~3-5 years with the lower limit of the menopausal transition the onset of irregular menstruation that occurs at ~47 years of age, with the upper limit being the final menstrual period (McKinlay et al., 1992). The World Health Organisation (WHO) defines the menopause as the permanent cessation of menstruation resulting from loss of ovarian follicular activity (WHO, 1996), that occurs between the ages of 45 and 55. Due to an increasing life expectancy females spend a larger portion of their lifespan in the postmenopausal state. In 2007, 35 million females in the United States were postmenopausal with the number increasing at a rate of over a million each year (Deecher and Dorries, 2007), while the Office for National Statistics reported there were ~5 million females aged between 45-55 in the UK in 2011 (ONS, 2012).

The menopause is characterised by a marked decrease in oestrogen production from the ovaries that become insensitive to FSH, and results in the loss of reproductive function and an increasing incidence of HFs (Figure 2.9). It has become clearer over the past 25 years that the ubiquitous presence of the oestrogen receptor in various body tissues and

organ systems provides the substrate for oestrogenic regulation or the modulation of multiple body functions (Yang et al., 1996). These hormonal changes affect the thermoregulatory and cardiovascular systems in addition to the ageing processes discussed previously.

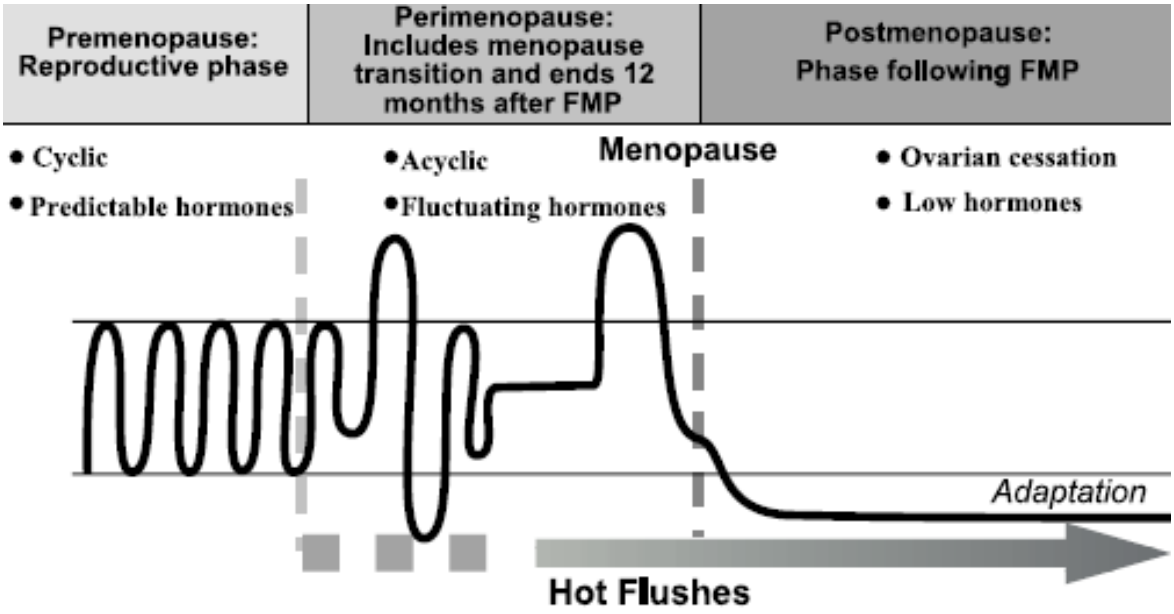


Figure 2.9. Relationship between oestrogen and reproductive phases. During perimenopause, hormones fluctuate and become acyclic. During this period, many females experience hot flushes; although severe the frequency is transient. During the postmenopausal period, females can experience severe and persistent hot flushes due to the declining levels of ovarian hormones. *FMP* final menstrual period (Deecher and Dorries, 2007).

2.6 Menopausal hot flushes

HFs, also known collectively as hot flashes or vasomotor symptoms, are a primary symptom of the menopause that can seriously disrupt the lives of symptomatic females (Kronenberg, 1990, Freedman, 2001). A HF is the subjective sudden sensation of heat that is associated with objective signs of rapid increases in cutaneous vasodilation, sweating and flushing that generally begin in the chest and radiate to the face, head and arms (Stearns et al., 2002, Freedman, 2002).

Symptomatic females also report a range of additional symptoms such as anxiety, embarrassment, depression, frustration and nausea (Freedman, 2005, Freedman, 2014a, Dormire, 2003). HF frequency and severity is individualised, with some females suffering in excess of 30 daily severe flushes, while HFs are even higher in females following surgically-induced menopause and in female oncology patients (Bachmann, 1999, Gupta et al., 2006, Carpenter et al., 2002). In addition, HFs can decrease quality of life by having adverse effects on concentration, quality of sleep, mood and sexual function resulting in chronic fatigue and elevated stress levels (Kronenberg, 1990, Daly et al., 1993, Katz-Bearnot, 2010).

HF prevalence among naturally menopausal females has been reported to be between 60-82% in the United States and Australia (Feldman et al., 1985, Guthrie et al., 1996). Kronenberg *et al.* (1990) reported ~70% of females experience HFs for the first 1-5 years after the onset of the menopausal transition, with 10-20% of symptomatic females finding them intolerable. Importantly, HFs can persist for ~15 years in 20% of females (Kronenberg, 1994). In the UK, there are currently over 10 million females over the age of 50 (ONS, 2014), thus over 7 million females in the UK today may have had, or currently be suffering from HFs that effect overall quality of life in this population.

2.6.1. Thermoregulatory dysfunction and hot flushes

The precise mechanisms underlying the pathophysiology of HFs are unclear; however it is well acknowledged that they are a result of central temperature dysfunction (Deecher and Dorries, 2007, Freedman, 2001, Stearns et al., 2002). It is suggested that the menopausal reduction in oestrogen lowers the upper threshold of the thermo-neutral zone in symptomatic females, with Freedman *et al.* (1999) reporting the thermo-neutral

zone to be 0.0°C in symptomatic females compared to 0.4°C in asymptomatic females. Thus, it is suggested that HFs are triggered by small core body temperature elevations acting within a greatly narrowed thermo-neutral zone (Freedman, 2001). This is further acknowledged by the fact that peripheral warming is used to provoke HFs, thus HFs may be triggered when core body temperature exceeds a narrowed sweating threshold (Kronenberg et al., 1984, Low et al., 2010, Low et al., 2008a, Freedman et al., 1995) (Figure 2.10).

A narrowing of the thermo-neutral zone due to increased sympathetic nervous activity with the menopause has been suggested (Figure 2.10) (Freedman, 2014a), while Low *et al.* (2010) reported acute increases in sympathetic neural activity to the skin during a HF, which subsequently returned to baseline after HF cessation. Furthermore, HFs were severely attenuated under local sympathetic cholinergic nerve blockade supporting the conclusion that cutaneous vasodilation during a HF is neurally mediated (Low et al., 2010). Alongside this, Hubing *et al.* (2010) reported that NO, but not prostaglandins, is involved in active cutaneous vasodilation during a HF. Whether the effect of NO is neuronally released, acetylcholine-induced or shear rate mediated is currently unknown. Additionally, NO or prostaglandin inhibition had no effect on sweat rate during HFs, however sweat rate during moderate heat stress is also unaffected by NO inhibition (Holowatz et al., 2005).

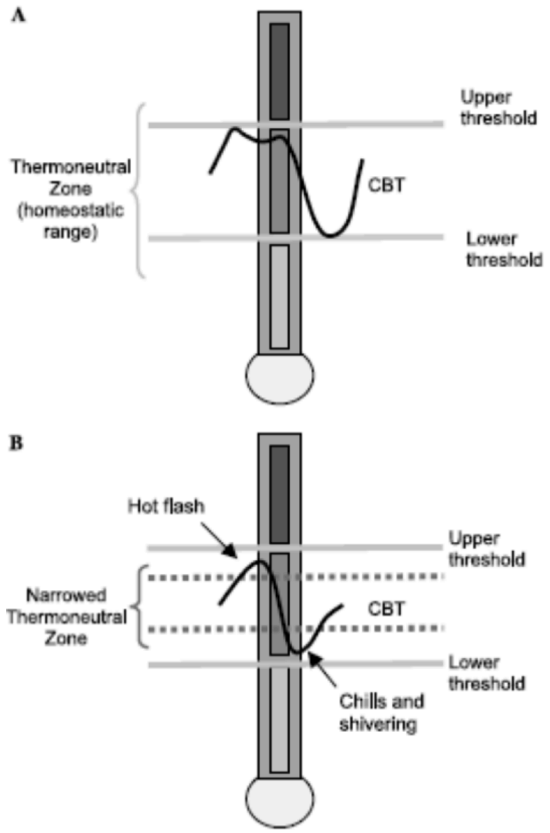


Figure 2.10 Maintenance of core body temperature (CBT). It has been hypothesised that CBT is regulated between an upper threshold for sweating and a lower threshold for shivering. Within the thermoneutral zone, major thermoregulatory responses do not occur. These mechanisms maintain CBT within the designated thresholds. A, normal temperature regulation; B, dysfunctional temperature regulation (Deecher and Dorries, 2007).

Alternatively, HFs are suggested to relate to neuroendocrine mechanisms (de Boer et al., 2009), specifically increases in luteinising hormone (LH) pulses (Tataryn et al., 1979), and to the hypothalamic neural circuitry controlling pulsatile gonadotropin-releasing hormone (GnRH) secretion (Casper et al., 1979, Freedman, 2014a). HFs were reduced by ~30% following LH receptor-agonist treatment (van Gastel et al., 2012) however, the effect of LH is inconsistent as females with no LH pulses due to gonadotropin deficiency (Gambone et al., 1984) or females who are hypophysectomised (pituitary gland removal) (Mulley et al., 1977) still report HFs. Recently, it has been hypothesised that marked changes in hypothalamic kisspeptin neurokinin-B and dynorphin (KNDy) neurons play a role in HFs, as

they relay oestrogen signals to preoptic structures regulating heat peripheral vasodilation (Rance et al., 2013, Mittelman-Smith et al., 2012).

It is also postulated that disturbances to reflex and/or local cutaneous vasodilation may contribute to temperature dysfunction, and subsequently HFs, as changes in vascular reactivity may interfere with the ability of the blood vessels to respond rapidly to temperature changes, resulting in exaggerated responses and HFs (Charkoudian, 2003). This is supported by low levels of estradiol post-menopause, contributing to reduced elasticity of the blood vessels that may result in dysfunctional responses to changes in core temperature (Joswig et al., 1999, Holowatz et al., 2010).

2.6.2 Physiological changes during a hot flush

Elevated skin temperature and skin blood flow cause a rapid increase in peripheral cutaneous vasodilation during a HF (Kronenberg et al., 1984, Freedman, 1998, Low et al., 2010). This is followed by an increase in sweating in ~90% of females who suffer from HFs (Freedman, 1998, Low et al., 2010) (Figure 2.11). Freedman *et al.* (1995) found small elevations (~0.04°C) in core temperature ~30 mins prior to recorded HFs and replicated these findings in two subsequent studies (Freedman, 1998, Freedman and Krell, 1999). However, possible explanations for the increases in core temperature prior to a HF are limited, with increases in metabolic rate (heat production) and/or peripheral vasoconstriction (decreased heat loss) currently unfounded (Freedman, 2014a). Furthermore, small variations in mean core temperature are not different between symptomatic and asymptomatic post-menopausal females (Freedman, 2002). HFs induce modest increases in ventilation and heart rate by approximately 10-15 beats per minute (Freedman, 1989, Low et al., 2008a) and induce short-term reductions in arterial blood

pressure in many symptomatic females (Low et al., 2008a). Recently, it was also demonstrated that ~76% of HFs are accompanied by a significant reduction CBF, that are not related to the observed drop in arterial blood pressure (Lucas et al., 2013). These data indicate that a HF can present a significant physiological challenge to brain blood flow, and that feeling of faintness and nausea during a HF may be associated with these short-term reductions (Dormire, 2003, Lucas et al., 2013).

In summary, the mechanisms of HFs are still unclear; but are primarily a consequence of reductions in oestrogen during the menopause that is known to have direct effects on thermoregulatory control mechanisms, the sympathetic nervous system and vascular function (Figure 2.12), while prior elevations in core body temperature alongside cutaneous vasodilation and sweating during a HF coincide with reduced blood pressure and brain blood flow during a HF *per se*. A treatment targeting these systems may potentially alleviate HFs (see HF treatment 2.7).

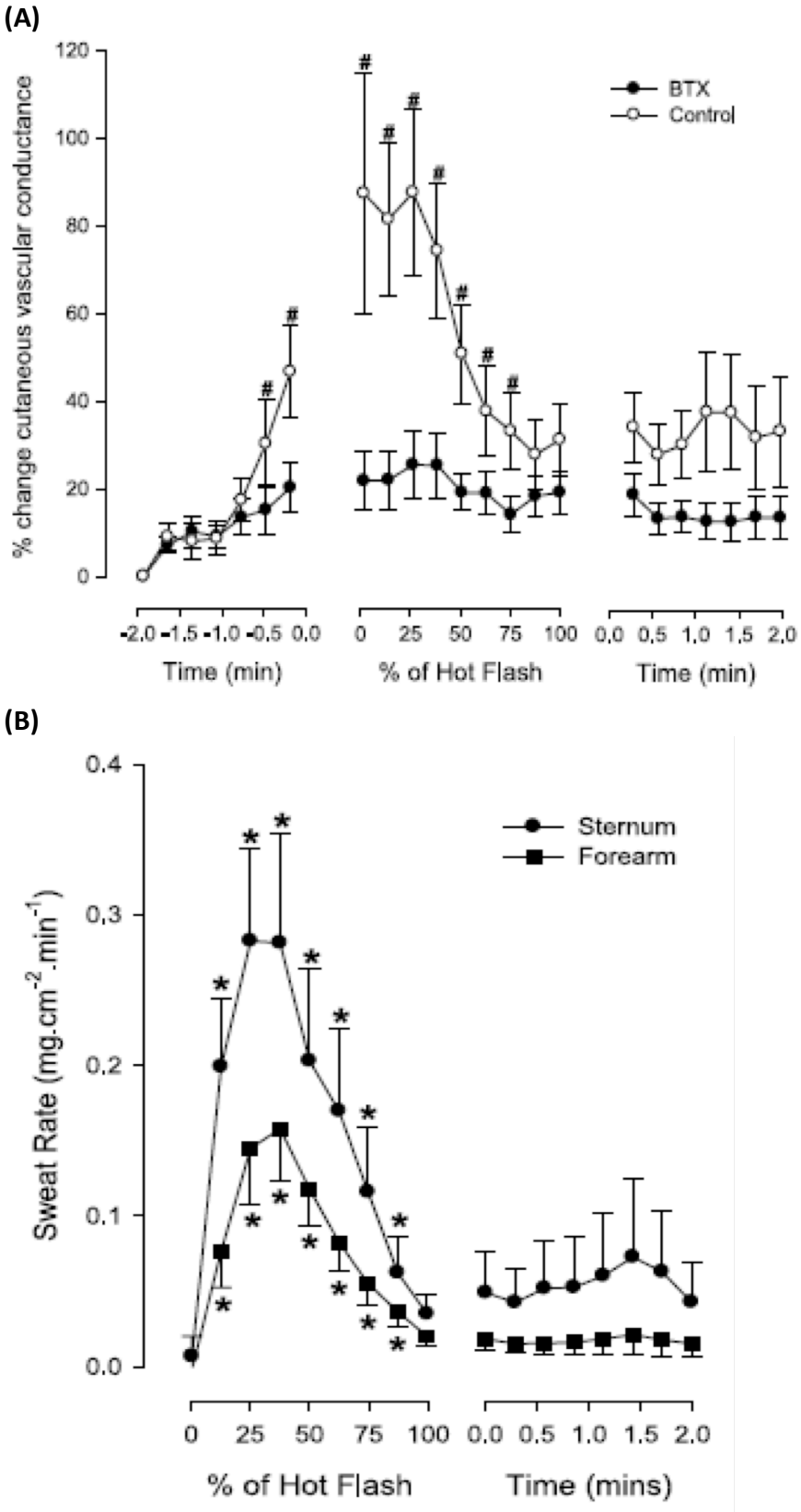


Figure 2.11 Changes during a hot flush in (A) forearm cutaneous vascular conductance at control and BTX treated sites (B) sweat rate at the sternum and forearm (Low et al., 2010).

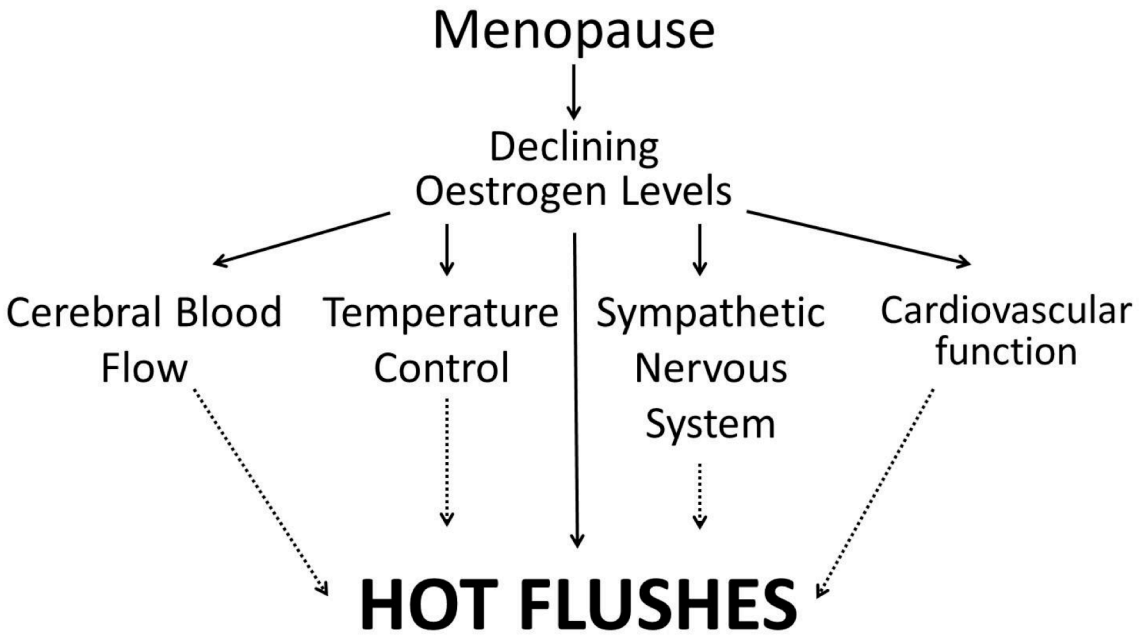


Figure 2.12 Diagram summarising the potential physiological mechanisms causing hot flushes

2.6.2.1 Hot flushes and cardiovascular risk

Cardiovascular disease risk largely increases following the menopause due to oestrogen reduction (van der Schouw et al., 1996, Ossewaarde et al., 2005, Zaydun et al., 2006). The menopause is accompanied by unfavourable levels of cardiovascular risk factors, including increases to low-density lipoproteins, blood pressure (Zaydun et al., 2006, Zanchetti et al., 2005), visceral adipose tissue and body mass index profiles (Akahoshi et al., 1996, Casiglia et al., 2002).

HFs are viewed primarily as a symptom of quality of life but little attention has been focussed on the clinical implications of females that suffer from HFs. However, emerging research suggests underlying vascular changes amongst females who suffer from HFs, with the Women's Health Initiative (WHI) indicating that coronary heart disease diagnosis was concentrated among post-menopausal females reporting HF symptoms at study

baseline (Rossouw et al., 2007). Furthermore, females with HFs have reduced endothelial-dependent vasodilation and greater aortic calcification (Thurston et al., 2008b) that have been prospectively linked to increased cardiovascular disease morbidity and mortality (Yeboah et al., 2007, Widlansky et al., 2003, Greenland et al., 2004). Additionally, increased vascular inflammation has been linked to HF severity, indicating increased atherosclerotic risk in this symptomatic group (Bechlioulis et al., 2012). This suggests HFs may be a marker of adverse vascular changes and that the vasculature may play an important role in the physiology of HFs (Thurston et al., 2008b). Endothelial dysfunction was found to be the main determinant of HF severity in early post-menopausal females (Bechlioulis et al., 2010). This supports the potential role of HFs in cardiovascular risk prognosis, whilst it is unknown if the targeted alleviation of HF severity coincides with reductions in cardiovascular risk factors.

2.7 Hormone Replacement Therapy

Due to the causal association between HFs and oestrogen depletion, hormone replacement therapy (HRT), which involves exogenous administration of oestrogen, with or without progesterone, is the first line of treatment for HFs. HRT can be administered orally, intravaginally or transdermally (Stearns et al., 2002). In large, randomised clinical trials HRT decreased HFs by 50-100% in post-menopausal females (McNagny, 1999, Maclennan et al., 2004, Utian et al., 2004).

Despite the benefits of HRT in reducing HFs, the prescription rates of HRT have plummeted in recent years (Isaacs et al., 2005, Townsend and Nanchahal, 2005, Hersh et al., 2004) and by ~50% in the UK since 2002 (Watson et al., 2007). This is due primarily to the results of the WHI (Rossouw et al., 2002) and other clinical trials (Sare et al., 2008,

Archer and Oger, 2012, Manson et al., 2003) which reported increased risk of several vascular outcomes including myocardial infarction, stroke and venous thromboembolism. Furthermore, an increased risk of hormone-dependent cancer is associated with HRT (Holmberg et al., 2008, Manson, 2014, Hou et al., 2013, Beral, 2003, Tworoger et al., 2005). HRT treatment is also limited by time since menopause (Hage and Oparil, 2013), whilst females with a prior history of cardiovascular disease or cancer are not recommended to use HRT (NICE, 2009), as a result females are declining HRT or not visiting their G.P. for treatment because of the associated risk of cardiovascular disease and cancer (Rossouw et al., 2002, Luo et al., 2013). As such, symptomatic females are attempting to live everyday life with these debilitating symptoms with little help because of a lack of therapeutic alternatives.

The administration of HRT is known to affect body temperature control. The exogenous administration of oestrogen reduces basal core body temperature and in response to heat stress in some, but not all, post-menopausal females (Brooks et al., 1997, Freedman and Blacker, 2002, Cagnacci et al., 1992). Freedman and Blacker (2002) further investigated the mechanism by which oestrogen effects temperature control and HFs, with symptomatic post-menopausal females receiving either 1mg·day 17β-estradiol or placebo for 3 months. The sweating threshold was significantly elevated alongside reductions in HF frequency following 17β-estradiol, compared to placebo (Freedman and Blacker, 2002). Dacks and Rance (2010) established that ovariectomised rats without estradiol treatment exhibited a shift of the thermo-neutral zone to lower temperatures and an elevated skin vasodilation. Conversely, an estradiol-induced shift in the thermo-neutral zone resulted in a delayed threshold for skin vasodilation, whilst core body temperature was lowered when exposed to high ambient temperatures following

estradiol treatment compared to ovariectomised rats receiving no treatment (Dacks and Rance, 2010). Thus, in both animal and human studies, the exogenous administration of oestrogen following menopause directly effects core body temperature and heat loss responses involved in the control of core body temperature, although the precise mechanisms responsible for this are not yet understood. Interventions, such as HRT, may ameliorate HFs through improvements in this control system.

2.8 Exercise training and hot flushes

Based on the evidence that menopausal-aged females have low participation rates in exercise (Daley et al., 2009) and severe menopausal symptoms correlate with lower levels of physical activity (Gold et al., 2000, Wilbur et al., 2005, Ivarsson et al., 1998, Karacan, 2010), recent research studies have begun to investigate the feasibility of exercise training as a non-pharmacological alternative to HRT. Several longitudinal studies have reported HFs to be more common in females reporting lower levels of physical activity (Gold et al., 2000, Di Donato et al., 2005, Ivarsson et al., 1998), conversely, habitual physical activity has been reported to have no effect on HFs (Sternfeld et al., 1999, Mirzaiinjmabadi et al., 2006). However, comparisons between studies are difficult due to differences in definitions of physical activity and/or exercise history in these female cohorts.

A Cochrane Review on the evidence for the effect of exercise training on HFs conducted in 2009 was inconclusive primarily due to the lack of robust studies (*n*=6) (Daley et al., 2009). More recently, randomised control trials have reported a reduction in HF symptoms (Moilanen et al., 2012, Luoto et al., 2012, Karacan, 2010, Reed et al., 2014,

Lindh-Astrand et al., 2004), or no effect (Wilbur et al., 2005, Sternfeld et al., 2014). Six-months of unsupervised aerobic exercise (~60% HR_{max}), that consisted predominantly of walking and/or aerobics reported via participant diary-logs improved the number of self-reported HFs, alongside improvements to mood and sleep quality compared to a control condition receiving lifestyle advice (Mansikkamaki et al., 2012, Luoto et al., 2012, Moilanen et al., 2012). However only a single study reported physiological responses to exercise training, with little differences observed in both aerobic fitness (Pre exercise 31.0 vs. 32.5 $ml^{-1}.kg.min^{-1}$ post exercise) and fat-free mass following the exercise intervention (Luoto et al., 2012). Conversely, Sternfeld *et al.* (2014) reported short-term (12 weeks) supervised exercise training (3 sessions per week at ~50-70% HRR) did not significantly reduce HF frequency in peri- and post-menopausal females compared to a control group that maintained their usual activity, but did improve depression symptoms, sleep quality and insomnia following exercise training. However no differences were observed in pedometer steps or BMI along with no direct measure of cardiorespiratory fitness following the exercise training study compared to control, making it difficult to elucidate any physiological adaptations from the exercise intervention.

Significantly, no study to date has investigated the effect of exercise training on objective measures of HFs, or the potential mechanisms responsible for how exercise-mediated changes in thermoregulatory and/or cardiovascular control might reduce HFs in symptomatic post-menopausal females.

2.8.1 Exercise training and thermoregulatory control in post-menopausal females

It is well known that HFs are a consequence of severe temperature dysfunction, with elevated basal core temperature and a narrowed thermo-neutral zone suggested to be primary explanations (see 2.6.1 thermoregulatory dysfunction and hot flushes). Therefore, the thermoregulatory system may be a useful target in alleviating HFs in post-menopausal females using aerobic exercise training that targets central and peripheral thermoregulatory adaptations. Studies investigating thermoregulatory control mechanisms i.e. core body temperature thresholds for cutaneous vasodilation and sweating in post-menopausal females do not currently exist without prior intervention at baseline i.e. HRT (Freedman and Blacker, 2002, Freedman and Dinsay, 2000), nor following a period of exercise training. The literature has established a body of evidence for a blunted effect of sweating and cutaneous vasodilation in older females (~65 years old; see *2.2.6 ageing and temperature control*), however the direct effect of the menopause on temperature control is difficult to elucidate as the studies do not specify about precise menopausal status. A study investigating the temperature thresholds in early post-menopausal females when HF symptoms are most commonly reported, and the effects of ageing are less prominent, is warranted.

Currently, the research evidence suggests improvements in thermoregulatory control via exercise training in post-menopausal females may be linked to changes in sympathetic nerve activity and/or the responses of the peripheral vasculature to exercise training. Sympathetic nerve outflow has been shown to increase following the menopause (Freedman, 2014b, Hart et al., 2011) and during HFs (Low et al., 2010) and is suggested to affect the thermoregulatory and vascular control systems (Charkoudian and Wallin,

2011). However, sympathetic outflow is reduced in post-menopausal females following an acute cycling exercise bout (45 min at 50% $\dot{V}O_{2peak}$) (Oneda et al., 2008) and chronic aerobic exercise training (Oneda et al., 2014) that may have direct effects on thermoregulatory and cardiovascular function in this population.

Oneda *et al.* (2014) reported an increase in forearm blood flow following 12-weeks of moderate intensity exercise training (HR at ~anaerobic cycling threshold) in healthy post-menopausal females compared to a no-exercise control group. Maximal cutaneous vasodilation and microvascular reactivity were enhanced after 24 weeks of moderate intensity exercise (~45-70% HRR) in post-menopausal females with improvements correlating to aerobic fitness (Tew et al., 2012a, Hodges et al., 2010), highlighting that vasodilatory responses of the cutaneous vasculature to exercise training is not lost in post-menopausal females, suggesting the training effects on endothelial function are potent, and not blunted by the absence of oestrogen or ageing (Oneda et al., 2014). Post-menopausal females with a history of exercise training exhibited similar basal core body temperatures and sweat rate thresholds and sensitivities to pre-menopausal females during both exercise-induced and passive heat stress (Kenney and Anderson, 1988, Drinkwater et al., 1982), but sweat rate sensitivities are blunted in both pre and post-menopausal cohorts that demonstrated sedentary lifestyles (Drinkwater et al., 1982).

2.8.2 Exercise training and conduit function in post-menopausal females

A reduced brachial artery FMD in early and late menopausal females is common (Moreau et al., 2012). This is primarily due to the loss of protective effects of oestrogen on the vascular endothelium in post-menopausal females (Nyberg et al., 2014, Celermajer et al.,

1994) (see *2.3 conduit artery function*). However, distinguishing reduced function as a consequence of the menopause and/or ageing is difficult.

Interestingly, a recent study investigated the effects of exercise training on pre- and post-menopausal females that were only separated by ~5 years, reducing the effect of ageing on the vasculature (Nyberg et al., 2014). At baseline, post-menopausal females had higher levels of vascular inflammatory markers that were indicative of vascular dysfunction and early-stage atherosclerosis; however 12-weeks of exercise training (Indoor hockey, 30min twice per week characterised by 4-6 min >85% HR_{max} separated by 1-3 mins of recovery) improved biomarkers of vascular function and cardiovascular disease risk profiles in both pre- and post-menopausal females (Nyberg et al., 2014). The exercise training-induced reduction in markers of vascular inflammation suggests that improved function and integrity of the vasculature is possible even in the absence of oestrogen.

However, unsupervised moderate intensity exercise training in post-menopausal females had no effect on brachial artery FMD compared to age-matched men and pre-menopausal females (Pierce et al., 2011, Moreau et al., 2013), whilst post-menopausal females receiving exogenous oestrogen treatment and exercise training improved FMD (Moreau et al., 2013). The authors suggested that the lack of vascular improvement in oestrogen-deficient post-menopausal females was due to increases in oxidative stress that had a direct influence on endothelial function and potentially outweighed the effect of exercise training. The explanation for unresponsive conduit vessels following exercise training in post-menopausal females is unknown, but may be due to reductions in NO bioavailability in the absence of oestrogen (Virdis et al., 2000).

However, acute exercise (~60% VO2max for 60 minutes) increased brachial artery FMD in post-menopausal females to pre-menopausal levels 60 minutes following the exercise bout, and was proposed to be due to exercise-induced increases in NO bioavailability (Harvey et al., 2005), while the level of immediate endothelial dysfunction induced by exercise may also play a role in the functional adaptation of that artery (Goto et al., 2003). On this evidence, there is the potential to suggest that brachial artery dysfunction may be immediately reduced in post-menopausal females following exercise training but is intensity and duration-dependent, as currently only predominantly self-paced walking exercise has been used lasting ~40 minutes (Moreau et al., 2013, Pierce et al., 2011). Kretzchmar *et al.* (2014) also revealed that mild-intensity exercise training (~50% VO_{2max}) improved LDL and early stage endothelial-dysfunction markers ($CD62E^{+}$ endothelial microparticles) in post-menopausal females (Kretzschmar et al., 2014). The usefulness of exercise training to offset menopause induced changes in vascular function warrants further exploration. Furthermore, the ability of the conduit vessels to dilate may improve the distribution of blood flow during heat stress and enhance thermoregulatory control.

2.8.3 Exercise training and cerebral blood flow in post-menopausal females

The effect of exercise training on CBF in symptomatic post-menopausal females at rest and during passive heat stress is unknown. Although previous research has incorporated post-menopausal females into study design and suggests improved CBF during and following exercise training (Murrell et al., 2013) (see 2.3.2 ageing and cerebral blood flow), gender and/or menopause-related differences in CBF in response to exercise training were not reported. It is plausible to suggest that enhancements in brain blood flow may serve to reduce HFs in post-menopausal females via improved central thermoregulatory functioning. Additionally, improvements in brain blood flow at rest

following exercise training may also attenuate reductions in brain blood flow during objectively measured HFs.

2.8.4 Exercise training and body composition in post-menopausal females

The impact of a reduction in BMI with exercise training on HFs also deserves consideration. The relationship of adiposity and HFs is complex, with a higher body fat traditionally assumed to protect against HFs via peripheral aromatisation of androgens into estrogens in body fat (Thurston et al., 2013). Notably, findings from large cohort studies suggest that higher adiposity is indeed a risk factor for HFs earlier in the menopausal transition, and may actually be protective later in menopause (Gold et al., 2006). Preliminary evidence suggests an existing relationship between HFs and higher levels of leptin, a key pro-inflammatory adipokine that is down regulated with obesity. Furthermore, another key adipokine adiponectin is inversely related to BMI and exerts anti-inflammatory effects (Thurston *et al.* 2013). Recently, Thurston *et al*. (2013) associated an adverse adipokine profile attributed to increased BMI levels, with a higher HF frequency in a longitudinal cohort study over 8 years. It is suggested that the proinflammatory properties of adipose tissue may act directly on the central nervous system, with a potential thermoregulatory effect that increases HFs (Luheshi et al., 1999)

To date, it is unknown if improving the ability of central temperature control and vascular function mechanisms with exercise training to respond to increases in core body temperature and/or improved peripheral blood flow during increases in core temperature, reduces the frequency and/or severity of HFs in post-menopausal females.

2.9 Summary

In summary, the hypothalamic region of the brain can be related to a thermostat that controls internal core body temperature. When the body is too hot heat dissipation responses of cutaneous vasodilation and sweating are initiated (Charkoudian and Stachenfeld, 2014). During the menopause, reductions in oestrogen lead to thermoregulatory dysfunction and consequently HFs in post-menopausal females. Reductions in oestrogen also leads to increases in cardiovascular risk factors and reduced vascular function in this population. Exercise training improves thermoregulatory function in pre-menopausal females, and vascular function in pre- and post-menopausal females (Hodges et al., 2010, Ichinose et al., 2009). Currently, it remains uncertain if exercise training improves cardiovascular and thermoregulatory function in symptomatic females that leads to reductions in HFs.

CHAPTER 3: GENERAL METHODS

The majority of measurements and protocols undertaken in this thesis are adopted throughout chapters 4 to 6. Therefore, this methods chapter describes general information for the techniques used for physiological measurements, and will briefly discuss the reliability and limitations of these techniques. The specific protocols employed for each study, including participant cohort, can be found within the methods section of their respective chapters.

3.1 Participants

A meaningful change in resting core body temperature of 0.20^{o}C has been observed following 12-weeks of exercise training in young healthy females (Ichinose et al., 2009), and is used as the primary mechanistic variable in this thesis. Using dedicated software (NQUERY, statistical solutions, Ireland) it was estimated that a sample size of 7 in each intervention group will have 80% power to detect a pre-post intervention difference in core body temperature of 0.20^{o}C assuming a common standard deviation of 0.15^{o}C using a t-test with a 0.05 two-sided significance level. To see participant specificity, please see individual *Chapters 4-6*.

3.2 Anthropometric Measurements

Height was measured in a freestanding position to the nearest 0.1 cm using a stadiometer measuring device (SECA, model 230, Germany). Body mass was measured to the nearest 0.05 kg using calibrated electronic digital scales (SECA, model 767, Germany). From these variables, Body Mass Index (BMI; mass (kg) / height (m)^{2}) was calculated. Resting blood pressure (mmHg) and resting heart rate (beats·min^{-1}) were also determined from an average of three measures in the seated position using an automated blood pressure monitor (Dinamap, G&E Medical, Tampa, Florida).

3.3 Vascular Function

All vascular function assessments were performed in a quiet, temperature-controlled (21-23^{o}C) laboratory. Upon arrival, participants rested in the supine position for approximately 20 minutes in order to gain an accurate assessment of baseline mean arterial pressure (MAP) and resting heart rate (HR). Following the rest period, MAP and HR were determined from an average of three measures on the left arm. Participants were then positioned with their right arm extended, comfortable and relaxed at an angle of ~80° from the torso.

3.3.1 Brachial Artery Flow-Mediated Vasodilation

Brachial artery endothelium-dependent function was measured using the flow-mediated dilation (FMD) technique. Participants were fasted for 12h and free from exercise, alcohol and caffeine for 24h previous. All measurements were recorded at the same time of day to avoid the effects of circadian rhythm vascular function. A rapid inflation and deflation pneumatic cuff (D.E. Hokanson, Bellevue, WA) was positioned on the forearm, immediately distal to the olecranon process to provide a stimulus to forearm ischemia (Thijssen et al., 2011). A 10-MHz multi-frequency linear array probe, attached to a high-resolution ultrasound machine (T3000; Terason, Burlington, MA) was then used to image the brachial artery in the distal third of the upper arm. When an optimal image was obtained, the probe was held stable and the ultrasound parameters were set to optimize the longitudinal, B-mode image of the lumen-arterial wall interface. Settings were identical between FMD assessments. Continuous Doppler velocity assessments were obtained using ultrasound and were collected using the lowest isonation angle (<60°). Endothelial-dependent function was assessed by measuring FMD in response to a 5 minute ischaemic stimulus, induced by forearm cuff inflation (Thijssen et al., 2011). All

images were recorded using specialised recording software (Camtasia Studio, Techsmith, US). Following a 1 min recording period of diameter and flow, the cuff was inflated (>220 mmHg) for 5 minutes. Artery diameter and velocity recordings resumed 30 seconds prior to cuff deflation and continued for 3 min thereafter, in accordance with recent technical specifications (Black et al., 2008b, Woodman et al., 2001, Thijssen et al., 2011). Peak brachial artery diameter and blood flow velocity, and the time taken to reach these peaks following cuff release were recorded.

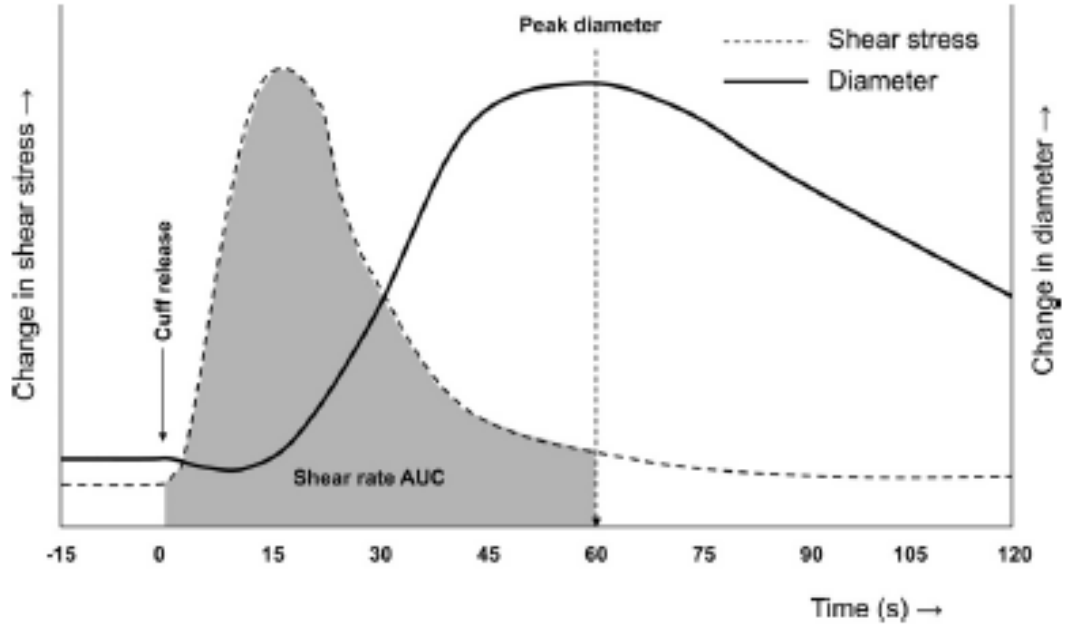


Figure 3.1 Schematic representations of diameter and shear stress responses following cuff deflation, in response to a 5-min ischemic stimulus, during a flow-mediated dilation (FMD) assessment. The grey area represents the shear rate under the curve (SRAUC). Taken from (Thijssen et al., 2011).

3.3.2 Brachial artery diameter and blood flow analysis

Analysis of brachial artery diameter was performed using custom-designed edge-detection and wall-tracking software, which is largely independent of investigator bias. The image was taken directly from the ultrasound machine and saved as an AVI file. Subsequent software analysis of this data was performed at 30Hz using an icon-based graphical programming language and toolkit (LabVIEW 6.02, National Instruments). The

initial phase of image analysis involved the identification of regions of interest (ROI) on the first frame of every individual study. These ROI's allowed automated calibration for arterial diameter on the B-mode image and velocities on the Doppler strip (Figure 3.2). A ROI was then drawn around the optimal area of the B-mode image and within this ROI a pixel-density algorithm automatically identified the angle-corrected near the far wall e-lines for every pixel column within the ROI. The algorithm begins by dividing the ROI into an upper half, containing the near wall lumen-intima interface, and a lower half containing the far wall interfaces. The near-wall intimal edge is identified by a Rake routine that scans from the bottom to the top of the upper half of the ROI. The position of the edge is established by determining the point where the pixel intensity changes most rapidly. Typical B-mode ROI's therefore contained approximately 200 to 300 diameter measures per frame, the average of which was calculated and stored. This process occurred at 30 frames per second. A final ROI was drawn around the Doppler waveform and automatically identified the peak of the waveform. The mean diameter measures derived from within the B-mode ROI were then synchronised with the velocity measure derived from the Doppler ROI at 30Hz.

From synchronised diameter and velocity data, blood flow (the product of lumen cross-sectional area and Doppler velocity) was calculated at 30 Hz. Shear rate (an estimate of shear stress without viscosity) was calculated as 4 times mean blood velocity/vessel diameter (Black et al., 2008a). All data were written to file and retrieved for analysis in the custom-designed analysis package. Reproducibility of diameter measurements using this semi-automated software is significantly better than manual methods and reduces observer error significantly (Woodman et al., 2001). Reproducibility of the FMD using this semi-automated software possesses a CV of 6.7% (Thijssen et al., 2009, Woodman et al.,

2001). Additionally, this method of blood flow assessment is closely correlated with the actual flow through a “phantom” arterial flow system (Green et al., 2002).

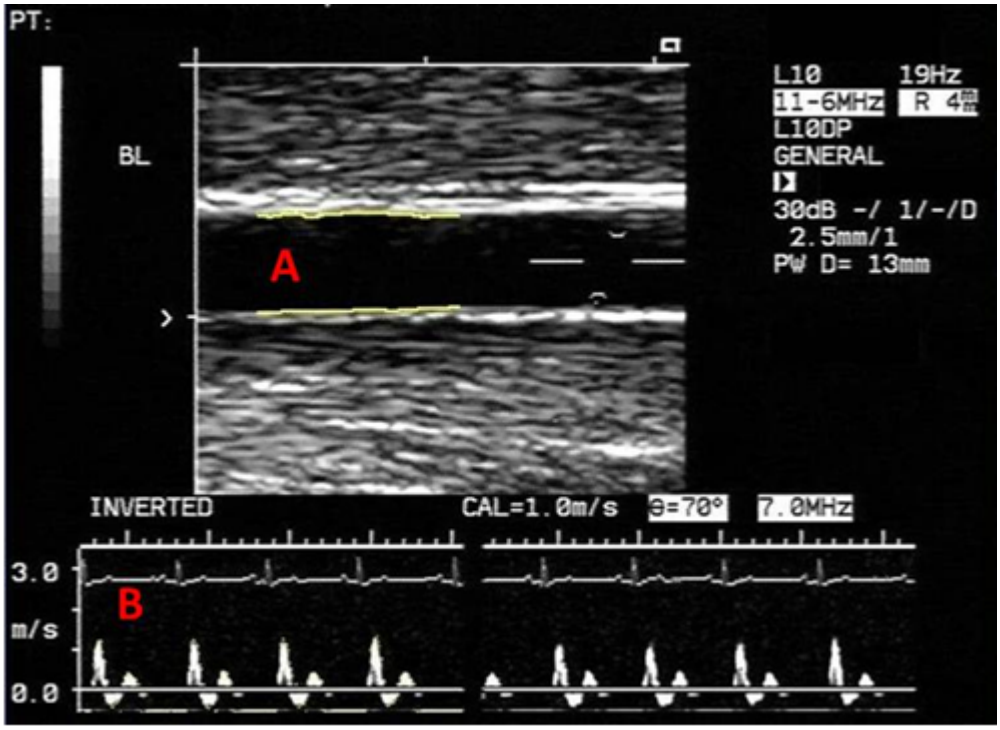


Figure 3.2 Example of Doppler ultrasound of a brachial artery allowed automated calibration for arterial diameter on the B-mode image (A) and velocities (B) on the Doppler strip

3.4 Passive Heat Stress Challenge

Experiments were performed in a temperature-controlled laboratory (22±1 ^{o}C) in the morning or early afternoon and were performed at least 2 hours post-prandial. Upon arrival to the laboratory participants were asked to wear a vest and shorts, and were clothed in a tube-lined jacket and trousers (Med-Eng; Ottawa, Canada), which covered the entire body except for the head, feet and one forearm (Figure 3.3). The suit permitted the control of skin and core body temperature, thereby mean body temperature, by changing the temperature of the water perfusing the suit. Participants rested quietly in a semi-recumbent position while water (34°C) was perfused through the suit at 5.5 L/min, using a heated water-bath (Eco Silver, Lauda; New Jersey, USA) and electronic water-

pump (Magnetically coupled pump FL5.5, RS components; Northants, UK) for a 15 minute resting baseline period. Participants were then exposed to a moderate heat stress by perfusing ~48^{o}C water through the suit for 60 minutes or until a rise of ~1°C in core body temperature. All data during the heat stress challenge were sampled at 50Hz with an analogue-to-digital converter data acquisition system (PowerLab/16SP ML795, ADInstruments, Oxford UK), and displayed in real-time on a PC laptop using commercially available software (Chart version 7.02, ADinstruments; Figure 3.4). All data were then stored for subsequent analysis. During the passive heat stress challenge, a host of physiological measures were recorded. All data were sampled as a 60 seconds average at baseline, and at every 0.1°C increase in core/mean body temperature during passive heating. These variables are discussed below.

3.5 Electrocardiogram

Heart rate was obtained from a 3-lead electrocardiogram (ECG) (ADInstruments, Oxford, UK), and recorded continuously (Antonicelli et al., 2012). The electrode sites were prepared and cleaned with an antiseptic medical swab before the application of positive and negative ECG electrodes (Tyco Healthcare, Mansfield: MA), on the right and left clavicle respectively, close to the acromioclavicular joint. The earth electrode was positioned between the seventh and eighth intercostal space. The ECG signal was displayed in real-time in the Chart software and heart rate was calculated from the beat-to-beat R-R interval values by using the formula:

$$HR = 60 / \text{R-R interval}$$

Where HR is in beats·min^{-1} and R-R is in seconds

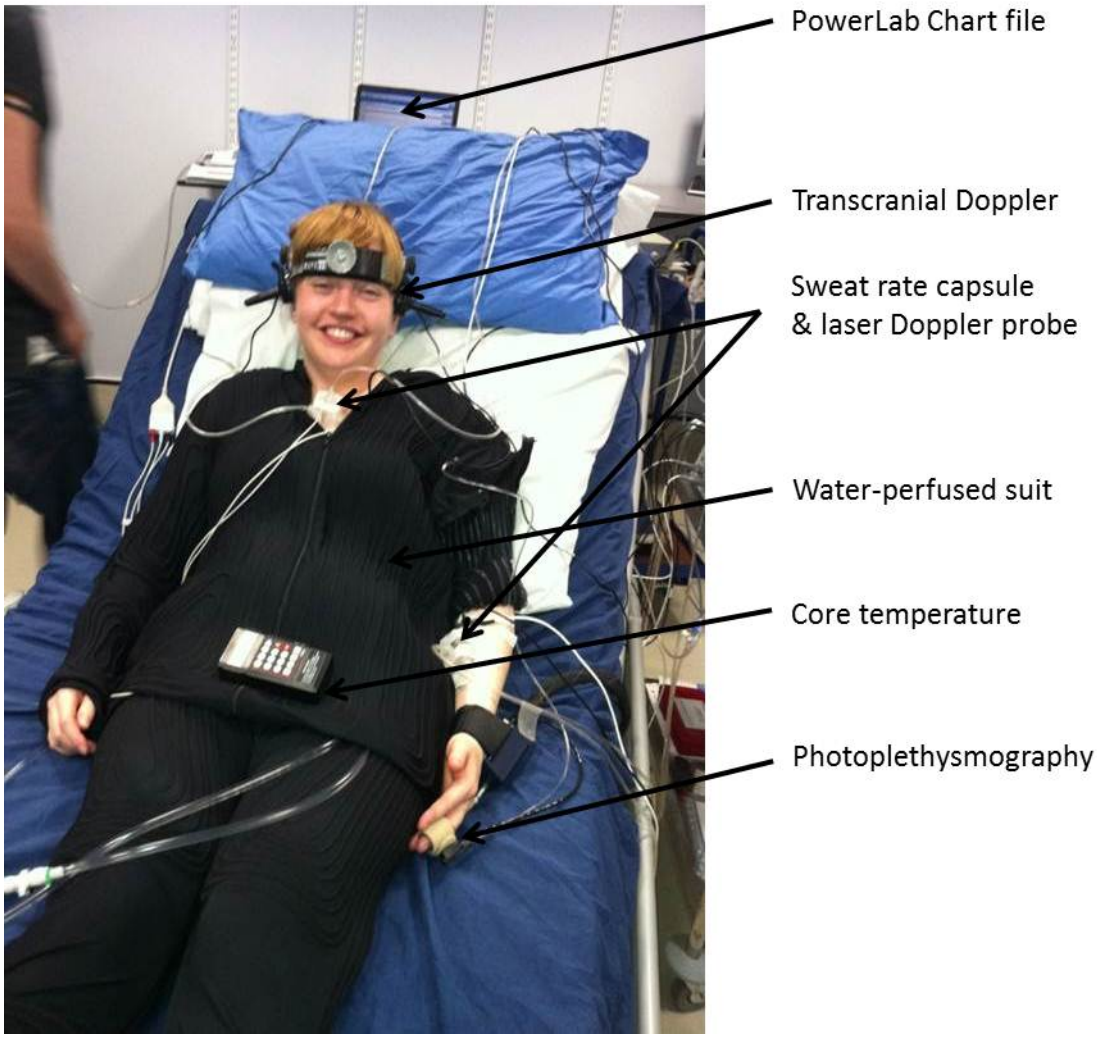


Figure 3.3 Passive heat stress challenge using the water-perfused suit

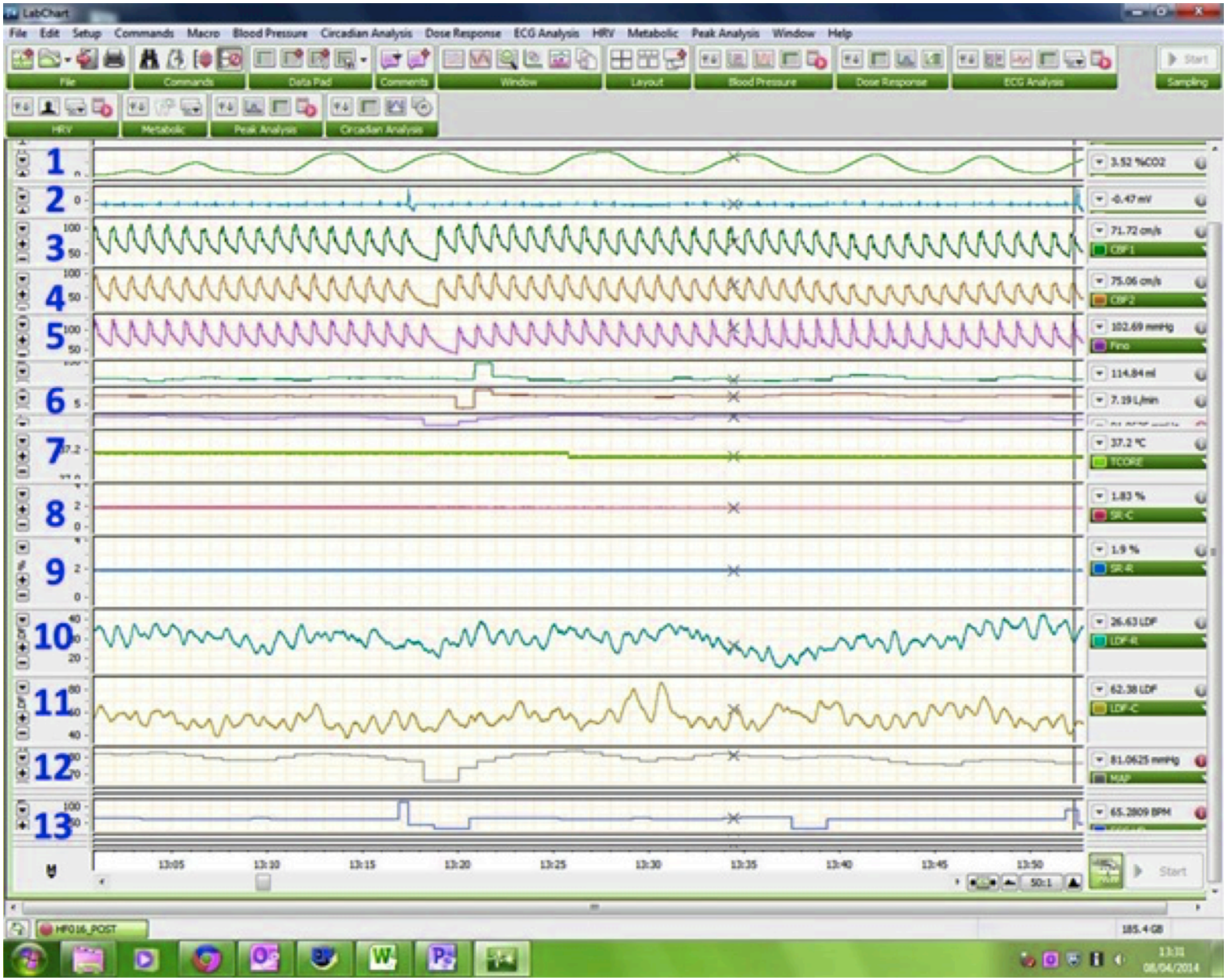


Figure 3.4 Chart file of a real-time PowerLab trace during a passive heat stress challenge. Variables measured on Chart were (1) Partial pressure of end tidal carbon dioxide (2) Electrocardiography (3-4) Right and left middle cerebral artery velocity (5) Blood pressure trace (6) Stroke volume, cardiac output and total peripheral resistance (7) Core temperature (8-9) Chest and arm sweat rate (10-11) Chest and arm laser Doppler flux (12) Mean arterial pressure (13) Heart rate from electrocardiogram

3.6 Beat-to-beat arterial blood pressure

Continuous beat-to-beat arterial blood pressure (BP) was measured by finger photoplethysmography (Finometer Pro, Finapress Medical Systems, Biomedical Instruments, Amsterdam: The Netherlands). The Finometer was developed by Wesseling *et al.* (1995) and uses the voltage-clamp method (Penaz, 1973) to clamp the diameter of the digital artery in a constant 'unloaded' condition, thereby tracking variations in intra-arterial pressure (Bogert and van Lieshout, 2005, Imholz et al., 1992, Imholz et al., 1998). To achieve this, the Finometer apparatus has three main components that includes; a finger cuff containing an inflatable air bladder and infrared plethymograph (light detector and light source), a fronted unit containing a fast-acting servo-controller system, and a main unit containing an air pump and electronics (Figure 3.5A).

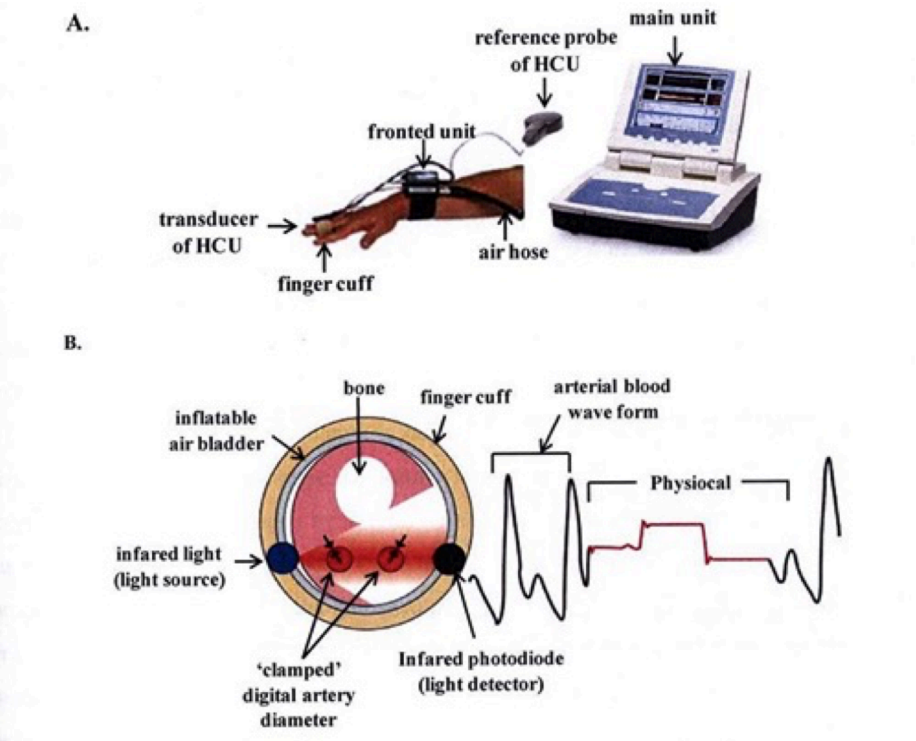


Figure 3.5 (A) Finometer apparatus including finger cuff, fronted unit containing servo-controller, and a main unit containing the air pump and electronics. (B) Finger-cuff cross-sectional view.

The method is based on the development of the dynamic pulsatile unloading of the finger arterial walls. The diameter of the artery under a cuff wrapped around the finger is kept clamped (constant) at a specific diameter (set point), despite any changes to arterial pressure during each heartbeat. Changes in diameter are detected by means of an infrared photo-plethysmograph built into a finger cuff (Figure 3.5B), with the servo-controller system detecting a difference between the light detector signal and the set point, sending a signal to the microcontroller within the unit. If an increase in arterial diameter is detected during systole the finger cuff is immediately inflated via increased air delivery into the inflatable air bladder of the cuff to prevent the diameter change in the digital artery. Therefore, the transmural pressure of the artery is maintained at zero (unloaded). At this point the pressure of the finger cuff pressure equals the intra-arterial pressure (Bogert and van Lieshout, 2005).

To ensure accurate measurement it is essential to define the correct unloaded diameter of a finger artery, however this is not straightforward, due to changes in haematocrit, stress and the tone of smooth muscle in the arterial wall that directly affect arterial diameter (Bogert and van Lieshout, 2005). The optimal unloaded diameter is close to the average diameter at a pressure where the amplitude of the pulsations in the plethysmogram is largest (Bogert and van Lieshout, 2005, Imholz et al., 1998, Wesseling et al., 1995). This is achieved using an expert inbuilt physiologic calibration system (Physiocal, Finometer Medical Systems, Amsterdam), consisting of a dynamic servo set point adjuster used to define and maintain the diameter at which the finger is clamped (Wesseling et al., 1995).

The calibration explores part of the pressure-diameter relationship by analysing the plethysmogram at a number of steady pressure levels, and is able to track the unloaded diameter of a finger artery even if smooth muscle tone changes, and adjusts the finger cuff accordingly (Bogert and van Lieshout, 2005, Imholz et al., 1998).

Once the correct finger cuff size was selected, it was placed around the mid-phalanx on the middle finger of the left hand. Care was taken to position the emission and detection light sensors symmetrically and to wrap the cuff firmly around the finger. Hand height was corrected to heart level using the hydrostatic height correction unit (FMS Height Correction Unit). The height correction unit comprises of a liquid-filled tube with one end attached to an adjustable reference component and the other end connected to a transducer (Figure 3.5A).

Prior to data collection participants were in the supine position with the arm resting flat, supported by a pillow. The reference component and the transducer were both placed upon the middle finger cuff (attached with Velcro), and a 'zeroing' procedure was performed on the transducer to the hydrostatic reference. In preparation for data collection, the reference component was then moved from the finger cuff and placed at the level of the heart on the upper arm. This process ensures that any changes in the vertical displacement of the finger cuff, relative to the heart are corrected for by the reference probe and artery pressure is reported.

To prevent peripheral vasoconstriction in the situation of participants experiencing cold hands and/or fingers, the hand was gently warmed using a warm compress (when warranted). During the experimental session the raw artery pressure wave was visualised

and recorded using the PowerLab© software. From the waveform profile, SBP, DBP and MAP were obtained which was calculated by:

$$MAP = 1/3\ SBP + 2/3\ DBP\ (mmHg)$$

Where MAP is mean arterial blood pressure, SBP is systolic blood pressure and DBP is diastolic blood pressure.

A limitation with using finger photoplethysmography is that the arterial waveform varies throughout the arterial tree, causing augmentation between intrabrachial and finger BP due to distortion of the waveform and gradient along the arterial tree, caused by transmission of the pressure pulse along the arm arteries (Imholz et al., 1990). Peripheral reflection amplifies the pressure wave influencing an over-estimation of systolic pressure when compared to intra-arterial measurements in healthy participants (Imholz et al., 1992).

Furthermore, MAP in the hand tends to be lower than in the arm as a result of a pressure gradient across the vascular tree caused by flow, as mentioned previously. The pressure decay related to flow in arteries is greatest for a high pulse-pressure at a low mean pressure, while the arterial pressure frequency transfer function between the hand and the arm has a significance of ~8Hz (Gizdulich et al., 1997). This difference translates to finger MAP being ~8-10 mmHg lower than brachial values (Gizdulich et al., 1997), while in the supine position the bias for systolic BP is ~2 mmHg (Bogert and van Lieshout, 2005). To correct this, one approach is to reference finger arterial pressure against brachial auscultation measures (Shi et al., 1993).

Several studies have compared finger photoplethysmography against a gold-standard, invasive intra-arterial measurement of blood pressure. Little differences are reported in photoplethysmography blood pressure measurements in comparison to intra-radial resting measurements, during standing or head-up tilt (Imholz et al., 1990), exercise (Silke et al., 1994), Valsalva (Imholz et al., 1988) or under pharmacological manipulation (Imholz et al., 1992). Additionally, photoplethysmography has been used to monitor alterations in BP during heat stress (Bain et al., 2013, Keller et al., 2009), manipulations in cerebral blood flow (Ainslie et al., 2008, Ainslie et al., 2007), and importantly, previous HF research in post-menopausal females (Low et al., 2010, Lucas et al., 2013, Hubing et al., 2010, Low et al., 2008a). These studies imply finger photoplethysmography is a suitable tool in monitoring dynamic changes in blood pressure. Alongside photoplethysmography, an automated blood pressure cuff (Dinamap, G&E Medical, Tampa, Florida) worn on the same arm (upper), was used to verify and calibrate finger blood pressure as previously demonstrated (Low et al., 2008b, Lucas et al., 2013, Nelson et al., 2011, Willie et al., 2013, Lewis et al., 2014).

3.6.1 Estimation of cardiac output and stroke volume

Despite various methods of measuring cardiac output and stroke volume, such as techniques that use thermo-dilution, CO_2 or rebreathing of inert gas, it is advantageous during clinical based research if measurements can be continuous and non-invasive (Bogert and van Lieshout, 2005). Doppler Ultrasound can be used in the non-invasive tracking of changes in stroke volume (Van Lieshout et al., 2003), but is methodologically limited due the probe being placed over the root of the aorta at a fixed angle (usually held) or by using a transesophageal approach (Bogert and van Lieshout, 2005). The

continuous monitoring of cardiac output and stroke volume allows for the detection of rapid changes in systemic flow and conductance that would otherwise be undetected by recording heart rate and arterial blood pressure alone (Leonetti et al., 2004). Stroke volume and cardiac output were calculated using the ModelFlow method, obtained from the BP waveform from photoplethysmography that uses age (years), sex, height (cm) and weight (kg) in its algorithm. The ModelFlow method is based on the Windkessel model (Figure 3.6) of arterial stress (Wesseling et al., 1995). The ModelFlow method uses a three-element model of the aortic input impedance to compute flow from the pulsation of the arterial pressure (Wesseling et al., 1993), and is based upon the relationship between SV and arterial pressure wave in the form of an algorithm, which then creates an aortic flow waveform from arterial blood pressure pulsation via simulation of a non-linear, self-adaptive model of the aortic input impedance (Harms et al., 1999) (Figure 3.6). Modelflow is a three element model that uses the following characteristics; impedance of the aorta and arterial compliance (Westerhof et al., 1971), and peripheral vascular resistance (ratio of mean pressure to mean blood flow), SV is subsequently calculated by integrating the aortic flow waveform per beat (Harms et al., 1999). Cardiac output is then calculated by multiplying stroke volume by HR. The Finometer uses ModelFlow by calculating proximal aortic impedance and arterial compliance using an in-built database of human aorta arctangent area-pressure relationships that rely on manually entered participant characteristics (mentioned above). The calculation of cardiac output and stroke volume from the ModelFlow technique has been seen to produce accurate measurements when compared to invasive intra-brachial methods (Harms et al., 1999) and when using thermodilution during cardiac surgery (Wesseling et al., 1993), but does slightly underestimate cardiac output and stroke volume during heat stress compared to the gold-standard thermodilution method (Shibasaki et al., 2011). However, the

underestimation is consistent and thus the technique can still track changes to cardiac output and stroke volume between trials and over time. Additionally, this methodology tracks rapid changes in cardiac output experimental protocols including static (van Dijk et al., 2005) stepping (Matsukawa et al., 2004) and dynamic cycling exercise (Sugawara et al., 2003, Tam et al., 2004).

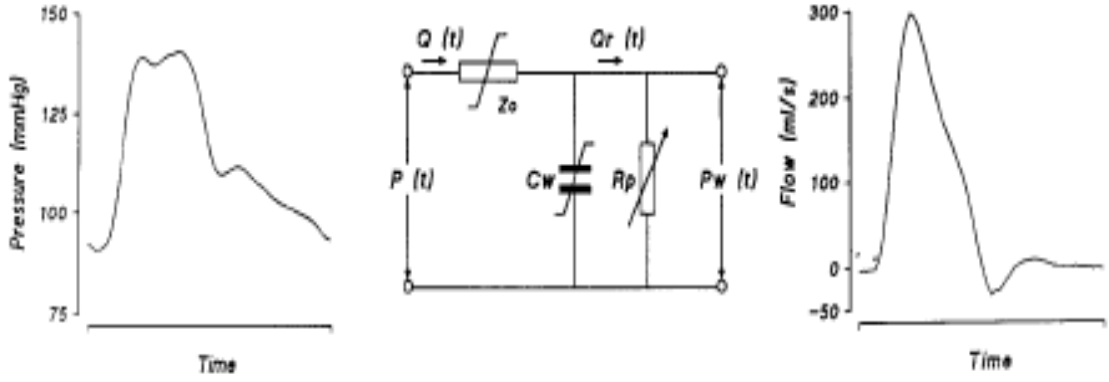


Figure 3.6 Diagram of flow modelling from measurements of arterial pressure. (A) Illustrates input to the model from the Finometer during one cardiac cycle. (B) Illustrates the three element model of the aortic input impedance used to compute flow from pressure. (C) illustrates the computer output from the model; the aortic flow as a function of time. Z0, proximal aortic characteristic impedence; Cw, arterial compliance; Rp, systemic vascular resistance; ∫, indicates the non-linear pressure-dependent properties of Z0 and Cw; the arrow indicates where Rp varies with Windkessel pressure. ModelFlow diagram adapted from Harms *et al.* (1999).

3.7 Middle cerebral blood velocity

The high temporal resolution and non-invasive nature of transcranial Doppler ultrasound (TCD) make it a useful tool in measuring blood velocities in cerebral vessels, in centimetres per second ($cm \cdot s^{-1}$) (Willie et al., 2011). In contrast to other methods of measuring CBF, including MRI and proton emission tomography, TCD has several advantages because it is non-invasive, allows repeated measures and continuous monitoring within the laboratory, is portable and has a high temporal resolution (<0.1

seconds) that rapidly monitors changes in CBF (van Beek et al., 2008). TCD is based on the use of a range-gated, pulsed Doppler ultrasonic beam of 2MHz frequency, with the probe emitting an ultrasonic beam that crosses the intact skull and the into the cerebral vessel of interest via 'windows' (Moppett and Mahajan, 2004). The ultrasonic beam is reflected off moving red blood cells in the field of transmission and detected by the transducer, which are both contained in the Doppler probe (Figure 3.7) (Willie et al., 2011). The resultant Doppler-shift is proportional to red blood cell velocity (DeWitt and Wechsler, 1988), and is the difference between the transmitted signal and the received signal, expressed by the following formula (Moppett and Mahajan, 2004):

$$\text{Doppler frequency shift} = 2 \cdot V \cdot Ft \cdot \cos\Theta / C$$

Where V is the velocity of the reflector (red blood cells), Ft is the transmitted frequency (2 MHz), C is the speed of sound in soft tissue (1540 $m \cdot s^{-1}$), and $\cos\Theta$ is the correction factor based on the angle of insonation (Θ).

The Doppler frequency shift depends upon the constant variables blood velocity and the angle of insonation of the TCD probe. TCD cannot measure CBF *per se*, but measures red blood cell velocity within the insonated vessel (Willie et al., 2011). For an overview of the cerebral circulation please see Figure 3.8. The middle cerebral artery (MCA) runs laterally and somewhat anteriorly after bifurcating from the internal carotid artery (ICA). When possible, it is best to insonate the MCA from the anterior window because it provides a near-zero insonation angle meaning absolute velocity measurements are obtainable (Willie et al., 2011).

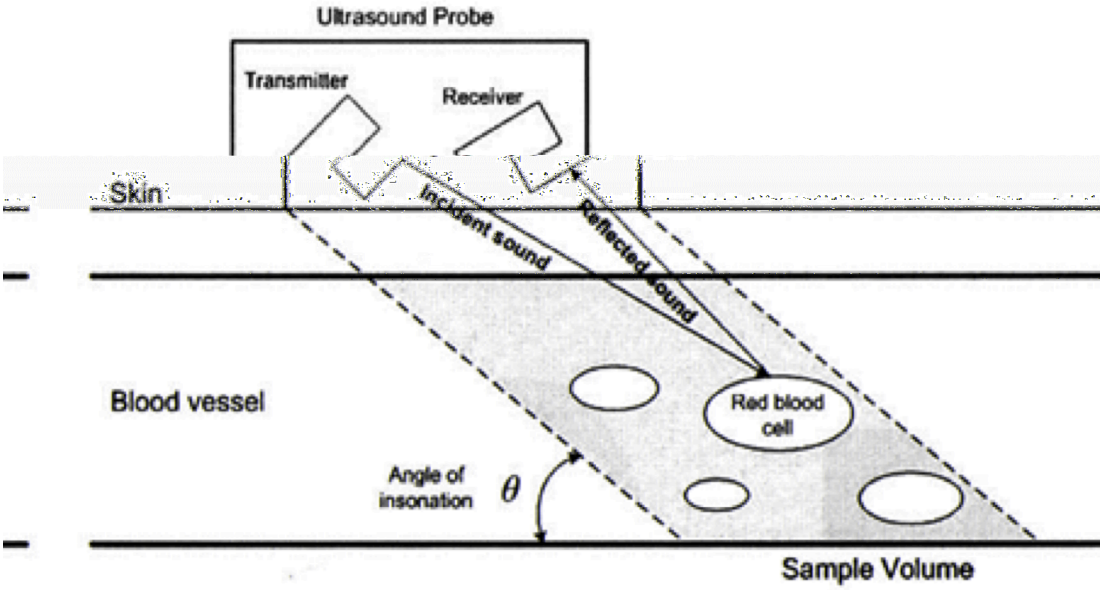


Figure 3.7 Schematic showing the components of the Doppler ultrasound probe and reflection and refraction of the ultrasound beam within the sample volume (grey).

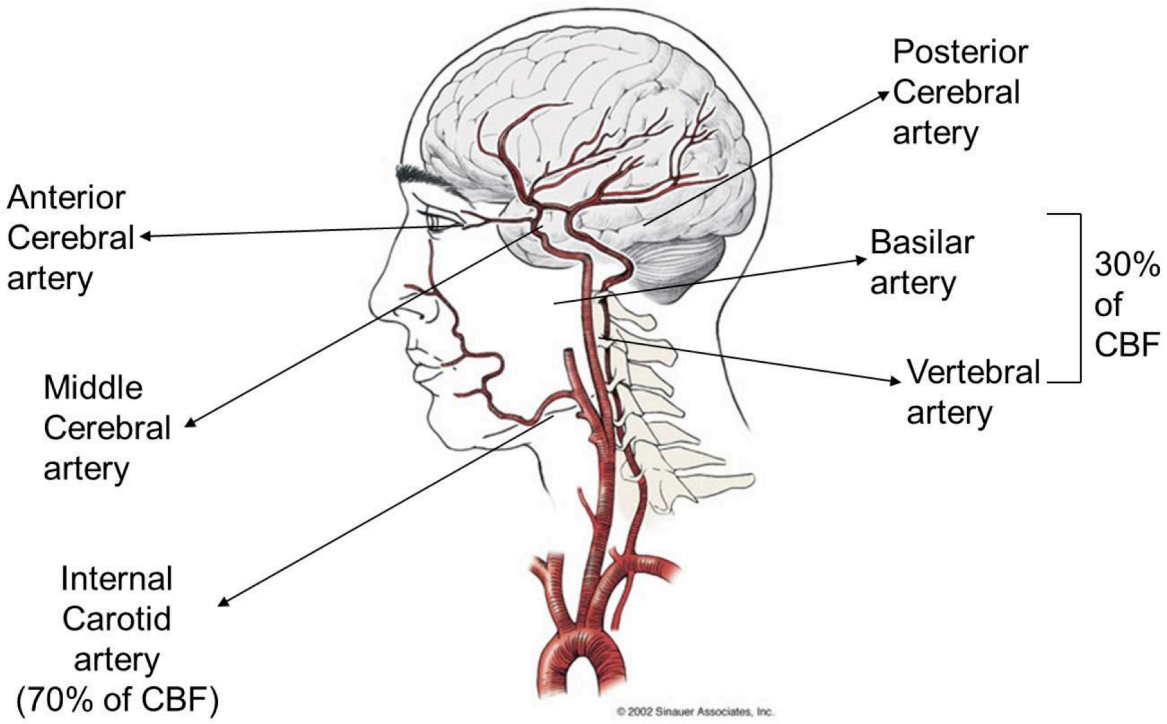


Figure 3.8 Overview of the cerebral circulation including percentage of cerebral blood flow (CBF)

The MCA is most commonly used because of the quality of the signal through the temporal window and the ease of access of insonation, whilst the MCA also carries 50-60% of the ICA blood flow (Moppett and Mahajan, 2004). Additionally, the MCA receives 80% of the blood volume delivered to the circle of Willis (Lindegaard et al., 1987), and can be taken to represent blood flow to the brain (Moppett and Mahajan, 2004). The technique for insonating the MCA has been described in detail elsewhere (Aaslid et al., 1982, Moppett and Mahajan, 2004, Willie et al., 2011).

In this thesis, middle cerebral artery blood velocity (MCA_V; 1 cm distal to the MCA-anterior cerebral artery bifurcation) was measured continuously, with the participant in the semi-supine position, through the right and left temporal window using TCD ultrasonography. Acoustic gel was applied to the temporal window (region just above the zygomatic arch) and a 2-MHz Doppler probe (Spencer Technologies, Seattle WA, USA) was adjusted, and applied gently with a consistent force, until an optimal signal was identified as described in detail previously (Willie et al., 2011), and held in place using a headband strap (1500, Spencer Technologies, Seattle WA, USA) to prevent subtle movement of the Doppler probe (Figure 3.9).

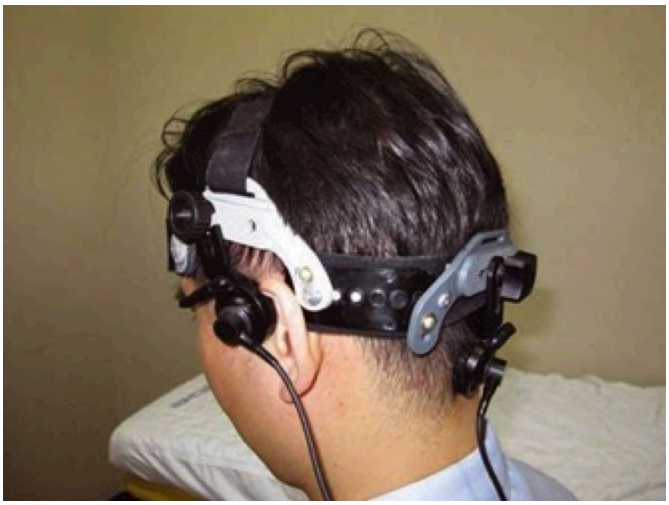


Figure 3.9 Transcranial Doppler probe held in place using headband and strap

The MCA was identified by firstly setting the probe to an insonation depth of 50mm (the MCA is usually viewed at depths between 35-60mm) and then moving the probe slowly perpendicular to the assumed vessel direction. Once a strong red signal (blood flow towards the transducer) was seen within the depth range on the Power M-mode screen, the signal was optimised by altering the gain of the signal and the insonation angle of the probe with slight corrections. During this technique, flow should be towards the probe (strong red signal) until the MCA trifurcation where there should be flow in both directions. The MCA-anterior carotid artery bifurcation is a useful anatomical landmark upon which to standardise a within-participant point of measurement during repeat tests (Willie et al., 2011). The spectrogram screen then shows a Doppler spectral waveform that indicates the velocity profile of the blood flow at the selected depth of the corresponding M-mode screen. Observing peak blood velocity also assisted in confirming the MCA, with velocities peaking between 85-130 cm/s, however there is large variability in peak blood velocity, specifically in patient groups and with ageing populations, and care should be taken when using this to identify the MCA (Moppett and Mahajan, 2004). Once the optimal MCA signal was attained on the right and left temporal windows, the probe and machine settings (depth, gain and power) were recorded to help identify the same imaging site during future assessments. To ensure angle accuracy with and between experimental sessions, the probe was constantly secured in place during the passive heat stress challenge, whilst the same researcher positioned the probe each time.

When bilateral MCAv was obtained, the average value was used unless there was a significant difference between the two sites. In the instance when there was a significant difference between signal velocities the strongest MCAv signal was used and repeated in the post-tests. The MCAv was transmitted and displayed in real-time on the PowerLab

Chart file. Within the Chart file, the mean middle cerebral artery velocity (MCAv; cm/s) was calculated for each cardiac cycle by:

$$\text{Mean MCAv} = 1/3\ \text{SMCAv} + 2/3\ \text{DMCAv}$$

Where SMCAv is the systolic middle cerebral artery and DMCAv is the diastolic middle cerebral artery.

As the anterior and posterior cerebral arteries can both be visualised through the temporal window, there are a number of identification procedures used to ensure that the insonated artery is the MCA (Table 3.1). The anterior cerebral artery lies deeper than the MCA (~65mm) and the velocity also flows away from the transducer (away – blue signal), whilst the posterior cerebral artery lies even deeper (~70mm) (Moppett and Mahajan, 2004, Willie et al., 2011).

A major limitation of TCD is that it measures blood velocity and not blood flow *per se*. However, as long as the diameter of the insonated vessel and the angle of insonation remain constant, blood velocity is a reliable index of blood flow (Giller et al., 1998):

$$\text{Flow velocity} = \text{blood flow volume} / \text{blood vessel diameter}$$

Table 3.1 Typical patterns for the identification of cerebral arteries with 'normal' Circle of Willis anatomy. Adapted from Moppett & Mahajan (2004).

Vessel	Probe Direction	Depth (mm)	Flow Direction	Ipsilateral carotid compression	Contralateral carotid compression
ACA	Anterior	60-75	Away	Flow reversal	Increased velocity
MCA	Perpendicular	35-60	Toward	Reduced velocity	No change
PCA	Posterior	55-70	Toward	No change or increased velocity	No change

Abbreviations; **ACA**, anterior cerebral artery; **MCA**, middle cerebral artery; **PCA**, posterior cerebral artery. This table was modified by Moppett & Mahajan (2004).

To date, there is no widely accepted, reliable method of assessing vessel diameter using TCD. However, collective evidence suggests that changes in MCAv are a reliable index of CBF (Schreiber et al., 2000, Serrador et al., 2000). The diameter of the MCA has been found to remain constant in response to a variety of physiological stimuli including changes in partial pressure (Peebles et al., 2007) pharmacologically induced changes in BP (Larsen et al., 1994), and handgrip exercise including post-exercise muscle ischemia (Pott et al., 1997). Additionally, TCD has been used in various heat-stress protocols (Low et al., 2008b, Nelson et al., 2011) and to measure CBF during HFs in post-menopausal females (Lucas et al., 2013). To ensure accurate measurement of MCAv it is important the angle of insonation is considered and kept constant, as the observed velocity is inversely proportional to the cosine angle of the incidence between the ultrasound beam and the vessel (Moppett and Mahajan, 2004). Insonation angles up to 30^{o} have been associated with an acceptable error of ~15% (Aaslid et al., 1982). However, an inherent advantage of

insonating MCA is that blood flows directly towards the probe allowing for easy signal acquisition with a small angle of insonation (Willie et al., 2014).

3.8 Cerebrovascular conductance

Cerebrovascular conductance (CBVC) measures the flow conductance in the cerebral vasculature and was calculated by:

$$CBVC = \text{mean MCAv} / MAP$$

Where CBVC is cerebrovascular conductance, mean MCAv is mean middle cerebral artery velocity and MAP is mean arterial pressure.

The CBVC index is used as it considers the response of arterial BP on CBF (Willie et al., 2014). Willie *et al.,* (2014) also suggest that few current research concepts incorporate the effects of both CO_2 and MAP on CBF, simultaneously. However, whilst MAP and arterial CO_2 should be considered, it is recommended they are expressed separately to provide a better impression of its effect on CBF (Willie et al., 2012).

3.9 The partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$)

Prior to experimental sessions, the PowerLab© gas analyser was calibrated with known oxygen and CO_2 beta grade gas concentrations. The pneumotachometer was also calibrated using a 3-Litre syringe (Hans-Rudolph 8980, Kansas City, Mo, USA) that was attached to a 3-way non rebreathing valve. An expiratory airline connected the facemask to the gas analysers (Model CD-3A CO_2 analyser, AEI Tech, Pittsburgh, USA) from which CO_2 concentrations at a flow rate of 30·mL·min^{-1} were sampled, and recorded in real-time using the Chart file. Total apparatus dead space ranged between 260-290 mL depending

on the size of the facemask (small-medium). Alongside mean CO_2, peak maximum cyclic CO_2 responses for end tidal CO_2 were identified via Chart and analysed.

3.10 Core body temperature

Core body temperature was measured from an ingestible pill telemetry system (CoreTemp, HQInc; Palmetto, FL, US). The pill was ingested ~5h before the participant arrived to the laboratory as recommended, due to the influence of ingestion timing on the consistency of core temperature measures (Goodman et al., 2009). Core body temperature measurement is fundamental to the study of human temperature regulation during heat stress (Byrne and Lim, 2007). However, the term 'core' does not describe a single anatomical location as the body interior is not at one uniform temperature. The temperature of the blood in the pulmonary artery is considered the best representation of the average internal temperature of the human body however this site is not accessible (Byrne and Lim, 2007). Due to its inaccessibility, core body temperature is often measured using an ingestible telemetric pill that transmits an internal temperature relative to the surrounding gastrointestinal temperature by radio waves to an external receiver for data logging (O'Brien et al., 1998).

The telemetry pill has been deemed reliable when using repeat assessments (Gant et al., 2006) and validated previously against other measures of core temperature (O'Brien et al., 1998), including both oesophageal and rectal temperature measures (Byrne and Lim, 2007, Sparling et al., 1993, Gibson et al., 1981). O'Brien *et al.* (1998) used a quantitative review of valid measures of core body temperature reliability and reported agreement between the ingestible pill and oesophageal temperature measurements, with a

systematic bias of <0.1ºC and 95% limits of agreement within ±0.04ºC. They also reported a bias against rectal temperature of >0.1ºC that was still deemed acceptable, particularly as rectal temperature elicited a time delay on true internal temperature (Byrne and Lim, 2007).

3.11 Mean skin temperature

Skin temperature plays a fundamental role in thermoregulation (Johnson et al., 2014) and was obtained from the weighted average of 4 regional temperatures (chest, forearm, thigh and calf) measured from wireless thermocouples (iButtons data logger, Maxim Integrated; San Jose, CA, US) .Skin temperature was automatically measured and recorded temperature every 60 seconds at each site. Measurements of skin temperature are often complicated due to the use of wired sensors, however the thermocouples are advantageous to traditional skin temperature recording devices in that they are wireless and do not interfere with other measurements i.e. wires, do not require auxiliary control, or a control-recording receiver that must remain within a set distance (Smith et al., 2010).

iButtons® have been validated previously using a waterbath and skin temperature measurement, with an accuracy of -0.09ºC and precision of 0.05ºC when compared to a reference thermometer (van Marken Lichtenbelt et al., 2006). The response time of the thermocouples on human skin is ~15 seconds compared to ~2 seconds with wired thermistors (van Marken Lichtenbelt et al., 2006), however because of the closely controlled elevation of skin temperature using a water-perfused suit the response time of the iButtons is acceptable. iButtons have also been validated for accurate measurements

of skin temperature during exercise, sleep research and clinical settings (Smith et al., 2010).

The wireless thermistors were secured to the lateral calf, lateral thigh, upper arm and chest. Mean skin temperature was calculated using the weighting system for mean surface temperature (Hardy and DuBois, 1938) and the equation (Ramanathan, 1964):

$$T_{sk} = (0.3*T_{chest}) + (0.3*T_{arm}) + (0.2*T_{thigh}) + (0.2*T_{calf})$$

Where T_{sk} is the mean skin temperature, T_{chest} is chest skin temperature, T_{arm} is arm skin temperature, T_{thigh} is thigh skin temperature and T_{calf} is calf skin temperature.

Ignoring the head and extremities in the weighting was not found to produce any error when compared to other methods (Ramanathan, 1964). The 4-point system (Ramanathan, 1964) has been found to correlate closely (r >0.97) (Mitchell and Wyndham, 1969) with 12- and 6-point systems previously proposed (Hardy and DuBois, 1938).

3.12 Mean body temperature

Mean body temperature was calculated as (Stolwijk and Hardy, 1966):

$$T_{body} = (0.8*T_{core}) + (0.2*T_{sk})$$

Where T_{body} is mean body temperature, T_{core} is core body temperature and T_{sk} is mean skin temperature.

The calculation of mean body temperature relates the average evaporative heat loss to mean body temperature and uses the assumption that primary thermoregulatory function is to maintain body temperature within a set physiological range. This implicates

both core temperature and skin temperature through weighting factors that when taken into account provides a mean body temperature (Hardy, 1961, Hardy and Dubois, 1937).

3.13 Local sweat rate

Local sweat rate was recorded continuously from the dorsal forearm and the mid-sternum (not covered by the water-perfused suit) using capacitance hygrometry (Brengelmann et al., 1975). Dry 100% nitrogen gas was supplied through acrylic capsules (surface area= 2.32cm^2) attached to the skin's surface at a flow rate of 150mL/min, with the humidity of the gas flowing out of the capsules measured by the capacitance hygrometer (Viasala HMP155, Helsinki, Finland). The flow rate of the gas was continuously monitored using a rotameter. Alongside humidity, the capacitance hygrometer also measured the temperature of the nitrogen gas as it exits the acrylic capsule. The relative humidity and temperature of the gas were collected via LabChart (in real-time), and local sweat rate was calculated from the ideal gas laws (Graichen et al., 1982):

$$SR = (RH / FR) / 3.464 * A * T_{cap}$$

Where SR is sweat rate (mg·cm^2·min^{-1}), RH is relative humidity (%) obtained from the capacitance hygrometer, FR is the flow rate of dry gas through the capsule (L·min^{-1}), 3.464 is the water vapour (dew point) gas constant, A is the area (2.32 cm^2) of the capsule enclosing the skin site and T_{cap} is the temperature (^{o}C) of the air leaving the capsule (Graichen et al., 1982).

Local sweat rate was sampled across 60 second segments, as this interval is well within the latency period that extends between sweat gland stimulation and sweat emergence (Bullard, 1971), and also produces less scatter and retains higher analytical certainty than averaging across 20 seconds (Motulsky and Brown, 2006).

3.13.1 Sweating threshold and sensitivity

Thermoregulatory sweating can be viewed as two line segments with a breakpoint, with sweating possessing an additional slope of zero that changes in abrupt monotonic fashion in response to heat stress (Vieth, 1989). The temperature threshold for the onset of local sweat rate is often defined from the intersection of the baseline flow (no sweating) of a linear regression of the effector response versus core body temperature (Brengelmann et al., 1994). In addition, visual estimation including any mean local sweat rate value in excess of the detection limit of the dew point capsule may also be used (Graichen et al., 1982, Sawka et al., 1989, Kolka et al., 1987, Montain et al., 1995). Very often however, the methodologies employed in the detection of temperature thresholds are not provided in detail.

For the purpose of this thesis, the temperature threshold for local sweat rate will be defined as:

"….the core temperature at which there is an abrupt, exponential rise in local sweat rate from baseline."

This method of local sweat rate threshold detection has been found to be reproducible (<0.1ºC) previously (Brengelmann et al., 1994). Cheuvront *et al.* (2009) requested 16 'raters' to calculate the local sweat rate thresholds and sensitivities for a complete data set using their own empirical approaches. Interestingly, the differences between 'rater' approaches were found to be both quantitatively and qualitatively acceptable, whilst also valid when compared with a standardised linear regression model using software (Cheuvront et al., 2009).

Sweating sensitivity was calculated from the slope of the relationship between sweat rate per unit change in mean body temperature beyond the core temperature thresholds, whilst any observed plateau (sweating <0.01 $mg \cdot cm^2 \cdot min^{-1}$/per ^oC) in sweat rate was excluded from the slope calculation. When comparing sweat rate sensitivity calculations using slope estimation (see above) and a standardised linear regression model, 324 inter-rater differences were within 73% of the qualitative limits of agreement (which define performance acceptability) (Cheuvront et al., 2009).

Sweating threshold temperature and sweating sensitivity are frequently reported to evaluate changes in thermoregulatory control (Cheuvront et al., 2009, Ichinose et al., 2009, Ichinose-Kuwahara et al., 2010, Inoue et al., 2005), yet currently no standardised method exists in thermoregulatory research (Vieth, 1989). A standard procedure would logically reduce inter-rater measurement error (Cheuvront et al., 2009), and focus the interpretation of sweating control around common factors of influence (Shibasaki et al., 2006). However, it should also be noted that improving threshold and sensitivity calculations are the responsibility, and in the care of, the end user (Cheuvront et al., 2009). Greater methodological details and more precise identification of analysis parameters are recommended to reduce inter-rater variability. For the purpose of this thesis, all temperature thresholds and sensitivities are reported by one observer in a blinded fashion (observer was unaware of assessment time or intervention).

3.14 Skin blood flow

An index of local cutaneous blood flow was obtained using Laser Doppler Flowmetry (LDF: Periflux System 5001, Perimed; AB, Sweden). Cutaneous red blood cell flux was measured

by affixing integrated laser-Doppler probes (each consisting of a seven-laser array) with an adhesive-ring in close proximity to the ventilated sweat rate capsule at both the chest and forearm sites. At both sites, care was taken to place the probe away from hairy skin or over veins. The laser-Doppler probe signals (laser Doppler flux) were continuously monitored using LabChart and averaged into 60-second segments; this produces less physiological scatter and retains higher analytical certainty than averaging across 20 seconds time-points (Motulsky and Brown, 2006). Absolute skin blood flux values from laser Doppler flowmetry provide an index (in arbitrary units, AU) of skin blood flow (Wingo et al., 2010).

LDF is based on the reflection of a beam of light that undergoes a change in wavelength (Doppler shift) when it is reflected by moving red blood cells. The magnitude and frequency of distribution of these changes in wavelength are related to both the number and velocity of red blood cells (Cracowski et al., 2006). LDF allows the evaluation of cutaneous microvessels over a specific time-period and its alterations during physiological challenges, such as during heat stress (Kuwahara et al., 2005b, Wingo et al., 2010) and post-menopausal HFs (Low et al., 2010, Low et al., 2008a). The major advantage of the laser Doppler technique is that it is continuous, has high temporal and spatial resolution beyond venous occlusion plethysmography (Charkoudian and Stachenfeld, 2014), and is sensitive in detecting relative changes in skin blood flow in response to a stimulus (i.e. heat stress) or an intervention (i.e. exercise training). Additionally, during heat stress, LDF at the forearm displayed a highly linear correlation (r = 0.9) with forearm blood flow as measured by venous occlusion plethysmography (Saumet et al., 1986). The major variation with LDF is the site of measurement (Johnson et al., 2014), but when it is standardised the day-to-day variability compares with flow-mediated dilation of the

brachial artery, with a coefficient of variation <10% (Kubli et al., 2000). At baseline, the site of skin blood flow was measured from the inguinal crease of the forearm and from the mid-sternum at the chest that was subsequently recorded for repeat assessments.

3.14.1 Cutaneous vascular conductance

Cutaneous vascular conductance (CVC) has proven useful in studies investigating the mechanisms of skin blood flow when using laser Doppler flux as it normalises blood flow in relation to mean arterial blood pressure, and enables comparison between areas of skin across a given participant and/or between participants (Kellogg et al., 1993). There is no theoretical maximum to skin blood flow, but there is a maximal CVC, when the resistance vessels are completely relaxed. This is commonly used in tests assessing mechanisms of control in the cutaneous circulation (Charkoudian and Stachenfeld, 2014). Cutaneous vascular conductance (CVC) was calculated as:

$$CVC = LDF / MAP$$

Where CVC (PU mmHg) is cutaneous vascular conductance, LDF (measured in perfusion units) is laser Doppler flux and MAP is mean arterial pressure derived from the finger photoplethysmography.

CVC values were also expressed relative to maximal CVC achieved during heating the skin to 42^{o}C, as %CVC_{max}, as this, along with CVC, is the preferred method of data expression adopted in the literature (Cracowski et al., 2006).

3.14.2 Local maximal skin blood flow

Following the passive heat stress protocol in the water perfused suit, a test for maximal NO-mediated vasodilation was performed. The temperature of the heating unit (that the

laser Doppler probe sits in) was set to 32°C and increased to 42°C, at a rate of 0.5°C·10s^{-1}, to obtain maximal skin blood flow (Tew et al., 2012a, Black et al., 2008b, Cracowski et al., 2006) following ~30-40 minutes of localised heating. This temperature-dependent increase in cutaneous blood flow achieves a maximal vasodilation between 42-44°C and corresponds to the maximal vasodilator capacity of the vessels (Charkoudian, 2003). Local heating–mediated cutaneous vasodilation is bi-phasic and evoked by two independent mechanisms, an initial peak in cutaneous blood flow is known as the axon reflex (relies on local sensory nerves), and a second longer peak (and plateau) after ~20-30 minutes that is largely NO-mediated (Minson et al., 2001). Peak laser Doppler flux was taken as a 60-second average of the highest point of the second curve (plateau) (Minson et al., 2001) at the chest and the forearm. Maximal cutaneous vasodilation (maximal cutaneous vascular conductance) was then calculated as:

$$CVC_{max} = LDF_{max} / MAP$$

Where CVC_{max} is maximal cutaneous vascular conductance, LDF_{max} is maximal Laser Doppler Flux and MAP is mean arterial pressure obtained at maximal LDF.

At baseline, and during the heat stress, CVC was then expressed relative to CVC_{max} as a percentage by:

$$\%CVC_{max} = (CVC / CVC_{max})*100$$

Where %CVCmax is cutaneous vascular conductance relative to maximal cutaneous vascular conductance, CVC is cutaneous vascular conductance and CVCmax is maximal cutaneous vascular conductance

3.14.3 Skin blood flow threshold and sensitivity

Similar to the calculation of the mean body temperature threshold for local sweat rate, the onset of cutaneous vasodilation can be viewed as two line segments with a breakpoint, with skin blood flow (laser Doppler flux, LDF) possessing baseline flow that

changes in an abrupt fashion (threshold) in response to heat stress. The temperature threshold for the onset of skin blood flow (vasodilation) is often defined from the intersection of the baseline flow of a linear regression of the effector response versus core temperature (Brengelmann et al., 1994). Very often however, the methodologies employed in the detection of temperature thresholds are not provided in detail.

A non-linear relationship exists between skin blood flow and local temperature, such that small increments in blood flow at lower local skin temperatures (20-35^{o}C) become much more pronounced at skin temperatures >37^{o}C (Johnson and Kellogg, 2010a). Warming of core body temperature (and locally at the skin), such as during heat stress using the water-perfused suit (~38 ^{o}C) causes an initial vasoconstriction followed by vasodilation of the skin in response to heat stress. It is the core body temperature at this initial vasodilation that is set as the temperature threshold for cutaneous vasodilation (Minson et al., 2001, Hodges et al., 2009, Houghton et al., 2006), whilst this visual estimation technique to assess the threshold for cutaneous vasodilation has been used previously in assessing thermoregulatory function following exercise training (Kuwahara et al., 2005a, Kuwahara et al., 2005b, Lorenzo and Minson, 2010).

For this thesis, the temperature threshold for the onset of cutaneous vasodilation was defined as:

"The core body temperature at the abrupt onset, and continuation of, cutaneous vasodilation from baseline."

Cutaneous vasodilation sensitivity was calculated from the slope of the relationship between skin blood flow and core temperature beyond the threshold (CVC per unit

change in core body temperature) (Wingo et al., 2010, Kuwahara et al., 2005a). The slope for CVC, per unit change in core body temperature beyond the core temperature thresholds was also calculated. In line with assessing thermoregulatory sweat rate thresholds and sensitivities, core body temperature thresholds and sensitivities are frequently reported for cutaneous vasodilation to evaluate changes in thermoregulatory control (Cheuvront et al., 2009, Ichinose et al., 2009, Ichinose-Kuwahara et al., 2010, Inoue et al., 2005).

3.15 Cardiorespiratory fitness ($\dot{V}O_{2peak}$)

Cardiorespiratory fitness ($\dot{V}O_{2peak}$) was assessed to quantify aerobic capacity. This comprised of a 2-minute warm-up followed by incremental exercise to volitional exhaustion on a treadmill (H/P Cosmos, Pulsar 4.0, Nussdorf-Traunstein, Germany) in a temperature-controlled environment (Bruce *et al.,* 1973). The *Bruce protocol* (1973) was used in female participants <40 years of age, whilst female participants >40 years of age followed a *modified Bruce protocol* that has been used in other sedentary, high-risk populations previously (Pugh et al., 2013, Sprung et al., 2013). $\dot{V}O_{2peak}$ during exercise was calculated from minute ventilation, measured using a pneumotach and simultaneous breath by breath analysis of expired gas fractions (Oxycon Pro, Jaeger, Germany). Gas analysers and flow probes were calibrated prior to each test. The results were expressed relative to body weight (ml/kg/min). Peak oxygen consumption was calculated as the highest consecutive 15-second period of gas exchange data occurring in the final minute before volitional exhaustion, which usually occurred due to breathlessness or leg fatigue. Physiological criteria for the assessment of $\dot{V}O_{2peak}$ included a plateau in $\dot{V}O_{2peak}$ and/or a

respiratory exchange ratio >1.15 combined with a heart rate in the region of >90% of age-predicted maximal heart rate (220-age) (Miller et al., 1993).

3.15.1 Bruce protocol

The fitness test was performed on a treadmill (H/P Cosmos, Germany) to quantify peak aerobic capacity. After a 2 min warm up, treadmill speed began at 6 kmh^{-1} for continuous 2-min stages, speed then increased by 2 kmh^{-1} per 2 min to a maximal running speed of 16 kmh^{-1} or until volitional exhaustion. Heart rate was measured continuously using short range telemetry (RS800, Polar, Finland) alongside subjective effort (RPE) using the 6-20 Borg scale (Borg, 1970).

3.15.2 Modified Bruce protocol

The fitness test was performed on a treadmill (H/P Cosmos, Germany) to quantify peak aerobic capacity. Following a 2-minute warm-up at 2.2km/h on a flat gradient, the initial workload was set at 2.7km/h at 5% grade. Thereafter, stepwise increments in speed and grade were employed every minute. Heart rate was continuously measured at rest, during, and in recovery from exercise via 12-lead ECG (Oxycon Pro, Jaeger, Germany), and the participant's RPE was monitored (Borg, 1970).

3.16 Statistical Analysis

Analyses were performed using the Statistical Package for Social Sciences Version 17.2 (SPSS Inc. Chicago, IL, USA). Data were presented in the text as mean (95% Confidence Interval), unless otherwise stated. Statistical significance was delimited at $P<0.05$ and exact P values are cited (values of P of "0.000" provided by SPSS are reported as "<0.001"). For specific statistical analysis please refer to each methodology section in *Chapters 4-6.*

CHAPTER 4: EXERCISE TRAINING REDUCES THE FREQUENCY OF MENOPAUSAL HOT FLUSHES BY IMPROVING THERMOREGULATORY CONTROL

4.1 Introduction

Menopausal HFs can seriously disrupt the lives of symptomatic females (Kronenberg, 1990, Freedman, 2001). Approximately 70% of females experience HFs 1 to 5 years after the onset of the menopausal transition (Shanafelt et al., 2002, Kronenberg, 1994). A HF is typically defined as the subjective sudden intense sensation of heat causing cutaneous vasodilation and profuse sweating (Stearns et al., 2002, Freedman, 2002). HRT is an effective treatment for HFs (McNagny, 1999, Maclennan et al., 2004, Utian et al., 2004) but has poor uptake (Hersh et al., 2004, Isaacs et al., 2005). Furthermore, not all females can be prescribed HRT due to their age, time since menopause and a history of cardiovascular disease or breast cancer (NICE, 2009). The current alternatives to HRT are limited but one non-pharmacological option is exercise training. A number of research studies, but not all, have shown exercise training can reduce the frequency of HFs (Moilanen et al., 2012, Luoto et al., 2012, Karacan, 2010, Reed et al., 2014, Lindh-Astrand et al., 2004) and reduce other non-vasomotor symptoms including depression, anxiety and insomnia (Daley et al., 2009, Ivarsson et al., 1998, Reed et al., 2014). To date these studies have solely relied on subjective questionnaires as the primary outcome and/or ambulatory skin conductance monitors (Daley et al., 2009, Sternfeld and Dugan, 2011, Luoto et al., 2012, Sternfeld et al., 2014). No study to date has examined the potential physiological, specifically thermoregulatory and vascular, mechanisms of how exercise training can improve menopausal HFs.

Although the mechanisms of HFs are not completely understood, it is thought that thermoregulatory dysfunction due to the reduction in oestrogen leads to HFs (Deecher and Dorries, 2007, Freedman, 2001, Rance et al., 2013). An elevation in basal core body

temperature and a narrowed thermo-neutral zone are thought to be primary explanations (Freedman (2001), and reduced skin vascular reactivity to increases in core body temperature is also proposed (Charkoudian, 2003, Deecher and Dorries, 2007). Therefore, examining the changes in the thermoregulatory control system and vasculature function following exercise training may provide insight into the potential mechanisms that cause reductions in the frequency of post-menopausal HFs.

Exercise training has been shown to enhance the efficiency of the thermoregulatory control system by decreasing core body temperature, and improving thermoregulatory control by changing both the threshold for the onset, and sensitivity, of sweating and cutaneous vasodilation in pre-menopausal females (Ichinose et al., 2009). HRT is reported to affect thermoregulatory control mechanisms via lowering core body temperature and altering the threshold at which cutaneous vasodilation and sweating responses are initiated (Freedman and Blacker, 2002, Dacks and Rance, 2010, Tankersley et al., 1992, Brooks et al., 1997). Moreover, exercise training improves endothelial function in the cutaneous and conduit vessels in post-menopausal females (Nyberg et al., 2014, Hodges et al., 2010), and CBF in older individuals (Ainslie et al., 2008, Murrell et al., 2013). Therefore, the aim of this study was to determine the effects of a structured progressive aerobic exercise training intervention on subjective HFs, and secondly, to explore whether these improvements are linked to positive thermoregulatory and vascular adaptations in symptomatic post-menopausal females. . We hypothesised that exercise training reduces HFs via improving sweat rate and skin blood flow efficiency to increases in core body temperature, as well as enabling enhanced (cerebro)vascular function.

4.2 Methods

4.2.1Participants

Twenty-one symptomatic post-menopausal females were recruited from the gynaecology and reproductive medicine clinic at Liverpool Women's Hospital, local G.P. practices and via local advertisement. Participants were 1-4 years since their last menstrual period suffering from >4 hot flushes over a 24h period. All participants presented with no history of type II diabetes, cardiovascular or respiratory disease, were non-smokers, drank <14 units of alcohol per week, and had no contraindications to exercise. Participants who had used HRT, metformin, vasoactive or BP lowering medications, or medication which could alter vascular structure and/or function, within the last 6 months were excluded from the study. Similarly, females who were currently taking part in regular exercise (>2h a week based on a self-reported questionnaire) were also excluded. Participants were informed of the methods verbally and in writing before providing written informed consent. The study conformed to the *Declaration of Helsinki* and was approved by the local research ethics committee.

4.2.2 Research Study Design

Participants reported to the laboratory on two separate occasions. Visit one included anthropometric measurements, assessment of brachial artery endothelial function using FMD and a cardiorespiratory test (Modified Bruce protocol). Visit 2 consisted of a passive heat stress challenge. For details of experimental procedures for these measurements please see *Chapter 3, General Methods.*

Fourteen (*n*=14, 52±4y, 29±6 kg/m^2) symptomatic females received a 16-week programme of supervised moderate-intensity aerobic exercise training while seven (*n*=7, 52±6y, 30±7 kg/m^2) symptomatic females were used as a no-exercise control group (Figure 4.1). All participants underwent visit 1 and 2 prior to and following either 16-weeks of exercise or the no-exercise control period. The control group continued with their normal everyday routine in terms of physical activity and diet, and received no contact with the research team at any point during the 16-week intervention period.

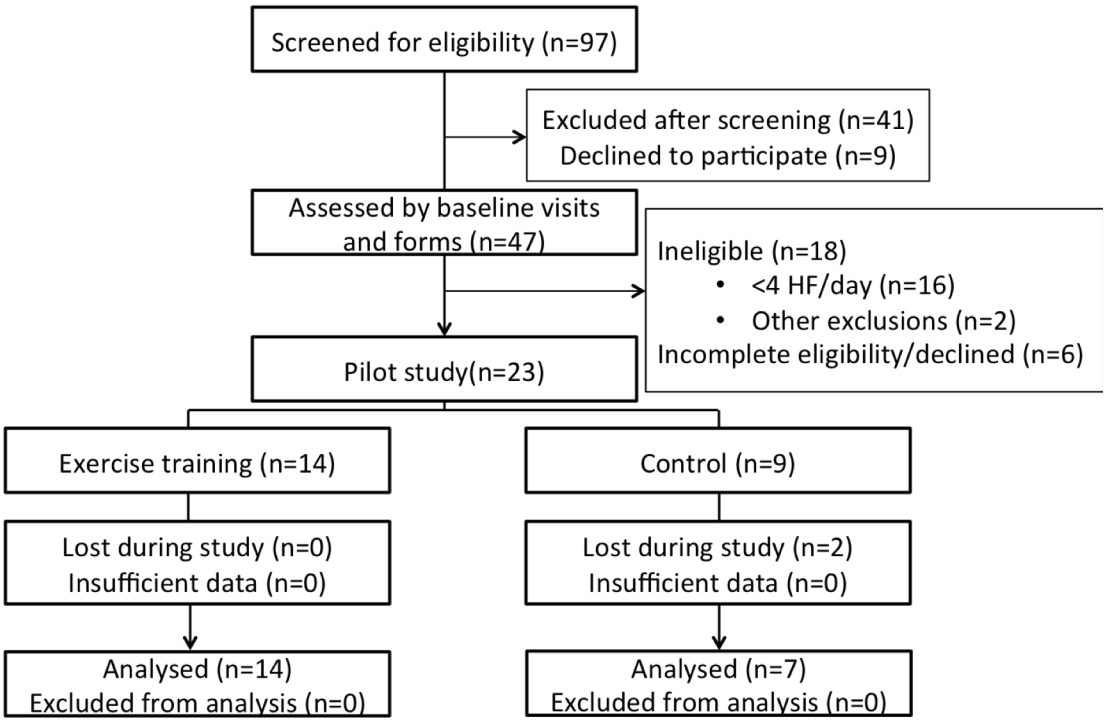


Figure 4.1 Consort diagram of the study participants

4.2.3 Hot Flush Frequency and Severity Questionnaire

Participants were asked to complete a 7-day HF frequency and severity diary (Sloan et al., 2001) prior to, and following the 16-week intervention period. Participants were asked to record on a daily basis how many HFs they experienced per day as well as information regarding the severity of each HF on a scale of 1-4 (1 being mild, 2 moderate, 3 severe and 4 very severe). From this, a 7 day sum of HFs provided a weekly HF score. A daily severity score was calculated by the sum of HFs recorded into each severity rating i.e. [(3*mild)+(4*moderate)+(1*severe)+(0*very severe) = daily severity score of 14]. A HF severity index was then calculated by the total sum of daily severity scores over the 7 day period. The use of subjective diaries has been established as a valid approach to obtaining data on subjective HFs when reporting patient symptoms and perceptions (Sloan et al., 2001) and in a number of HF research studies (Luoto et al., 2012, Moilanen et al., 2012, Carpenter et al., 1999, Carpenter et al., 2002, Sternfeld et al., 2014).

4.2.4 Supervised Exercise Training Intervention

Prior to commencing the exercise intervention, participants in the exercise intervention group attended a gym-based exercise familiarisation session at the Research Institute for Sport and Exercise Sciences research gymnasium. In the first instance, participants were required to undertake 30 minutes of moderate-intensity aerobic exercise consisting of treadmill walking/jogging, upright cycling, cross-training and rowing. Participants were required to attend the University gym at least once per week during which time they wore a heart rate monitor (Polar Fitness, Polar Electro Oy, Finland) and were provided with full exercise supervision and guidance from a trained exercise physiologist. During these sessions, participants were issued with a weekly progressive exercise programme that was specific to their own rate of progression. Exercise was primarily directed by

heart rate responses and utilisation of the Borg scale (Borg, 1970), with training protocol's specific to each participant's basal fitness level (Sprung et al., 2013, Pugh et al., 2013). During the initial 4 weeks of the intervention, participants underwent 30 minutes of exercise three times per week at ~30% of heart rate reserve (HRR). This routine continued from weeks 5-8, however during this period exercise workload was modified so that participants maintained ~45%HRR. From week 9 to 12 participants continued to maintain a 45% HRR workload whilst the duration of each session increased to 45 minutes. Finally, from week 13 to 16 the duration of each session lasted between 45-60 minutes at 60%HRR and participants underwent 5 sessions per week (Figure 4.2). HRR was calculated as [(Max HR-Resting HR) x Intensity] + Resting HR. Maximal and resting HR measures were calculated during the maximal oxygen consumption test completed prior to exercise training. Participants were closely monitored to ensure the maintenance of their individually prescribed RPE and HRR. Furthermore, to facilitate maximum compliance throughout the 16-week period, all participants used the Wellness Key© system (TechnoGym, UK). This is a software programme that enables remote and accurate tracking of exercise activity. No dietary modifications were made throughout the course of the exercise intervention, confirmed by use of a standard food diary at baseline and post intervention.

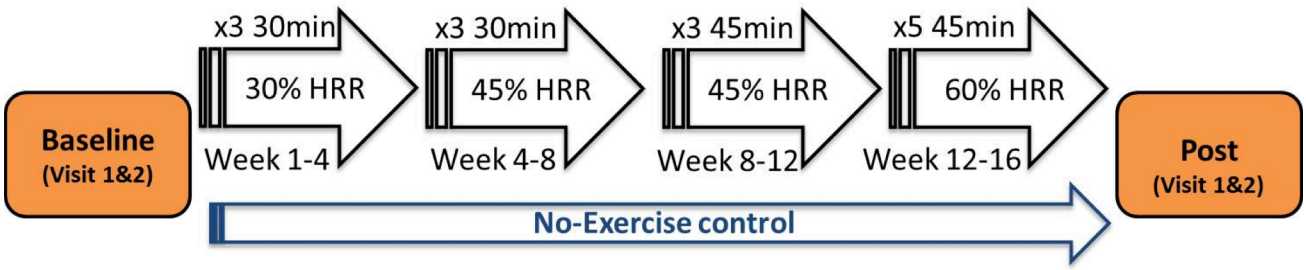


Figure 4.2 Schematic of study design including the progressive exercise training protocol

4.2.5 Statistical Analysis

The mean body/core temperature thresholds for the onset of sweating (mean body temperature) and cutaneous vasodilation (core body temperature) were calculated in a blinded fashion by the same analyst (Cheuvront et al., 2009). The sensitivity of the sweating responses were estimated from the slope of the relationships between sweat rate per unit change in mean body temperature beyond the mean body temperature threshold, and any sweat rate plateau, or increase during a hot flush episode, was excluded from the slope calculation. Skin blood flow sensitivity was estimated in the same way, instead using the rate of CVC per unit change in core temperature. A two (intervention*time) factor linear mixed model was employed to analyse HF frequency, HF severity index, $\dot{V}O_{2peak}$, and FMD [with baseline diameter entered as a covariate to control for exercise-induced changes in baseline (Atkinson and Batterham, 2013)], alongside resting baseline variables and temperature thresholds and sensitivities of sweat rate and skin blood flow during the passive heat stress. A three-way (intervention*time*temperature) linear mixed model was employed for the analysis of CBF in response to each 0.1°C increase in core body temperature, during the passive heat stress. Statistically significant interactions were followed up with the least significant difference (LSD) approach to multiple comparisons (Perneger, 1998). Due to variable increments in core body temperature during the passive heat stress, data up to an increase of 0.6°C were used for CBF analysis.

4.3 Results

Females that completed the exercise training intervention demonstrated 93% compliance to exercise sessions.

4.3.1 Cardiorespiratory Fitness

There was a significant intervention*time interaction (*P*<0.001) in cardiorespiratory fitness (VO_{2peak}). VO_{2peak} improved by 4.45 $ml \cdot kg^{-1} \cdot min^{-1}$ (95% CI, 3.40, 6.49; *P*<0.001) following exercise training compared to a negligible change of -0.60 $ml \cdot kg^{-1} \cdot min^{-1}$ (95% CI, -2.07, 0.88; *P*=0.41) in the no-exercise control group.

4.3.2 Hot flush frequency and severity

There was a significant main effect of time (P<0.001) and intervention*time interaction (P<0.001) for both HF weekly frequency and weekly HF severity index (Table 4.1). Exercise training reduced HF weekly frequency by 39 HF·wk (95% CI, 31-47; *P*<0.001) compared to a negligible change of 5 HF·wk (95% CI, -6 to 16; *P*=0.33) in the no-exercise control group. This coincided with a reduction in HF severity index by 101 AU (95% CI, 80 to 121; *P*=0.01) following exercise training compared a negligible change of 9 (95% CI, -20 to 37; *P*=0.56) in the no-exercise control group.

4.3.3 Conduit brachial artery endothelial function

There was a significant intervention*time interaction (*P*=0.008) for FMD%. FMD increased by 2.32% (95% CI 1.33, 3.31; *P*<0.001) following exercise training compared to a negligible change of -0.6% (95% CI, -1.3, 1.24; *P*=0.96) in the no-exercise control group. The intervention did not impact on brachial artery diameter, time-to-peak diameter and shear rate area-under-the-curve (Table 4.1).

Table 4.1 Baseline characteristics and changes in vascular function following exercise training or control

Characteristics	Pre Exercise	Post Exercise	Pre Control	Post Control	P value
Body mass index (kg/m^2)	29 (25, 32)	27 (24, 30)*	28 (23, 33)	28 (23, 34)	T= 0.01 IxT= 0.009
Weight (kg)	77.9 (67.5, 88.3)	74.5 (63.1, 84.9)*	75.6 (60.8, 90.3)	75.2 (60.5, 90)	T= 0.01 IxT= 0.03
Systolic (mmHg)	128 (124, 132)	126 (121, 130)	127 (122, 133)	128 (123, 134)	T= 0.74 IxT= 0.07
Diastolic (mmHg)	78 (74, 83)	75 (70, 79)	77 (71, 83)	77 (69, 81)	T= 0.92 IxT= 0.19
VO_{2peak} (ml/kg/min)	22.5 (20.6, 24.5)	27 (25.1, 28.9)*	23.2 (20.6, 25.9)	22.6 (19.9, 25.3)	T=< 0.001 IxT=< 0.001
Hot Flushes					
Frequency (HF·wk)	64 (51, 75)	23 (11, 35)*	48 (31, 65)	53 (36, 70)	T=<0.001 IxT=< 0.001
Severity Index (AU)	137 (110, 165)	37 (9, 63)*	98 (59, 136)	106 (68, 145)	T= 0.01 IxT= 0.03
Vascular measurements					
Flow-Mediated Dilation (%)	5.0 (4.2, 5.9)	7.4 (6.5, 8.2)*	5.6 (4.3, 6.7)	5.5 (4.2, 6.7)	T= 0.01 IxT= 0.008
Baseline Diameter (mm)	0.37 (0.35, 0.39)	0.37 (0.35, 0.39)	0.36 (0.33, 0.39)	0.35 (0.32, 0.38)	T= 0.91 IxT= 0.45
Peak Diameter (mm)	0.39 (0.37, 0.41)	0.40 (0.38, 0.42)	0.38 (0.35, 0.42)	0.37 (0.34, 0.40)	T= 0.4 IxT= 0.14
Shear rate$_{AUC}$ (s^{-1} x 10^3)	16.3 (8.8, 19.7)	17.8 (8.4, 21.3)	21.5 (13.9, 29.1)	20.4 (12.8, 27.9)	T= 0.72 IxT= 0.87
Time to Peak (s)	69.7 (52.5, 86.8)	54.2 (37.1, 71.4)	70.5 (46.9, 93.7)	76.7 (53.2, 100.2)	T= 0.59 IxT= 0.22

Data are presented as mean (95% CI.). *Significant difference between pre and post. T is time, IxT is intervention x Time interaction

4.3.4 Haemodynamic and thermoregulatory measurements prior to heating

4.3.4.1 Haemodynamics

Heart rate was 3 beats·min^{-1} (95% CI, 1, 5; *P*=0.03) lower following exercise training compared to no change in control [0 beats·min^{-1} (95% CI, -1, 1)]. There was no main effect of intervention or intervention*time interaction in mean arterial pressure or cardiac output. Stroke volume was 7 ml (95% CI, -3, 10) higher following exercise training compared to a difference of 2 ml (95% CI, -4.8, 2.5) in the no-exercise control group, however this did not reach statistical significance (*P*=0.14; Table 4.2).

4.3.4.2 Thermoregulatory

Skin temperature was not different between interventions or over time (*P*>0.05; Table 4.2). There was a significant intervention*time interaction (*P*=0.05) for basal core body temperature. Basal core body temperature was 0.14°C (95% CI, 0.03, 0.20) lower following exercise training compared to no change in control [0.01°C (95% CI, -0.01, 0.03)].

There were no significant main effects for resting skin blood flow at the chest or forearm (Table 4.2). There was a significant intervention*time interaction for forearm CVC_{max} (P=0.03) during local maximal heating, which was 0.70 AU/mmHg (95% CI, 0.25, 1.15; *P*=0.004) higher following exercise compared to no change following no-exercise control [0.18 AU/mmHg (95% CI, -0.21, 0.43; *P*=0.65)]. There were no significant main effects for chest CVC_{max} (Table 4.2).

4.3.4.3 Cerebral Blood Flow

There was a significant intervention*time interaction in basal MCAv (*P*=0.04). MCAv was 3.1 cm/s (95% CI, 1.2, 5.0; *P*=0.03) higher following exercise training compared to a negligible change of 0.2 cm/s (95% CI, -2.2, 2.3; *P*=0.98; Table 4.2). Cerebral vascular conductance (CBVC) was also 0.05 $cm \cdot s^{-1} \cdot mmHg^{-1}$ (95% CI, -0.01 to 0.07; *P*=0.02) higher following exercise training compared to no change following the no-exercise control [0.02 (95% CI, -0.02, 0.03; *P*=0.83)]. There was no significant main effects of intervention (*P*=0.21), time (*P*=0.88) or intervention*time interaction (*P*=0.85) for $P_{ET}CO_2$ at rest.

Table 4.2. Resting baseline data before and after exercise training (*n*=14) or control (*n*=7) (16-weeks)

Variable	Exercise (n=14)		Control (n=7)		*P value*		
	Pre	Post	Pre	Post	*Intervention Interaction*	*Time*	
Heart Rate ($b \cdot min^{-1}$)	64 (61, 68)	60 (57, 64)	66 (62, 69)	65 (61, 68)	0.11	0.03*	0.03*
MAP (mmHg)	75 (72, 78)	75 (72, 77)	76 (72, 80)	75 (71, 79)	0.63	0.82	0.83
Stroke Volume (ml)	107 (93, 117)	114 (102, 124)	105 (89, 120)	103 (88,119)	0.53	0.21	0.14
Cardiac Output ($l \cdot min^{-1}$)	7.2 (6.3, 8.1)	7.6 (6.8, 8.5)	7.1 (6.5, 7.9)	7.3 (6.7, 7.8)	0.46	0.77	0.46
Core Temperature (°C)	36.93 (36.79, 37.05)	36.79 (36.65, 36.93)	36.83 (36.63, 36.97)	36.84 (36.64, 37)	0.48	0.05*	0.04*
Skin Temperature (°C)	32.2 (31.8, 33.5)	32.9 (32.6, 33.4)	32.8 (32.2, 33.3)	32.9 (32.4, 33.5)	0.41	0.91	0.20
MCA*v* (cm/s)	51 (48, 55)	54 (51, 57)	51 (47, 55)	51 (46, 55)	0.60	0.05*	0.04*
CBVC ($cm \cdot s^{-1} \cdot mmHg^{-1}$)	0.69 (0.64, 0.74)	0.74 (0.68, 0.79)	0.68 (0.62, 0.74)	0.69 (0.62, 0.74)	0.39	0.05*	0.02*
End Tidal CO_2 (Torr)	42 (40, 44)	42 (41, 44)	41 (40, 43)	42 (40, 44)	0.23	0.88	0.81
CVC_{chest} (%CVC_{max})	11.1 (8.3, 13.9)	10.9 (8, 13.7)	9.5 (5.5, 13.5)	8.6 (4.6, 12.5)	0.82	0.88	0.50
CVC_{arm}, (%CVC_{max})	10.2 (7.6, 12.8)	9.7 (6.9, 12.4)	8.8 (5.1, 10.7)	9.5 (5.7, 13.3)	0.36	0.41	0.20
Chest CVC_{max}	5.1 (4.4, 5.9)	5.9 (5.1, 6.6)	5.4 (4.2, 6.5)	5.2 (4.9, 6.2)	0.49	0.18	0.14
Arm CVC_{max}	2.9 (2.5, 3.4)	3.6 (3.2, 4.1)	3.3 (2.5, 4.1)	3.4 (2.8, 4)	0.27	0.08	0.03*

Data are presented as mean (95% CI). *significant difference between pre and post

4.3.5 Haemodynamic and thermoregulatory responses during the heat stress challenge

6.3.5.1 Haemodynamics

Changes in heart rate, mean arterial pressure, stroke volume and cardiac output were not different between interventions or over time (*P*<0.05).

4.3.5.2 Thermoregulatory

Changes in core body temperature and skin temperature were similar between interventions and over time, whilst there was no interaction between intervention and time (P>0.05).

4.3.5.3 Sweat rate

There was a significant intervention*time interaction for chest and forearm sweating mean body temperature thresholds (*P*<0.05, Figure 4.3). The onset of chest sweating occurred at a 0.22°C (95% CI, 0.13, 0.32; *P*<0.001) lower mean body temperature following exercise training compared to a negligible change in control [0.01°C (95% CI, -0.08, 0.09; *P*=0.98); Figure 4.3A]. Similarly, the onset of forearm sweating occurred at a 0.21°C (95% CI, 0.12, 0.31; *P*<0.001) lower mean body temperature following exercise training compared to a negligible change in control [0.02°C (95% CI, -0.12, 0.13; *P*=0.89); Figure 4.3B].

There was a significant intervention*time interaction for sweat rate sensitivity at the chest (*P*=0.01; Figure 4.3). The rate of sweating at the chest increased by 0.26 $mg \cdot cm^{2} \cdot min^{-1}$ (95% CI, 0.10, 0.41; *P*=0.002) following exercise training compared to a negligible change in control [0.04 (95% CI, -0.06, 0.09; *P*= 0.35); Figure 4.3C]. Similarly at the forearm, there was a significant intervention*time interaction (*P*=0.01) with the rate

of sweating increased by 0.18 mg·cm^{2}·min^{-1} (95% CI, 0.09, 0.27; *P*=0.001) following exercise training compared to a negligible change in control [0.03 (95% CI, -0.06, 0.09; *P*= 0.59)].

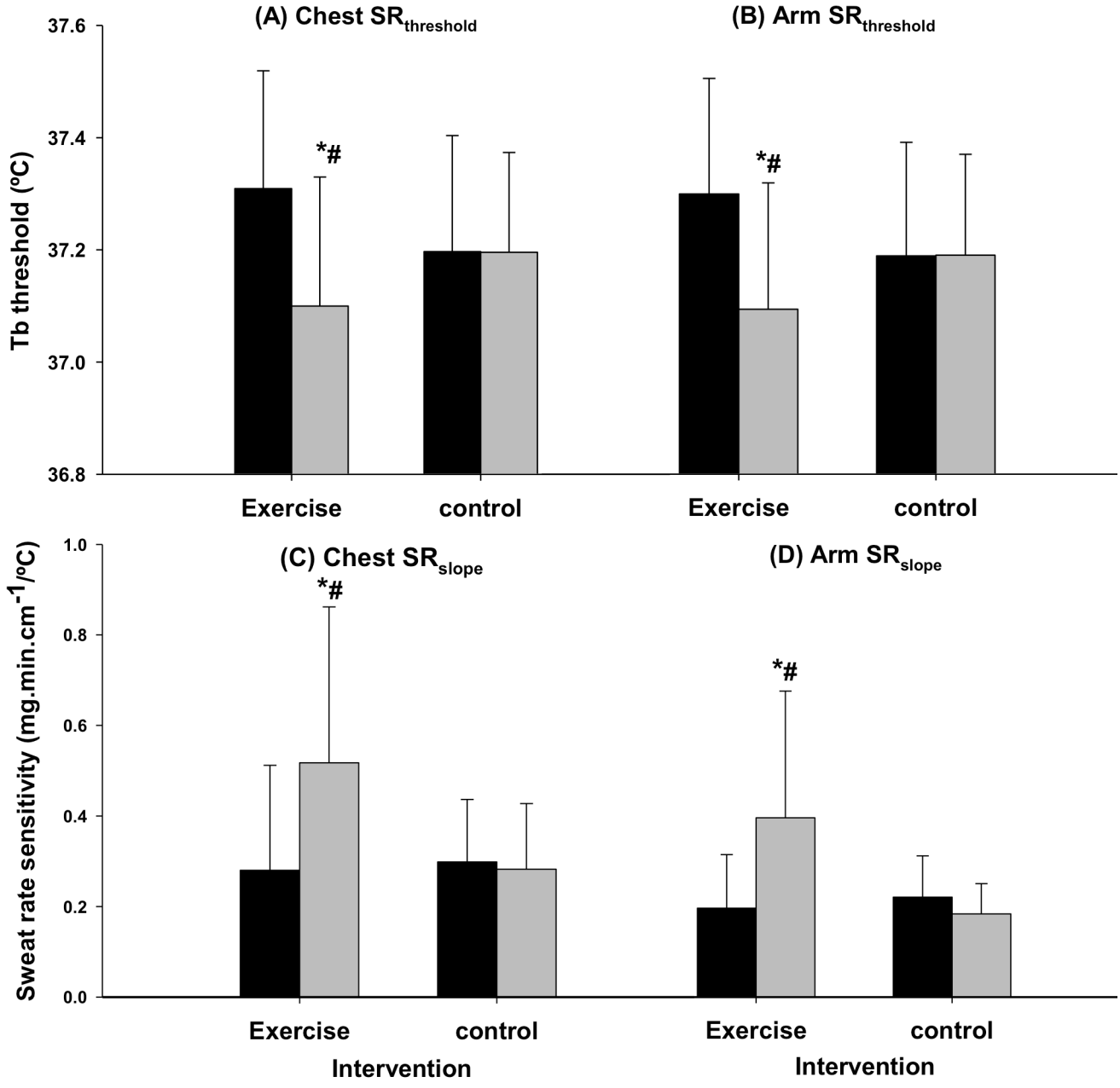


Figure 4.3 Mean body temperature threshold for the onset of chest (A) and forearm (B) sweating. Sweat rate sensitivity (slope) at the chest (C) and forearm (D). Error bars are *SD*.
*significant difference between pre (black bars) and post (grey bars); #denotes significant interaction between interventions (*P*<0.05).

4.3.5.4 Cutaneous blood flow

There was a significant interaction between intervention and time for core body temperature threshold for cutaneous vasodilation at the chest (*P*=0.004) and forearm (*P*=0.004). The onset of chest cutaneous vasodilation occurred at a 0.21°C (95% CI, 0.14, 0.29; *P*<0.001) lower core body temperature following exercise training compared to a negligible change following control [0.02°C (95% CI, -0.07, 0.09; *P*=0.70); Figure 4.4A]. Similarly, the onset of arm cutaneous vasodilation occurred at a 0.21°C (95% CI, 0.14, 0.27; *P*<0.001) lower core body temperature following exercise training compared to a negligible change following control [0.04°C (95% CI, -0.05, 0.09; *P*=0.70) Figure 4.4B]. The CVC slope was not significantly different between interventions (chest, *P*=0.29; forearm, *P*=0.67), over time (chest, *P*=0.64; forearm, *P*=0.26) with no significant intervention*time interaction (chest, *P*=0.90; forearm, *P*=0.22; Figure 4.4C&D).

4.3.5.5 Cerebral blood flow

CBF decreased during the heat stress (*P*<0.001). There was a significant intervention*time interaction (*P*<0.001), where the reduction in MCAv during heat stress was attenuated following exercise training compared to a negligible change in control (Table 4.3). MCAv was 4.5 cm/s (95% CI, 3.6, 5.5, *P*<0.001) higher during heating following exercise training compared to 0.6 cm/s (95% CI, -0.4, 1.4; *P*=0.24) in control. $P_{ET}CO_2$ decreased during heat stress (*P*<0.001), with no significant main effects or intervention*time interaction (Table 4.3; *P*<0.05). Similarly, CBVC decreased during the heat stress (*P*<0.001). There was a significant intervention*time interaction (*P*=0.01). CBVC was 0.07 $cm \cdot s^{-1} \cdot mmHg^{-1}$ (95% CI, 0.04, 0.09; *P*<0.001; Figure 4.5A) higher during heat stress following exercise training compared to no difference following the control intervention [0.01 $cm \cdot s^{-1} \cdot mmHg^{-1}$ (95% CI, -0.01, 0.02; *P*=0.49); Figure 4.5B].

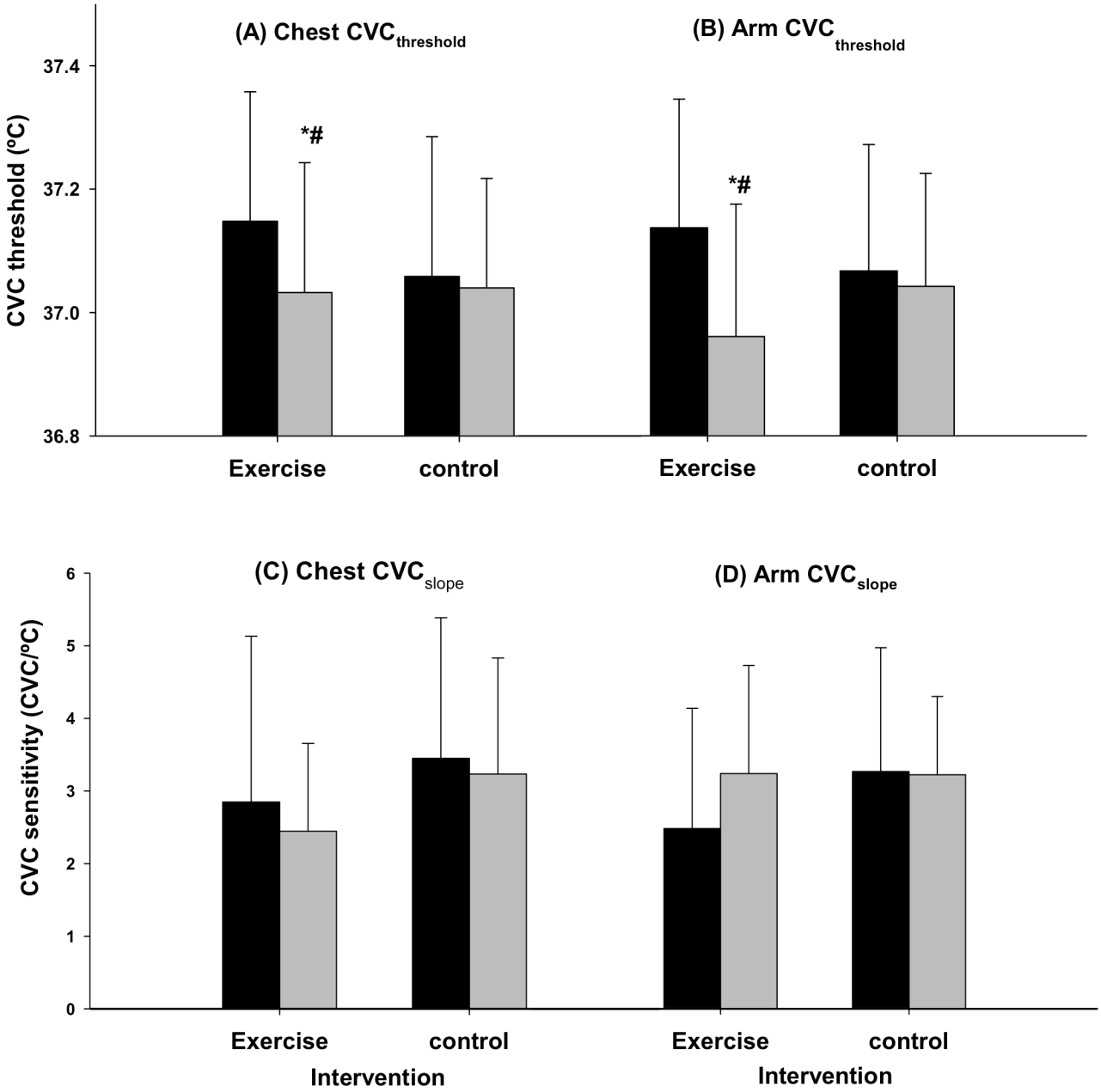


Figure 4.4 Mean core body temperature threshold values for onset of chest (A) and forearm (B) cutaneous vasodilation. CVC sensitivity at the chest (C) and forearm (D). Error bars are *SD*. *significant difference between pre (black bars) and post (grey bars); #denotes significant interaction between interventions (P<0.05).

4.3. Cerebrovascular responses to passive heat stress before and after 16 weeks of exercise training or no-exercise control. *P* values for MCAv (interventio 3, time *P*<0.0001, intervention*time interaction *P*<0.001, intervention*time*temp interaction *P*=0.52), and $P_{ET}CO_2$ (intervention *P*=0.79, time *P*< 0.00 ention*time interaction *P*=0.47, intervention*time*temp interaction *P*=0.59).

e														
	Exercise Training													
	Pre							***Post***						
	Rest	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**Rest**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**
cm/s)	51 (48, 55)	50 (46, 53)	47 (43, 50)	45 (42, 49)	43 (40, 46)	43 (39, 47	42 (37, 46)	54 (51, 58)	54 (50, 57)	51 (47, 54)	50 (47, 54)	49 (45, 52)	48 (45, 52)	48 (44, 52)
	42 (40, 44)	42 (39, 44)	41 (38, 43)	40 (36, 44)	40 (36, 43)	39 (37, 42)	39 (37, 42)	42 (41, 44)	42 (40, 44)	40 (37, 43)	40 (36, 44)	40 (38, 42)	39 (38, 41)	39 (37, 42)
	No-Exercise Control													
	Pre							***Post***						
	Rest	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**Rest**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**
cm/s)	51 (47, 55)	50 (46, 55)	49 (45, 53)	48 (44, 53)	47 (43, 51)	44 (40, 49)	41 (36, 47)	51 (47, 56)	50 (46, 55)	48 (44, 52)	46 (42, 51)	44 (40, 49)	44 (41, 50)	43 (38, 49)
	41 (39, 43)	41 (38, 44)	41 (38, 43)	40 (36, 44)	39 (36, 42)	39 (37, 42)	38 (37, 40)	42 (39, 44)	42 (39, 44)	41 (38, 43)	40 (36, 44)	40 (37, 42)	39 (36, 41)	39 (37, 42)

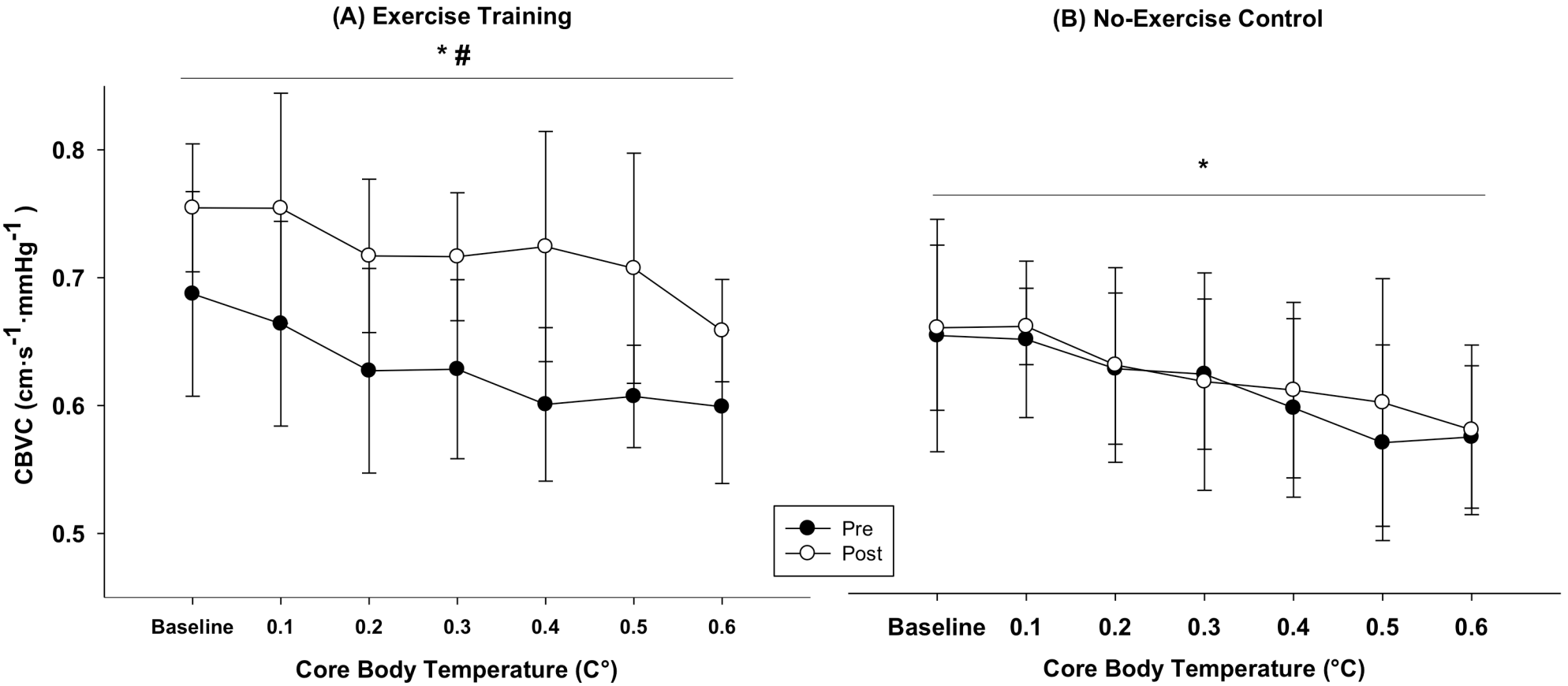


Figure 4.5 Cerebrovascular conductance (CBVC) during passive heating pre (black circles) and post (clear circles) exercise (A) or no-exercise control (B) training. Error bars are SD. *significant reductions from baseline. #significant interaction between intervention and core body temperature (P<0.05).

4.4 Discussion

The novel findings of the present study were that reductions in the subjective ratings of frequency and severity of HFs with exercise training coincided with improved thermoregulatory, conduit and cerebrovascular function in post-menopausal females. These findings provide evidence that improving thermoregulatory and (cerebro)vascular control with moderate-intensity aerobic exercise training can be effective in the treatment of HFs in post-menopausal females.

Exercise training has previously been shown to improve the subjective ratings of HFs in post-menopausal females (Moilanen et al., 2012, Luoto et al., 2012, Karacan, 2010, Reed et al., 2014, Lindh-Astrand et al., 2004), but the underlying physiological mechanisms of how this occurs has not been previously investigated. The results of the current study suggest that the improvements in the occurrence of post-menopausal HFs following 16-weeks of moderate-intensity aerobic exercise training are linked to improvements in thermoregulatory efficiency. The data suggest that exercise training improves thermoregulatory dysfunction via stabilisation of central thermoregulatory control i.e. lowering core body temperature and improving heat dissipation thresholds, alongside improvements in peripheral mechanisms that allow for greater heat dissipation (sweating sensitivity), and thus thermoregulatory efficiency. Importantly, these findings of improved thermoregulatory efficiency with exercise training support previous studies that suggest an improvement in VO_{2peak} in the range of ~15-20% mediates positive adaptations to thermoregulatory function (Pandolf, 1979, Ichinose et al., 2009). For example, this has been shown in pre-menopausal females following a similar intensity exercise training intervention (Ichinose et al., 2009). Nevertheless, this is the first study to demonstrate

that post-menopausal females can improve thermoregulatory function, and intriguingly, that this can contribute to alleviating the frequency and severity of HFs.

The precise mechanisms underlying the pathophysiology of HFs is unclear; however it is acknowledged that thermoregulatory dysfunction is a key contributing factor (Deecher and Dorries, 2007, Freedman, 2014a, Stearns et al., 2002). Elevated basal core body temperature and a narrowing of the thermo-neutral zone (null zone where shivering and sweating do not occur) are thought to be responsible for the large, rapid increases in skin blood flow, sweating and flushing that characterise HFs (Freedman, 2001). However, a narrowing of the thermo-neutral zone to ~0.0^{o}C as reported by Freedman *et al.* (2001) would suggest that any increase in core body temperature (>0.01^{o}C) would continually trigger cutaneous vasodilation and/or sweating, yet this does not characterise the rapid increases observed during HFs (Low et al., 2010, Low et al., 2008a). Instead, it is possible that a fluctuating core body temperature and threshold(s) for cutaneous vasodilation and sweating are responsible for HFs in symptomatic females. This study suggests that improving the control of the thermoregulatory system through lowering core body temperature and improving heat dissipation mechanisms *per se* reduces the occurrence of HFs.

The improvements in thermoregulatory efficiency (lowered thresholds, increased slopes), may be due a variety of central and/or peripheral adaptations as a result of the exercise training intervention. These mechanisms are likely mediated via changes in the sweating response and include increases in the number of sweat expulsions per minute (Ogawa and Sugenoya, 1993), sweat gland hypertrophy (Inoue et al., 1999), increased NO availability (Welch et al., 2009), and/or enhanced sweat gland recruitment at a given

internal temperature, or indeed, a combination of all of the above (Shibasaki et al., 2006). Regardless of the exact mechanism, this study suggests that improved thermoregulatory function in post-menopausal females following exercise training, leads to a reduction in the occurrence of HFs.

The ability of the blood vessels (including the cutaneous, conduit and cerebral vasculature) to vasodilate and thus deliver blood flow systemically also contributes to thermoregulatory control. The reduction in oestrogen associated with the menopause causes endothelial dysfunction via decreased NO bioavailability (Virdis et al., 2000) and/or increased reactive oxygen species scavenging NO (Kojda and Harrison, 1999). Exercise increases eNOS expression via similar mechanisms of transcriptional regulation to that of oestrogen (Chambliss and Shaul, 2002). Arterioles exposed to oestrogen *in vitro* exhibit an upregulation of eNOS and augmented NO-mediated vasodilation in response to increased flow and shear stress (Huang et al., 2000), similar to the responses observed with exercise training (Green et al., 2004b). A novel finding of the current study was that NO-mediated conduit artery endothelial function, measured using FMD, improved following the exercise training intervention. Previous studies in post-menopausal females have not observed exercise-mediated increases in endothelial function using FMD (Moreau et al., 2013, Pierce et al., 2011). The exercise stimulus (unsupervised walking (Moreau et al., 2013) vs supervised moderate intensity exercise in the current study) is likely responsible for these differing findings given that supervised moderate intensity exercise training has been shown to enhance cutaneous endothelial function and microvascular reactivity in post-menopausal females previously (Hodges et al., 2010, Tew et al., 2012a). Nevertheless, this is the first investigation of exercise mediated changes in endothelial function in symptomatic post-menopausal females. Recent research studies have

suggested that menopausal HFs are associated with greater vascular impairments including endothelial dysfunction (Thurston et al., 2008b), with endothelial dysfunction a main determinant of HF severity in early post-menopausal females (Bechlioulis et al., 2010). Therefore, it is likely that this group of females have greater impairments in endothelial function and cardiovascular disease risk, a condition that exercise training in this study appears to ameliorate.

Reductions in CBF are evident during a HF (Lucas et al., 2013) and during a passive heat stress challenge (Brothers et al., 2009, Low et al., 2009, Low et al., 2008b) and thus are implicated in thermoregulatory control, via a reduction in the delivery of blood flow to the thermoregulatory centre in the brain. Improved basal blood flow delivery to the cerebral circulation was observed with exercise training along with attenuation in the reduction of CBF typically observed with passive heat stress in the current study. Whilst, Murrell *et al.* (2013) recently suggested that a short-term exercise training intervention improved cerebrovascular health across the lifespan; this is the first study to establish that short-term exercise training enhances basal MCAv in post-menopausal females, and that this reduction remains evident when normalised for blood pressure (CBVC), with no differences in $P_{ET}CO_2$. No study to date has examined the exercise mediated change in CBF during passive heat stress, yet given that CBF reductions occur during a HF (which could be described as a heat stress response) it is plausible that an exercise mediated attenuation in CBF decreases during heat stress may positively impact on cerebrovascular control during a HF. The mechanisms responsible for these adaptions could include an exercise-mediated increases in stroke volume (Stratton et al., 1994) related to plasma volume expansion (Nielsen et al., 1993, Schlader et al., 2013) and/or improved endothelial function (Ainslie et al., 2007) (see *Chapter 6* for further discussion).

An alternative although not mutually exclusive explanation for the improvement in HF occurrence could be related to the central sympathetic nervous system that influences cutaneous, conduit and cerebral vasculature by activating decreases and increases in blood flow via the noradrenergic and cholinergic systems, respectively (Johnson et al., 2014, Ainslie and Duffin, 2009), whilst the latter also activates sweating onset (Shibasaki et al., 2006). Sympathetic noradrenergic nerve outflow increases following the menopause (Hart et al., 2011), and elevates peripheral vascular resistance (Charkoudian and Wallin, 2014), while sympathetic cholinergic nerve activity is also increased during HFs (Low et al., 2010). Moreover, Oneda *et al.* (2014) reported reduced muscle sympathetic nerve activity (MSNA), an index of sympathetic nerve activity measured using microneuography, in post-menopausal females following 6-months of moderate-intensity cycling exercise alongside improvements in forearm blood flow. Although not directly assessed in this study, reductions in sympathetic nerve activity are likely following exercise training that could have directly reduced the occurrence of HFs in this study, or indirectly, by reducing vascular resistance.

The impact of a reduction in BMI with exercise training on HFs also deserves consideration. Reductions in BMI were evident with exercise training in the current study in accordance with one previous study that reported lower BMI and HF symptoms following increases in self-reported physical activity (van Poppel and Brown, 2008). Whilst the role of BMI on HF prevalence is unclear, observational studies have reported that females with low (Schwingl et al., 1994) and high (Thurston et al., 2008a, van Poppel and Brown, 2008) body fat are at increased risk of HFs. Although speculative, increased adiposity may increase HFs due to elevated insulation and associated dysfunctional thermoregulatory control.

Despite the benefits of the exercise training intervention on reducing both the frequency and severity of HFs, HFs were not completely abolished (~62% reduction in weekly frequency) following exercise training. Nevertheless, these responses mimic those observed (~50-72% reduction in HFs) following HRT administration in symptomatic females over a short-time frame i.e. 12-16 weeks (Utian et al., 2004). HRT administration over a longer time-frame (i.e. 12 months) further reduces HFs (MacLennan et al., 1993, Maclennan et al., 2004) and thus the effects of exercise training may also further alleviate HFs in a similar dose-response manner, and should be considered in future studies. Despite a similar effect i.e. reduction in HFs, it is currently unknown if the effects of exercise and/or HRT act via the same mechanisms. A distinct difference with HRT is the re-introduction of oestrogen, known to be the primary cause of HFs (in its absence) during the menopause (Freedman, 2001), and thus potentially restoring 'normal function'. Conversely, exercise training may directly target the area of dysfunction i.e. thermoregulatory control in the absence of oestrogen. Whether the combined effects of exercise training and HRT further improve HFs and offset the increases in cardiovascular risk observed with HRT (Sare et al., 2008, Rossouw et al., 2002) is worth considering. Furthermore, whether the positive effects of exercise on reducing HFs remain following cessation of exercise training is currently unknown; however it can be speculated that the positive effects may be transient in the absence of exercise training i.e. ~4 weeks, in line with the reductions observed in thermoregulatory function following the cessation of exercise training in young females (Ichinose et al., 2009).

One limitation of this study is that it was not a randomised control trial, with participants free to choose which treatment group they entered. Whilst these findings are labelled preliminary and need to be confirmed in a larger randomised control intervention trial,

the reduction in HF frequency in the exercise group is similar to that observed in previous studies, and the HF frequency remained unchanged in the control group. Furthermore, thermoregulatory measurements are objective and cannot be influenced by the participant (Cheuvront et al., 2009), and were analysed in a blinded fashion. Nonetheless, it is acknowledged that the current findings are specific to early post-menopausal females (1-4y since last menstrual period) that were free of cardiovascular disease and not engaged in regular physical activity. The impact of exercise training in alleviating HFs in individuals with cardiovascular risk factors, or disease, or other populations who experience HFs (e.g. cancer patients) warrants further research.

In summary, improvements in the occurrence of HFs with short-term exercise training are mediated via thermoregulatory and (cerebro)vascular adaptation(s). This study provides mechanistic evidence that exercise training is indeed a useful non-pharmacological alternative intervention in the treatment of HFs. These findings suggest that targeting the thermoregulatory and (cerebro)vascular control systems with interventions may be useful in treating symptomatic post-menopausal females that suffer from HFs.

CHAPTER 5: EXERCISE TRAINING ALTERS THE ACUTE PHYSIOLOGICAL RESPONSES THAT OCCUR DURING MENOPAUSAL HOT FLUSHES

5.1 Introduction

A HF is broadly defined as a sudden subjective intense sensation of heat that is associated with rapid increases in skin blood flow and profuse sweating and flushing that begins in the chest and radiates to the face, head and arms (Stearns et al., 2002, Freedman, 2002). Thus, HFs are physiologically identified as a transient and pronounced increase in sweat rate that is preceded by a rapid surge in cutaneous vasodilation (Low et al., 2010, Low et al., 2008a, Freedman, 2001).

In order to establish mechanistic understanding of HFs, thermoregulatory, cardio- and cerebro-vascular responses have been observed prior to, during, and following acute HFs using peripheral warming in the laboratory (Kronenberg, 2010, Freedman, 2001, Low et al., 2008a) allowing for objective physiological insight. Elevations in limb blood flow during HFs were first observed using plethysmographic techniques (Ginsburg et al., 1981), however despite the suggestion of increased skin blood flow during a HF, technique constraints meant that regional differences in blood flow i.e. skin vs. muscle, could not be distinguished. Low *et al.* (2008) confirmed increases in cutaneous vasodilation and sweat rate during a HF alongside elevated HR and transient decreases in MAP (in certain individuals). In a series of elegant studies by the same group they found that increases in cutaneous vasodilation and sweating during HFs are mediated by sympathetic neurally-mediated mechanisms similar to those observed during heat stress (Hubing et al., 2010, Low et al., 2010). Hubing *et al.* (2010) established that NO, and not prostaglandins, contribute to cutaneous vasodilation during a HF and that a different pathway other than NO or prostaglandin is responsible for sweating during a HF (Hubing et al., 2010). In a separate study by the same group, acute reductions (~5-30%) in CBF were observed

during menopausal HFs, further highlighting the significant physiological challenge that HFs induce (Lucas et al., 2013).

Chapter 4, and others (Moilanen et al., 2012, Luoto et al., 2012, Karacan, 2010) suggest that exercise training can improve the frequency and severity of subjectively reported HFs compared to a no-exercise control group and also *chapter 4* has for the first time shown that this could be due to improvements in the thermoregulatory control system and the distribution/delivery of blood flow across the vascular tree. Despite the recent insight into the acute physiological changes that occur during a HF, only one study (that specifically investigated CBF) has examined if these changes are related to the severity of HFs (Lucas et al., 2013), whilst no study has investigated if any of the physiological changes during an acute HF are responsive to interventions (pharmacological or non-pharmacological). Such information may provide valuable insight into the treatment of menopausal HFs.

Importantly, profuse sweating, skin reddening (cutaneous vasodilation) and increases in HR typically define the severity of a HF, as shown in *chapter 4*. Therefore, the aim of this study was to compare the acute physiological thermoregulatory and (cerebro)vascular changes that occur during HFs prior to and following a 16-week exercise intervention compared to changes in a no-exercise control group. In line with improved thermoregulatory and (cerebro)vascular control observed in *chapter 4*, we hypothesised that exercise training would reduce the physiological responses during a HF, specifically attenuating cutaneous vasodilation, sweat rate and CBF responses compared to a no-exercise control group.

5.2 Methods

5.2.1Participants

Exercise training (*n*=14) and no-exercise control (*n*=7) participants from *chapter 4* were used as the population for the current study (see Table 4.1 & 4.2). Participants were informed of the methods verbally and in writing before providing written informed consent. The study conformed to the *Declaration of Helsinki* and was approved by the local research ethics committee (LREC).

5.2.2 Research Study Design

Participants reported to the laboratory for a passive heat stress challenge. For details of experimental procedures please see *Chapter 3, General Methods.* All participants underwent visit 1 at baseline and following either 16-weeks of exercise or a no-exercise control period. The exercise-training group took part in an exercise training intervention (see chapter *4.2.5 methodology*).

5.2.3 Hot Flush Analysis

A total of twenty-six HFs were recorded in 18 participants at baseline (11 exercise and 7 control participants). HFs recorded in participants both at baseline, and following the exercise training (*n*=9) and control (*n*=6) interventions were used for analysis. Mild heating was used to provoke the onset of HFs (Kronenberg, 1990, Low et al., 2010), with the onset of a HF objectively identified as a transient and pronounced increase in sternal sweat rate (>0.002 $mg \cdot cm^{-2} \cdot min^{-1}$ per second) as has been used previously (Low et al., 2010, Low et al., 2008a, Freedman, 2001). Participants also informed the research team of a subjective feeling of a HF and once the feeling had dissipated. The end of each HF was objectively recorded as the return of sweat rate to pre HF baseline. Because of the

variance in the length of HFs, each HF episode was divided into eight equal segments, with each segment representing 12.5% of HF duration (Low et al., 2010). Five-second periods of data at the end of each segment, and over a period of 2 minutes before i.e. baseline, and 2 minutes following the HF i.e. post HF recovery, were used for data analysis (Figure 5.1).

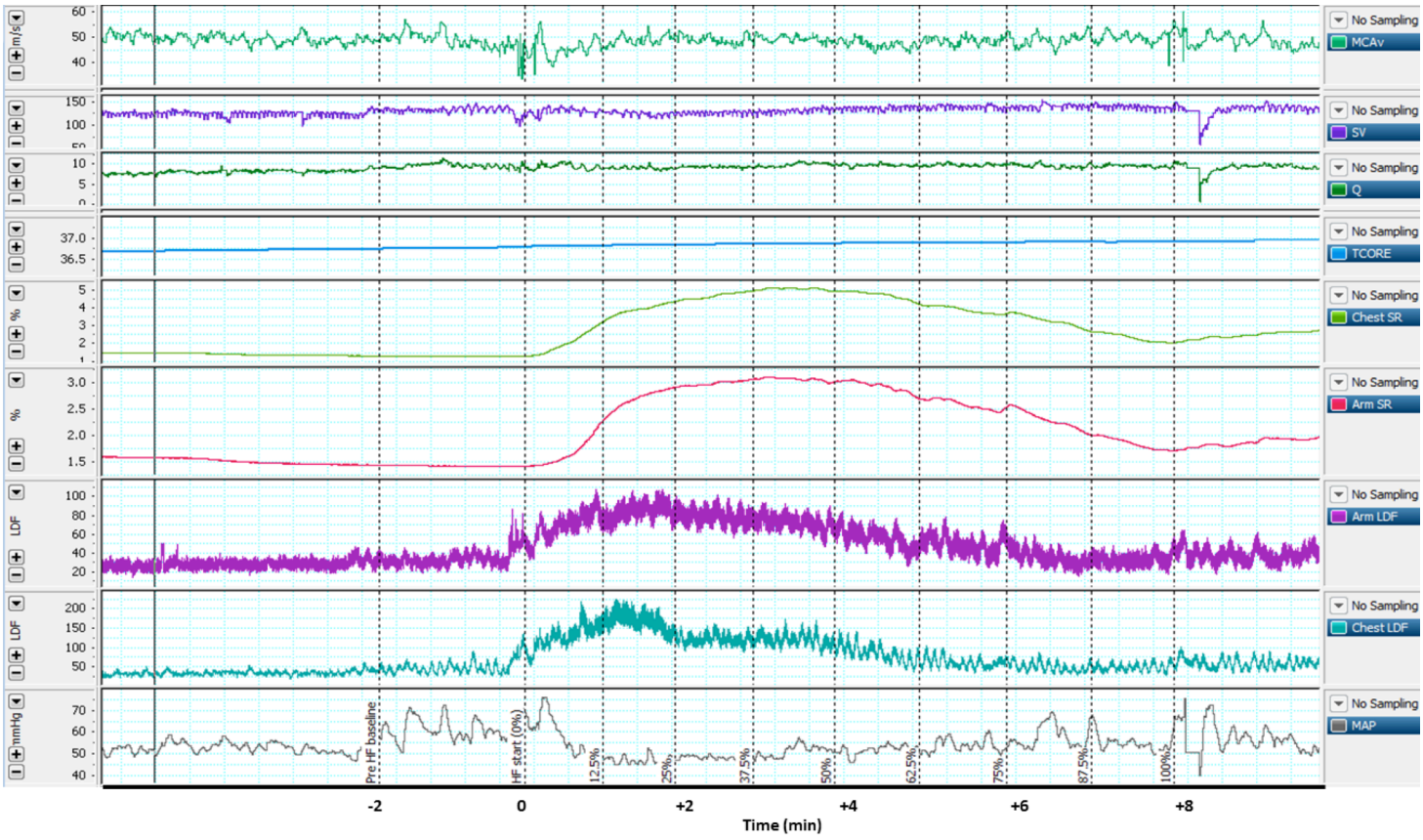


Figure 5.1 Representative data of middle artery cerebral velocity (CBF), stroke volume (SV), cardiac output (Q), core temperature (T_{core}) chest arm sweat rate (SR), chest and arm skin blood flow (LDF), and mean arterial pressure (MAP) from a participant during a hot flush.

5.2.6 Statistical Analysis

HFs were analysed in a blinded fashion by the same observer. A two (intervention*time) factor linear mixed model was employed to analyse HF duration prior to and following exercise training or no-exercise control. A three-way (intervention*time*segment) linear mixed model was employed for the analysis of HF variables during each 12.5% segment, following exercise training or no-exercise control. Statistically significant interactions were followed up with the least significant difference (LSD) approach to multiple comparisons (Perneger, 1998).

5.3 Results

5.3.1 Hot flush duration

HF duration reduced by 63 seconds (95% CI, 14, 113; Figure 5.2) following exercise training compared to a negligible decrease of 17 seconds (95% CI, -43, 66) in the no-exercise control group, however this did not reach statistical significance (P=0.08).

5.3.2 Haemodynamic responses during hot flushes

HR significantly increased by 8 $b \cdot min^{-1}$ (95% CI, 6, 11; P<0.001; Table 5.1 and Table 5.2) during all HFs. There was no main effect of intervention, time, or intervention*time interaction for HR responses during HFs (P>0.05). MAP significantly decreased by 5 mmHg (95% CI, 1, 10; P<0.001) during the HFs with no main effect of intervention, time or intervention*time interaction (P>0.05). Cardiac output and stroke volume did not change during the hot flushes (P>0.05).

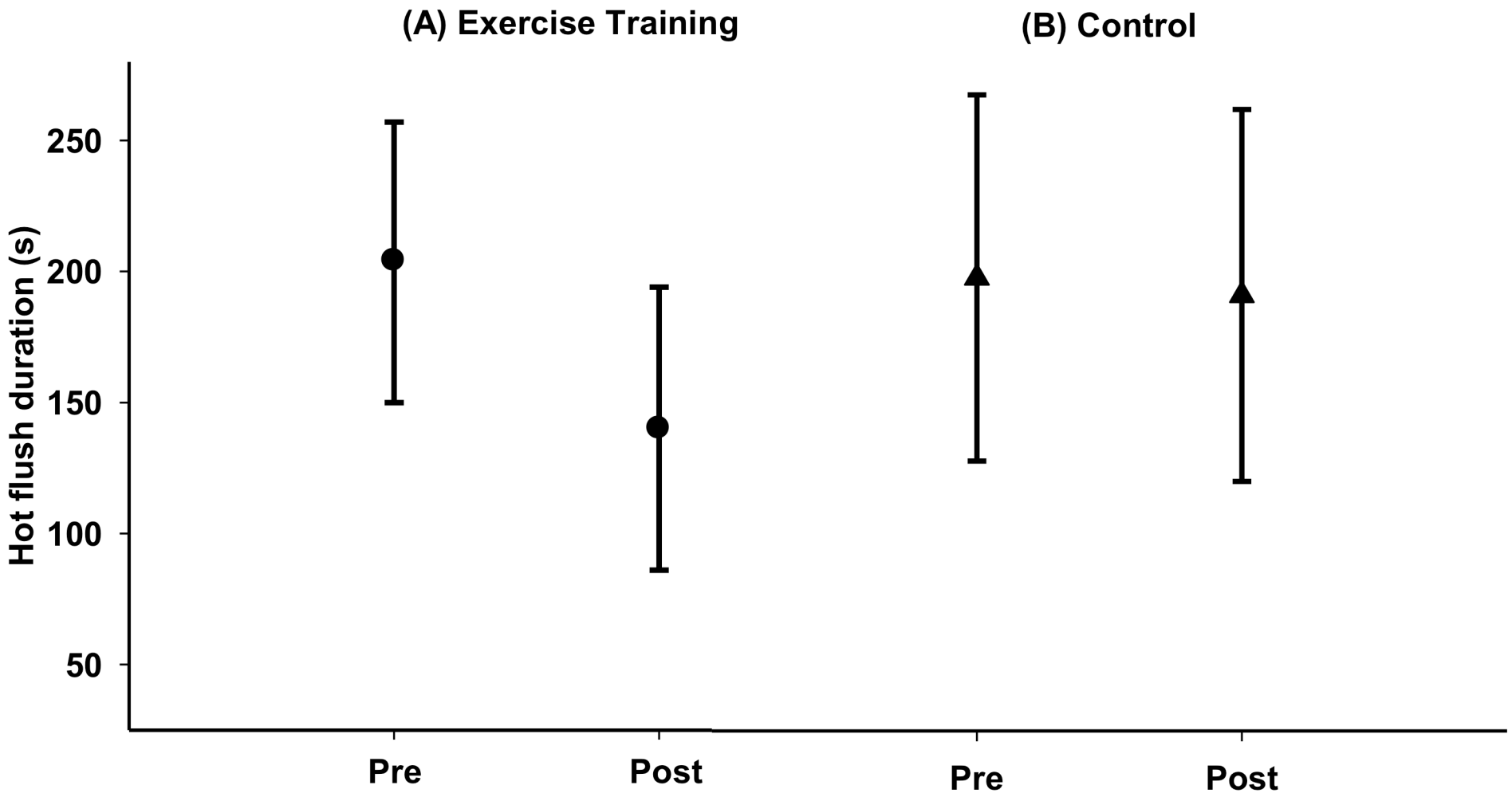


Figure 5.2 Hot flush duration (seconds) before and after (A) exercise training or (B) no exercise control. Error bars are 95% CI.

Table 5.1 Haemodynamic and thermoregulatory responses during hot flushes before (pre) and after (post) exercise training.

Variable	Pre Exercise Training					Post Exercise Training				
	Hot flush (%)					Hot Flush (%)				
Haemodynamics	**Baseline (2min)**	**0**	**50**	**100**	**Post (2 min)**	**Baseline (2 min)**	**0**	**50**	**100**	**Post (2 min)**
Heart rate ($b \cdot min^{-1}$)*	68 (65, 72)	76 (67, 86)	74 (65, 83)	72 (66, 77)	69 (64, 75)	66 (63, 69)	74 (64, 83)	73 (65, 81)	71 (64, 72)	67 (63, 72)
MAP (mmHg)*	75 (71, 79)	70 (61, 78)	71 (60, 79)	73 (64, 85)	73 (64, 83)	74 (69, 80)	71 (60,82)	70 (60, 81)	72 (62, 86)	73 (67, 80)
Cardiac Output (L·min)	7.4 (6.5, 8.2)	7.9 (6.1, 9.1)	7.6 (6.6, 8.7)	7.2 (6.0, 8.4)	7.5 (6.8, 8.2)	7.8 (6.9 8.0)	8.0 (6.4, 9.3)	7.8 (6.3, 9.2)	7.6 (6.6, 8.5)	7.3 (6.3, 8.2)
Stroke Volume (ml)	109 (97, 120)	108 (94, 122)	106 (91, 125)	110 (94, 124)	107 (97, 117)	112 (95, 123)	110 (92, 129)	107 (94, 121)	109 (97, 120)	108 (95, 122)
Thermoregulatory										
Core temperature (°C)	37.1 (36.9, 37.3)	37.1 (36.9, 37.3)	37.1 (36.9, 37.3)	37.0 (36.8, 37.3)	37.0 (36.9, 37.2)	37.0 (36.7, 37.3)	37.0 (36.7, 37.3)	37.0 (36.8, 37.3)	36.9 (36.6, 37.2)	36.9 (36.6, 37.1)
CVC_{chest} (AU/mmHg)*#	1.3 (0.5, 1.8)	2.5 (1.9, 3.2)	1.8 (1.1, 2.5)	1.7 (0.9, 2.4)	1.4 0.6, 2.0)	1.1 (0.5, 1.7)	1.9 (1.3, 2.6)	1.4 (0.8, 2.1)	1.3 (0.7, 2.1)	1.2 (0.5, 1.9)
CVC_{arm} (AU/mmHg)*	0.5 (0.1, 1.9)	1.4 (0.8, 2.0)	0.9 (0.4, 1.6)	0.8 (0.4, 1.2)	0.8 (0.3, 1.3)	0.4 (0.1, 0.8)	1.3 (0.5, 2.0)	0.7 (0.3, 1.2)	0.6 (0.3, 1.1)	0.6 (0.2, 1.0)
CBVC ($cm/s^{-1} \cdot mmHg$)*#	0.66 (0.58, 0 .76)	0.62 (0.48,0 .77)	0.59 (0.47, 0.74)	0.60 (0.50, 0.85)	0.63 (0.52, 0.84	0.70 (0.65, 0.76)	0.69 (0.58, 0.77	0.67 (0.61, 0.77)	0.71 (0.62, 0.80)	0.72 (0.62, 0.81

Data are presented as mean (95% CI). *Significant main effect of time #Significant intervention*time interaction ($P<0.05$). NB. Hot flushes were statistically analysed over 8 segments, but are represented over 3 time segments (0, 50, 100%) above.

Table 5.2 Haemodynamic and thermoregulatory responses during hot flushes before (pre) and after (post) the control intervention

Variable	Pre Control					Post Control				
	Hot flush (%)					Hot Flush (%)				
Haemodynamics	**Baseline (-2min)**	**0**	**50**	**100**	**Post (+2 min)**	**Baseline (-2 min)**	**0**	**50**	**100**	**Post (+2 min)**
Heart rate ($b \cdot min^{-1}$)*	69 (66, 72)	77 (67, 88)	76 (65, 87)	72 (66, 77)	70 (67, 74)	68 (66, 70)	77 (66, 89)	74 (63, 85)	72 (63, 81)	70 (65, 76)
MAP (mmHg)*	75 (71, 80)	70 (62, 77)	71 (62, 79)	72 (62, 81)	73 (64, 83)	75 (69, 82)	69 (60, 79)	71 (62, 80)	72 (64, 78)	74 (64, 83)
Cardiac Output (L·min)	7.2 (6.3, 8.4)	7.3 (6.5, 8.4)	7.4 (6.5, 8.5)	7.2 (6.3, 8.2)	7.3 (6.2, 8.3)	7.4 (6.5, 8.4)	7.6 (6.5, 8.8)	7.4 (6.5, 8.6)	7.2 (6.3, 8.4)	7.2 (6.2, 8.2)
Stroke Volume (ml)	103 (97, 111)	107 (95, 120)	104 (93, 115)	106 (91, 119)	105 (92, 117)	106 (96, 118)	110 (92, 129)	108 (95, 121)	109 (93, 124)	108 (96, 121)
Thermoregulatory										
Core temperature (°C)	37.0 (36.7, 37.2)	37.0 (36.7, 37.3)	37.0 (36.7, 37.2)	36.9 (36.7, 37.1)	36.9 (36.6, 37.1)	37.0 (36.8, 37.3)	37.0 (36.8, 37.3)	37.0 (36.7, 37.3)	36.9 (36.7, 37.2)	36.9 (36.7, 37.2)
CVC_{chest} (AU/mmHg)*	1.6 (0.8, 2.4)	2.7 (1.9, 3.6)	1.9 (1.1, 2.8)	1.8 (1.0, 2.7)	1.3 (0.4, 2.2)	1.3 (0.5, 2.1)	2.6 (1.8, 3.4)	1.7 (0.9, 2.6)	1.6 (0.8, 2.5)	1.3 (0.4, 2.2)
CVC_{arm} (AU/mmHg)*	0.7 (0.3, 1.2)	1.4 (0.5, 1.9)	0.9 (0.4, 1.4)	0.8 (0.3, 1.2)	0.9 (0.4, 1.4)	0.8 (0.3, 1.4)	1.5 (0.6, 2.1)	0.8 (0.3, 1.3)	0.7 (0.4, 1.4)	0.7 (0.2, 1.2)
CBVC ($cm/s^{-1} \cdot mmHg$)*	0.67 (0.57, 0.76)	0.65 (0.43, 0.75)	0.62 (0.53, 0.75)	0.68 (0.55, 0.87)	0.66 (0.52, 0.84)	0.68 (0.65, 0.76)	0.65 (0.58, 0.76)	0.63 (0.51, 0.77)	0.67 (0.54, 0.83)	0.65 (0.50, 0.81)

Data are presented as mean (95% CI). *Significant main effect of time ($P<0.05$). NB. Hot flushes were statistically analysed over 8 segments, but are represented over 3 time segments (0, 50, 100%) above.

5.3.3 Thermoregulatory responses during hot flushes

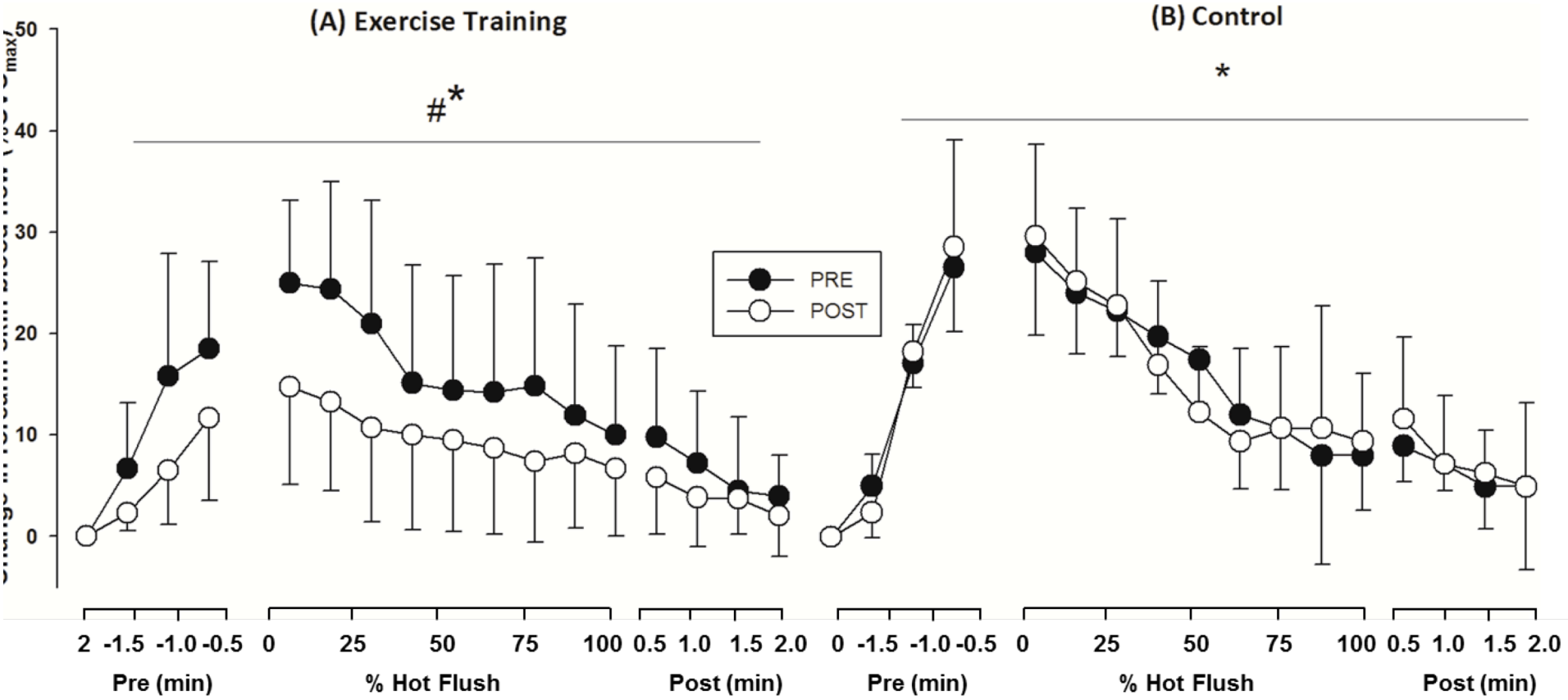
5.3.3.1 Core body temperature responses during hot flushes

The changes in core body temperature during HFs prior to, and following, exercise training or control are summarised in Table 5.1 and Table 5.2, respectively. Core body temperature tended to decrease [by 0.06°C (95% CI, 0.02, 0.11)] during HFs, however this did not reach statistical significance (*P*=0.15). There was no significant intervention*time interaction for core body temperature.

5.3.3.2 Skin blood flow responses during hot flushes

Skin blood flow significantly increased during all HFs (*P*<0.001; Figure 5.3). At the chest, there was a significant intervention*time interaction with a reduction of 9 %CVC_{max} (95%CI, 6, 11; *P*=0.01; Figure 5.3A) during HFs following exercise training compared to a mean difference of 4 %CVC_{max} (95%CI, -6, 8; *P*=0.34; Figure 5.3B) in no-exercise control. There was also a significant intervention*time interaction for CVC at the chest during HFs, with a reduction of 0.5 AU/mmHg (95% CI, 0.2, 0.8; *P*=0.01) following exercise training compared to a negligible change in control [0.2 AU/mmHg (95% CI, -0.3, 0.4; *P*=0.32; Table 5.1 and 5.2).

Similarly, at the forearm there was a significant intervention*time interaction with a reduction of 7 %CVC_{max} (95%CI, 4, 9; *P*=0.05; Figure 5.4A) during HFs following exercise training compared to a mean difference of 3 %CVCmax (95% CI, -3, 6; *P*=0.44; Figure 5.4B). This difference at the forearm was not significant when expressed as CVC, with no intervention*time interaction (*P*<0.05; Table 5.1 and 5.2).



gure 5.3 Changes in chest skin blood flow during hot flushes before and after (A) exercise training or (B) no exercise control. Error bars are SD. ignificant change in sweat rate during a hot flush #significant interaction between intervention and time (P<0.05).

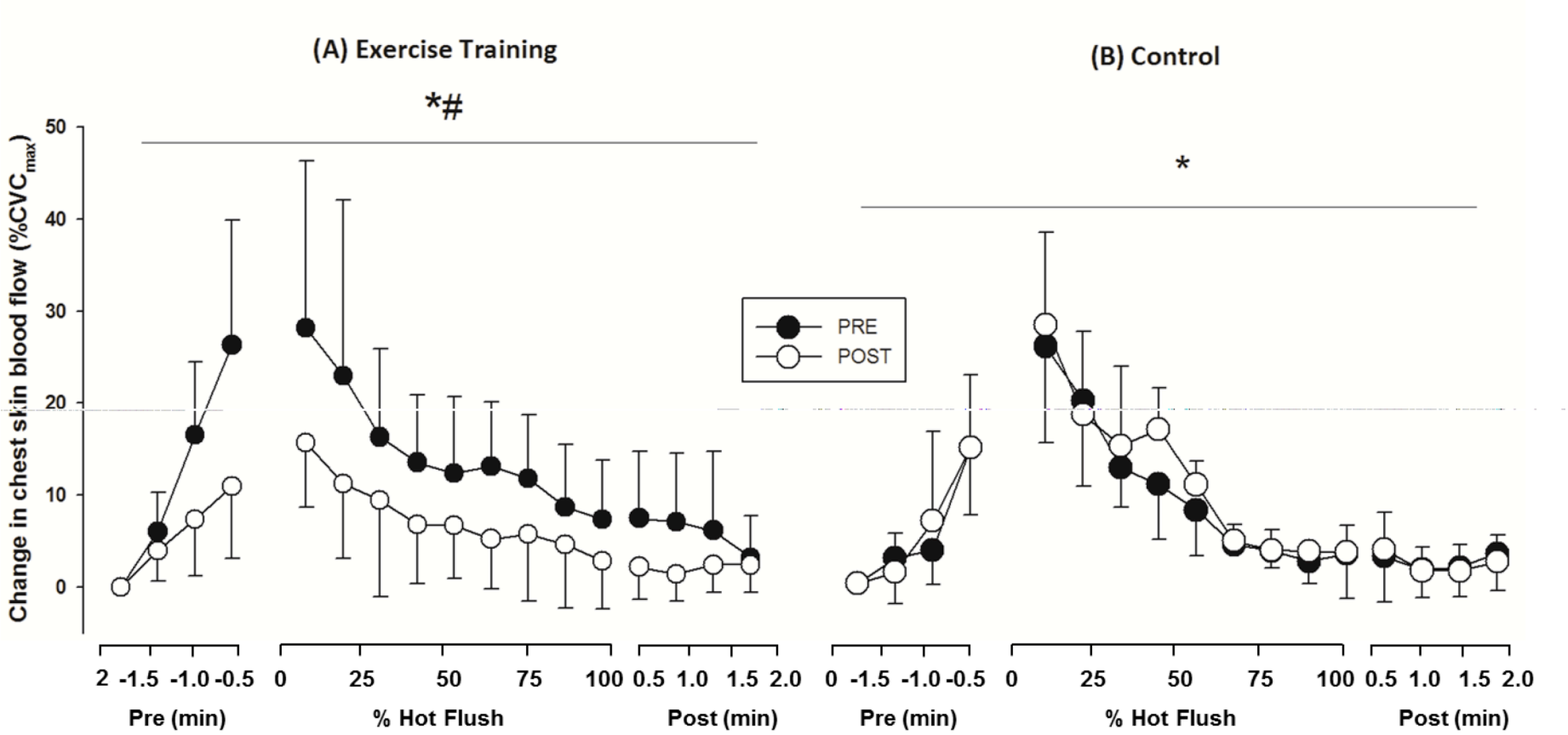


Figure 5.4 Changes in forearm skin blood flow during hot flushes before and after (A) exercise training or (B) no exercise control. Error bars are SD. *significant change in skin blood flow during a hot flush $^{\#}$significant interaction between intervention and time (P<0.05).

5.3.3.3 Sweat rate responses during hot flushes

Sweat rate significantly increased at the chest during HFs (P<0.001) before returning to pre baseline levels. There was a significant intervention*time interaction with a reduction of 0.04 mg·cm^2·min^{-1} (95% CI, 0.02, 0.06; P=0.01; Figure 5.5A) in chest sweat rate following exercise training compared to no change in control [0.01 mg·cm^2·min^{-1} (95% CI, -0.02, 0.03; P=0.19; Figure 5.5B)]. The largest difference was observed in peak chest sweat rate that decreased by 0.11 mg·cm^2·min^{-1} (95% CI, 0.05, 0.17; P=0.001) following exercise training compared to a negligible difference of 0.03 mg·cm^2·min^{-1} (95% CI, -0.03, 0.09; P=0.34) in the control group.

Similarly, sweat rate significantly increased at the forearm during HFs (P<0.001) before returning to baseline levels. There was a significant intervention*time interaction with a reduction of 0.03 mg·cm^2·min^{-1} (95% CI, 0.02, 0.05; P=0.01; Figure 5.6A) in forearm sweat rate following exercise training compared to negligible change in control [0.01 mg·cm^2·min^{-1} (95% CI, -0.01, 0.02; P=0.78); Figure 5.6B]. The largest difference was seen in peak forearm sweat rate that decreased by 0.07 mg·cm^2·min^{-1} (95% CI, 0.02, 0.12; P=0.01) following exercise training compared to a negligible difference of 0.03 mg·cm^2·min^{-1} (95% CI, -0.03, 0.08; P=0.22) in the control group.

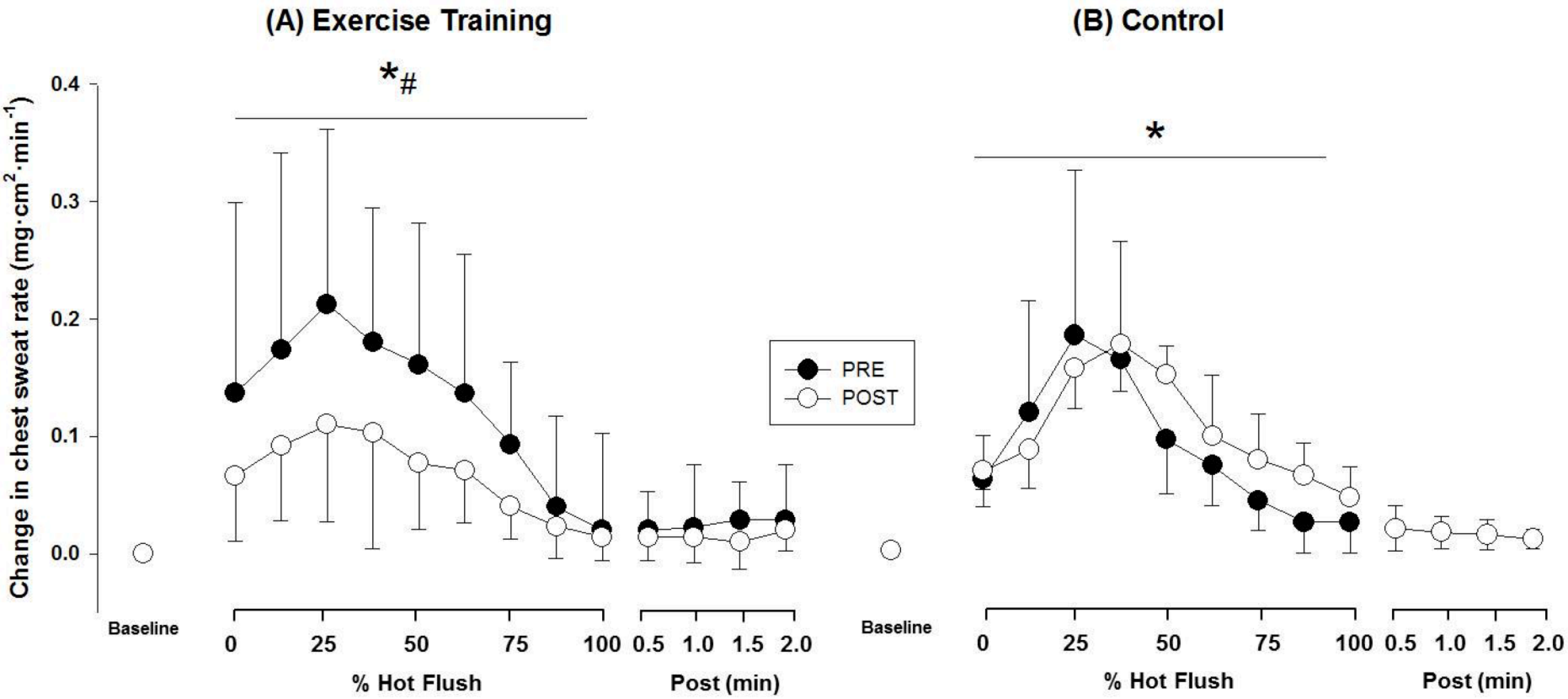


Figure 5.5 Changes in chest sweat rate during hot flushes before and after (A) exercise training or (B) no exercise control.
Error bars are SD. *significant change in sweat rate during a hot flush #significant interaction between intervention and time (P<0.05).

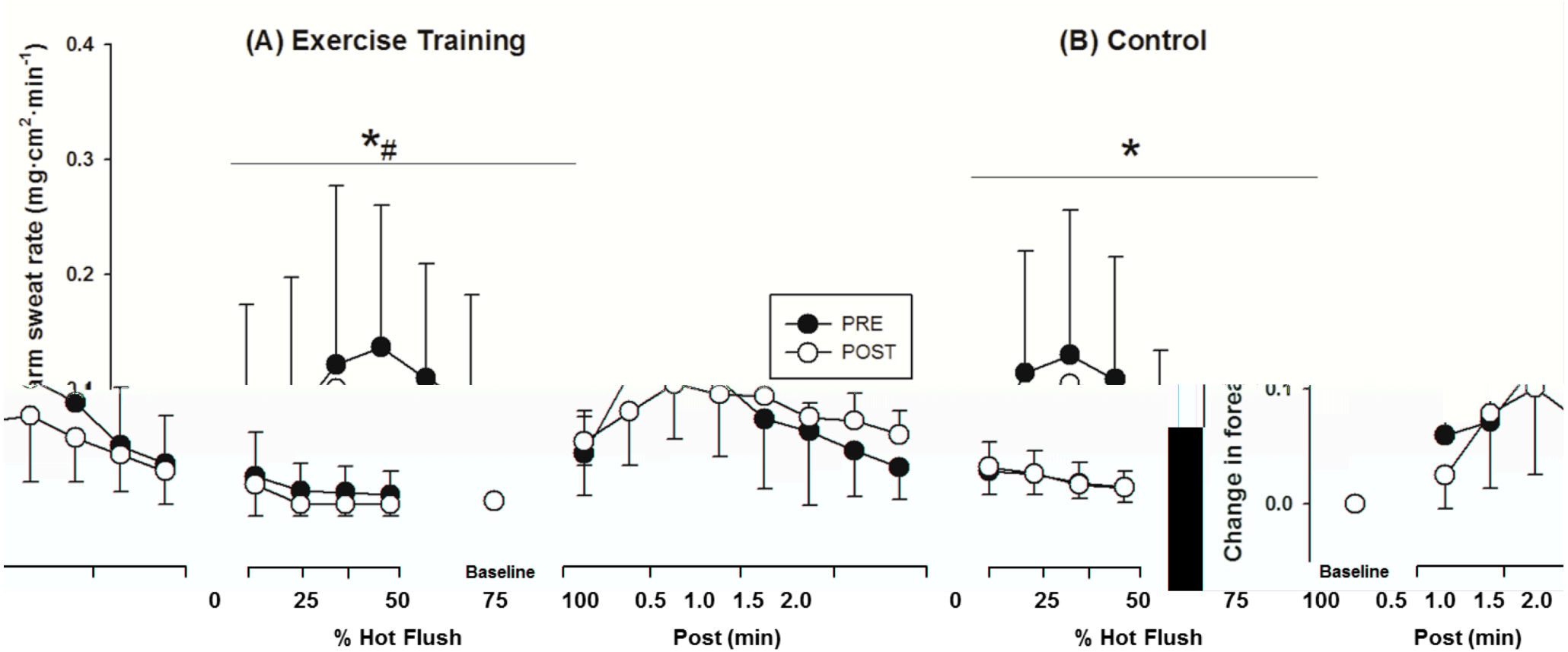
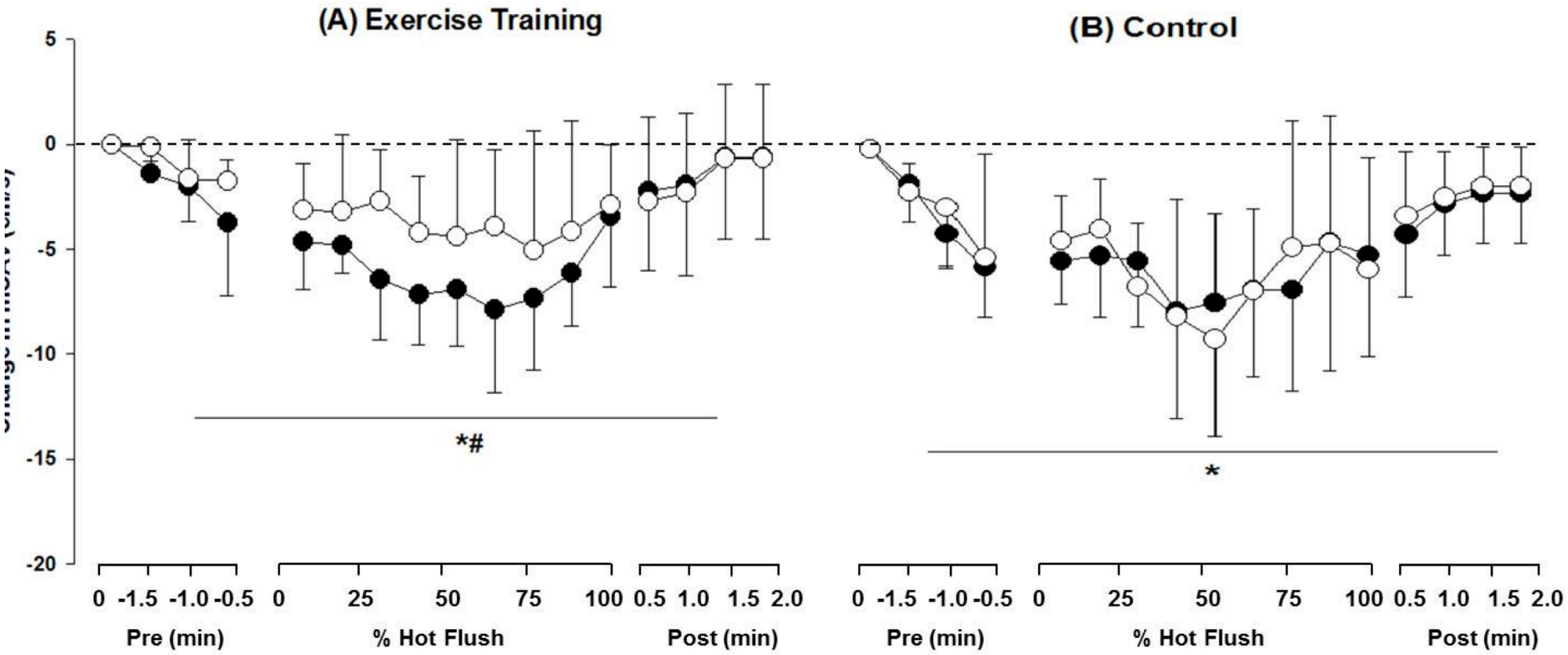


Figure 5.6 Changes in forearm sweat rate during hot flushes before and after (A) exercise training or (B) no exercise control. Error bars are SD.
*significant change in sweat rate during a hot flush #significant interaction between intervention and time (P<0.05).

5.3.4 Cerebral blood flow responses during hot flushes

MCAv significantly decreased during HFs (P<0.001) and returned to pre-baseline levels following HF cessation. There was a significant intervention*time interaction in changes in MCAv during HFs (P=0.04), with the size of the decrease reduced by 3.4 cm/s (95% CI, 0.7, 5.1; P<0.001; Figure 5.7A) following exercise training compared to no differences in control [mean difference 0.6 cm/s (95% CI, -0.7, 1.8; P=0.41; Figure 5.7B). The largest improvements in CBF during hot flushes following exercise training was an attenuation in the peak MCAv decrease of 4.7 cm/s (95% CI, 1.1, 7.4; P=0.005) compared to a negligible difference of 1.6 cm/s (95% CI, -2.2, 4.4; P=0.93) in the control group.

Similarly, CBVC decreased during HFs (P=0.01) and returned to pre baseline levels. There was a significant intervention*time interaction in CBVC (P=0.04) during HFs. CBVC was 0.04 $cm \cdot s^{-1} \cdot mmHg^{-1}$ (95% CI, 0.02, 0.08; P=0.03; Table 5.1) higher during HFs following exercise training compared to a difference of 0.01 $cm \cdot s^{-1} \cdot mmHg^{-1}$ (95% CI, -0.03, 0.04; P=0.12; Table 5.2) in the control group.



gure 5.7 Changes in cerebral blood flow during hot flushes before and after (A) exercise training or (B) no exercise control.
·ror bars are SD. MCA*v* = middle cerebral artery velocity. *significant change in sweat rate during hot flush #significant interaction between intervention ıd time (P<0.05).

5.4 Discussion

The novel findings of the present study were that a short-term exercise training intervention reduced the amount of sweating and cutaneous vasodilation that occurs during a HF in post-menopausal females. Importantly, sweating and skin reddening (cutaneous vasodilation) typically define the subjective severity of a HF. An attenuation of the reduction in CBF typically observed during a HF was also detected. Taken together these findings suggest that exercise training can positively impact upon the acute physiological responses that occur during a HF. These data provide evidence that exercise training directly reduces the severity of a HF by improving thermoregulatory and vascular responses in the cerebral and cutaneous circulations and provides support to the decreased subjective frequency and severity of HFs with exercise training observed in *chapter 4*.

Chapter 4, alongside others (Luoto et al., 2012, Moilanen et al., 2012), established that a short-term exercise training intervention reduced the subjective frequency of HFs. *Chapter 4* also suggests that exercise training can reduce the subjective severity of HFs. Physiologically, sweating and cutaneous vasodilation typically define the severity of a HF on the subjective scale. This is the first study to investigate if the physiological responses, and thus potentially severity, can be altered with an intervention. The results of the current study suggest exercise training mediates acute amelioration of physiological perturbations (sweating and cutaneous vasodilation) observed during HFs which potentially explains the improvements in HF severity corresponding with reduced skin reddening and sweating responses as described on the subjective severity scale in *chapter 4*.

The onset of a HF was objectively identified in the current study as a transient and pronounced elevation in sternal sweat rate (Hubing et al., 2010, Low et al., 2010, Low et al., 2008a). Nevertheless, a HF typically begins with a pronounced cutaneous vasodilation closely followed by profuse sweating; these responses are likely neurally mediated, and are preceded by, a large increase in sympathetic nerve activity (Low *et al.,* 2010). Although not directly measured in the current study, a reduction in basal sympathetic nerve activity has been observed in post-menopausal females following short-term exercise training (Oneda et al., 2014), and may translate into a reduction in sympathetic nerve activity prior to and during HFs in this study, and/or reductions in sympathetic cholinergic vasodilator activity that can directly affect cutaneous vasodilation and sweating during a HF (Low et al., 2010).

The NO system is also likely involved in the exercise mediated attenuated sweating and cutaneous vasodilation during a HF, although the impact of shear stress mediated NO production would occur later in the HF rather than immediately. HF induced cutaneous vasodilation is proposed to cause an increase in shear stress and NO within the cutaneous vasculature that further facilitates vasodilation (Hubing et al., 2010). Whilst NO does not influence sweating during a HF directly (Hubing et al., 2010), the attenuated sweating response during HFs following the exercise training may be due to the decrease in cutaneous vasodilation and a subsequent effect on sweat glands. Shear-related NO release associated with a higher skin blood flow amplifies sweating (Lee and Mack, 2006), while NO inhibition decreases the sweating response (Welch et al., 2009) to increases in core body temperature. Taken together, it is probable that the reduced cutaneous vasodilation (and potentially sweating) may be a consequence of a smaller engagement of

the sympathetic cholinergic system, potentially reducing the direct or ACh-induced release of NO from the endothelium during HFs.

During passive heat stress both cutaneous vasodilation and sweating are neurally mediated via the sympathetic cholinergic system and the release of ACh, and subsequently NO (Charkoudian and Wallin, 2014, Shibasaki et al., 2006). Although a HF mimics these heat dissipation responses and a functional relationship between cutaneous vasodilation and sweating is suggested (Love and Shanks, 1962, Smith et al., 2013b), it is possible that cutaneous vasodilation via cholinergic nerves is partially mediated via additional putative vasodilator peptides separate to sweating, such as other co-transmitters i.e. TRVP and VIP (see section 2.2.1.1). This concept is potentially supported by the current data as there was a reduction in the sweating response at both the chest and forearm with only significant reductions to chest cutaneous vasodilation. Other explanations for the lack of change in forearm cutaneous vasodilation could be due to regional differences in exercise-mediated changes to cholinergic sensitivity (Smith et al., 2013b) or eNOS activity (Del Pozzi et al., 2013). Regional differences exist in impaired cutaneous vasodilator systems with ageing (Inoue et al., 1998) that may respond differently to exercise training. The forearm also has a larger surface area (long and thin) compared to the chest (flat) for cutaneous blood flow and thus heat loss, which may suggest when heat dissipation mechanisms are triggered during a HF the forearm is a priority in thermoregulatory control compared to the chest. Additionally, chest cutaneous vasodilation has a higher basal blood flow than the forearm (irrespective of pre or post intervention) in both *chapter 4* and this study, suggesting there is more scope for a reduction in skin blood flow following a training response.

The acute reductions in CBF during HFs observed in this study were similar to those observed previously (Lucas et al., 2013). In line with the findings in *chapter 4* of improved basal CBF and maintenance during passive heat stress following exercise training, this study established, for the first time, that the acute reductions in CBF during HFs in post-menopausal females are attenuated following a short-term exercise training intervention. Importantly, the exercise-mediated attenuated reductions in CBF during HFs were not explained by alterations in the blood pressure responses in the current study, in agreement with Lucas *et al.,* (2013). Other modulators of CBF consist of cardiac output, arterial CO_2 content and sympathetic nerve activity (Ainslie and Duffin, 2009). Cardiac output (along with stroke volume) remained unchanged during HFs following exercise training in the current study. A hyperventilatory-induced reduction in arterial CO_2 during HFs is possible but this was not measured during HFs in the current or previous studies (Lucas et al., 2013) and warrants further investigation. A reduction in basal or HF-induced sympathetic nerve activity (as discussed above) may also reduce cerebral vasoconstriction and elevate CBF during HFs.

Feelings of faintness during HFs are common (Ekblad et al., 2000, Dormire, 2003), and are associated with the short-term reductions in brain blood flow observed during HFs (Lucas et al., 2013), and is included as a symptom of subjective severity. Despite this, peak reductions in CBF observed during HFs did not correlate with subjective ratings of severity, nor sweat rate or skin blood flow in a previous study although the number of HFs was small to interpret the correlations (Lucas et al., 2013). Regardless of whether a relationship exists between CBF and HF severity, improvements in the control of CBF during a HF with exercise training may be important for reducing feelings of faintness, and also in maintaining cerebrovascular health as reductions in CBF with the menopause

are associated with reduced cerebral reactivity and cerebral microvascular tone in the absence of oestrogen (Ohkura et al., 1995, Belfort et al., 1995, Matteis et al., 1998).

This study supports the findings of *chapter 4* that exercise training reduces the severity of HFs in post-menopausal females via thermoregulatory and cerebrovascular adaptations, and uses objective measures that cannot be influenced by the participant and were analysed in a blinded fashion. A limitation of this study is that it is currently unclear if the passive heat stress stimulus is a reproducible model that can be used to assess objective severity of HFs following intervention. In this study, objective HF severity was unchanged in the control intervention following a 16-week no-contact period that serves some preliminary evidence to suggest HFs can be reliably monitored using the protocol in this study. Also, subjective HF severity was not measured during the acute HFs assessed in the laboratory, and thus it is currently unknown if a reduction in subjective HF severity coincides with a similar reduction in objective HF severity following exercise training. Nonetheless, exercise training reduces the severity of post-menopausal HFs assessed in the community by 101 (95% CI= 80, 121; *P*=0.01) in *chapter 4*, thus it is likely that the current findings support the notion that exercise training reduces the severity of post-menopausal HFs through physiological adaptations that directly affect the acute responses observed during HFs. Such information may provide valuable insight into the treatment of menopausal HFs.

In summary, this is the first study to demonstrate an improvement in objective physiological markers (sweating and cutaneous vasodilation) of HF severity using exercise training as a non-pharmacological treatment in symptomatic post-menopausal females. These findings confirm that exercise training has a direct influence on thermoregulatory

and cerebrovascular responses during HFs *per se*, which may explain reductions in subjective HF severity following exercise training.

CHAPTER 6: WARM WATER IMMERSION TRAINING INDUCES SIMILAR THERMOREGULATORY AND CEREBROVASCULAR RESPONSES TO EXERCISE TRAINING IN YOUNG FEMALES

6.1 Introduction

The function of the thermoregulatory control system (i.e. sweating and cutaneous vasodilation) during internal or external heat stress can be enhanced with exercise training as observed in *chapter 4,* and previously (Armstrong et al., 2005, Ichinose et al., 2009). For example, 3 months of moderate intensity endurance training can enhance the efficiency of the thermoregulatory control system by decreasing the core body temperature threshold for the onset (central function) of, and the sensitivity (slope; peripheral function) of sweating and cutaneous vasodilation (Ichinose et al., 2009). Improvement of thermoregulatory function is important in a sporting context for athletes exercising in the heat but also in a clinical context for individuals with heat related illnesses (e.g., heat induced syncope) or thermoregulatory dysfunction (e.g. symptomatic postmenopausal females in *chapters 4 and 5*, heart failure patients or spinal cord injured individuals). In addition, in some individuals exercise may be contraindicated, and therefore research examining alternative interventions that may improve thermoregulatory control is warranted.

Heat acclimation, involving repeated exposure to internal heat stress (during exercise in a temperate environment for example) has been shown to improve thermoregulatory control by decreasing the core body temperature threshold for the onset of sweating and cutaneous vasodilation (Armstrong et al., 2005, Kuwahara et al., 2005a, Kuwahara et al., 2005b, Lorenzo and Minson, 2010) and/or the slope of the sweating and cutaneous vasodilation response (Inoue et al., 2005, Lorenzo and Minson, 2010, Takeno et al., 2001, Sawka et al., 1989, Sawka et al., 2000). It is also possible that targeting the thermoregulatory control system with a specific exercise-independent heat stress

intervention may induce similar thermoregulatory improvements in the efficiency of sweating and cutaneous vasodilation. Repeated exposure to external heat stress (e.g. resting in a hot and humid environment) has only been employed in a small number of studies using a short time-frame i.e. 2 weeks of heat acclimation to induce thermoregulatory (Eichna et al., 1950, Fox et al., 1963a, Fox et al., 1963b, Shido et al., 1999, Armstrong and Kenney, 1993), vascular and/or cardiac (Imamura et al., 2001, Kihara et al., 2002) adaptations. This strategy is challenging in terms of inducing an appropriate level of hyperthermia as participants have often been allowed to dress minimally in order to promote heat loss and/or it has taken unacceptable amounts of time to reach an appropriate level of hyperthermia to subsequently induce thermoregulatory and/or vascular adaptations. In addition, the beneficial thermal adaptations can be lost soon after the end of the intervention (Ichinose et al., 2009). One external heating intervention that could be more effective than these previous attempts in enhancing thermoregulatory function is repeated episodes of warm water immersion where skin temperatures are maintained high enough to favour heat gain. Few studies have examined the impact of acute warm water immersion (Nguyen and Tokura, 2003) or repeated warm water immersion 'training' on thermal and vascular responses (Green et al., 2010, Naylor et al., 2011). One recent study investigated the local (forearm) responses of skin blood flow to repeated forearm warm water immersion (42^{o}C) over an 8-week period and found improvements in skin blood flow responses to local heating that were attributed to increased blood flow and subsequent shear stress during the forearm water immersion bouts (Green et al., 2010). Therefore, it is possible that a whole body warm-water immersion training intervention could cause similar favourable thermoregulatory and vascular changes to that of exercise training and thus might be a useful alternative intervention to exercise.

The preservation of brain blood flow (CBF) is critical for maintaining appropriate cognitive function. In contrast to elevations during acute bouts of exercise (Ide et al., 2000, Ogoh et al., 2007), CBF is significantly reduced during passive heat stress (Brothers et al., 2009, Low et al., 2010). Precipitous reductions in cerebral perfusion can have significant clinical consequences (Van Lieshout et al., 2003). In addition, chronic reductions in CBF, as observed in aging, are linked to an increased risk of cerebrovascular disease (de la Torre, 2012, Ivey et al., 2011, Ainslie et al., 2008, Stoquart-ElSankari et al., 2007). Murrell *et al.,* (2013) have recently shown that 12-weeks of aerobic exercise training enhances cerebrovascular reactivity (for the same changes in carbon dioxide content there is a greater change in cerebral perfusion) suggesting an improvement in the control of CBF and consequently cerebrovascular health. Furthermore, Ainslie *et al.,* (2008) also found that lifelong endurance exercise training may offset the age-related decline in CBF, whilst *chapter 4* established that CBF at rest and during heat stress improved following exercise training in post-menopausal females. However, no study to date has examined the impact of repeated heat stress exposure on CBF at rest or during passive heat stress. It is unclear if repeated bouts of heat stress and likely reductions in CBF result in a modification of the cerebrovascular response to passive heat stress. Therefore, the aim of this study was to employ a randomised control intervention to compare the effects of 8-weeks of exercise training with 8-weeks of warm water immersion training in young females on thermoregulatory, conduit and cerebrovascular function. We hypothesised that a passive heating intervention using warm-water immersion elicits similar thermoregulatory, conduit and cerebrovascular adaptations to exercise training.

6.2 Methods

6.2.1 Participants

Eighteen healthy females (aged, 25±5 y; BMI, 24.2±4.1 kg.m^2) were recruited. Participants were recreationally active and typically engaged in low (e.g., walking) and moderate (e.g., running, stationary cycling) intensity aerobic activities (2–3 days/week) assessed using a self-report questionnaire. Participants reported regular menstrual cycles (~28 days), had no history of cardiovascular or metabolic disease, were non-smokers and not taking any form of medication, including hormonal contraceptives. Participants were informed of the methods and study design verbally and in writing before providing written informed consent. The study conformed to the Declaration of Helsinki and was approved by the institutional ethics committee.

6.2.2 Research Study Design

Participants underwent two initial visits to the laboratory, and were asked to fast overnight, refrain from alcohol and exercise for 24h and caffeine for 12h before each visit. Visit 1 consisted of assessment of brachial artery endothelial function (FMD) followed by a maximal cardiorespiratory fitness test ($\dot{V}O_{2peak}$). Visit 2 consisted of a passive heat stress challenge to assess thermoregulatory and (cerebro)vascular responses. For details of experimental procedures please see *Chapter 3, General Methods*. Both visits were completed within 7 days and assessments were conducted in a temperature-controlled laboratory (23±1°C). Participants were then randomly assigned to either an exercise [*n*=9; mid-follicular 6; mid-luteal 3)] or warm water immersion (*n*=9; mid follicular 5; mid-luteal 4) intervention. All assessments described in visits 1 and 2 were repeated (repeat assessment in same menstrual phase) 2-5 days following the 8-week training program.

6.2.3 Training interventions

6.2.3.1 Exercise training programme

Participants exercised on a cycle ergometer (Ergo Bike Premium8i, Daum Electronic; Germany) at ~70% of maximal heart rate (HR_{max}) [average exercise heart rate (HR) during training 135±12 beat·min^{-1}], which was achieved by manipulating power output (watts) until the target HR was attained. Each session was supervised and lasted for 30 min, was conducted 3 times per week (94% compliance) for 8-weeks in a temperature-controlled laboratory (21°C and relative humidity (RH) of 45%). Pilot data from the laboratory illustrate these exercise bouts typically increase core body temperature by 0.4-0.7ºC which is similar to previous studies (Kuwahara et al., 2005b, Kuwahara et al., 2005a).

6.2.3.2 Water Immersion training programme

Participants were supervised and immersed (seated) in a water tank (ECB, Gloucester, UK) to top-sternal level (arms were not immersed) at a constant temperature (42ºC) for 30 min, 3 times per week (91% compliance) for 8-weeks. Laboratory temperature was controlled (21°C and 45% RH). Pilot data from the laboratory illustrate that these water immersion sessions increase core body temperature by 0.5-0.8ºC, which is similar to previous studies (Fox et al., 1963b, Armstrong and Kenney, 1993, Nguyen and Tokura, 2003, Inoue et al., 2005, Carter et al., 2014a).

6.2.4 Statistical analysis

For analysis of thermoregulatory variables see *Chapter 4.2.6.* A two (intervention*time) factor linear mixed model was employed to analyse VO_{2peak} and FMD [with baseline diameter entered as a covariate to control for variability in baseline (Atkinson and Batterham, 2013)], alongside resting baseline variables and temperature thresholds and

sensitivities of sweat rate and skin blood flow during the passive heat stress. A three-way (intervention*time*temperature) linear mixed model was employed for the analysis of CBF in response to each 0.1°C increase in core body temperature, during the passive heat stress. Statistically significant interactions were followed up with the least significant difference (LSD) approach to multiple comparisons (Perneger, 1998). Due to variable increments in core body temperature during the passive heat stress, data up to an increase of 0.6°C were used for CBF analysis (*n*=18). Data are presented in the text as mean (95% confidence interval) unless otherwise stated.

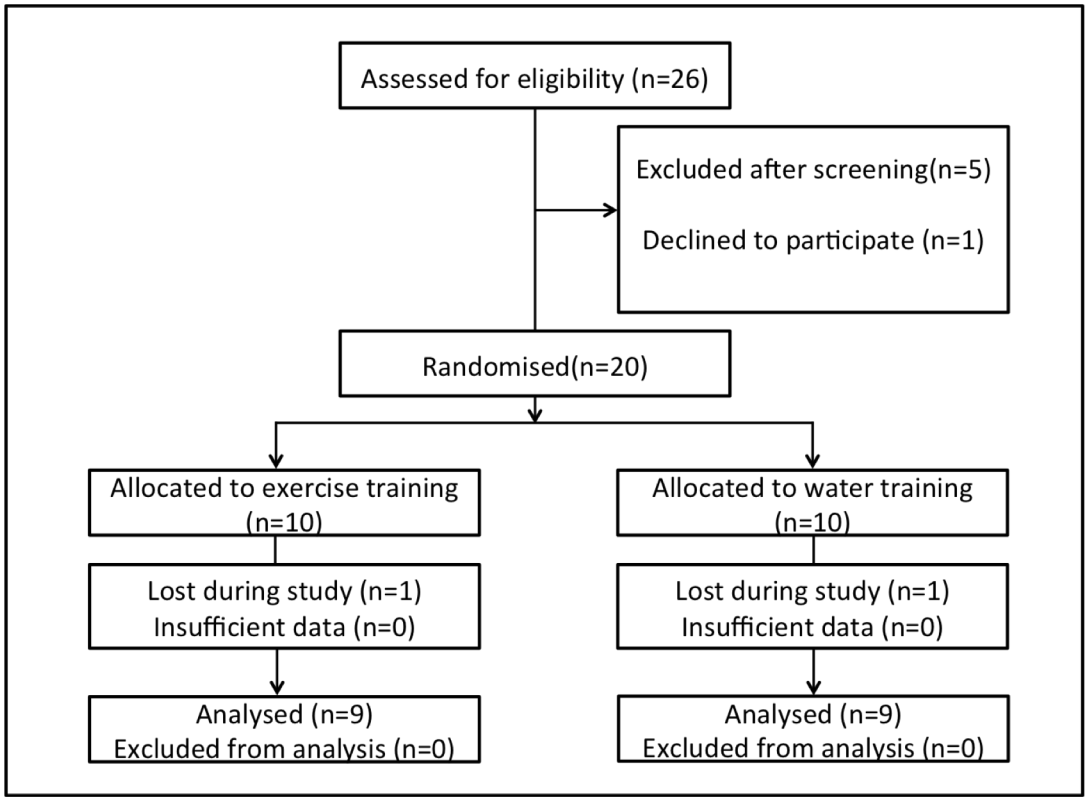


Figure 6.1. Consort-style flowchart regarding inclusion, randomisation and drop-out

6.3 Results

6.3.1 Conduit brachial artery flow-mediated dilation

Brachial artery FMD increased by 1.71 % (95% CI, 0.56, 2.19) following both interventions (*P*=0.003; Figure. 6.2A). There was no main effect of intervention (*P*=0.11) or interaction between intervention and time (*P*=0.18).

6.3.2 Cardiorespiratory Fitness

VO_{2peak} improved by 2.26 ml·kg^{-1}·min^{-1} (95% CI, 1.27, 3.25) following both interventions (*P*<0.001; Figure 6.2B). There was no main effect of intervention (*P*=0.78) or interaction between intervention and time (*P*=0.43).

6.3.3 Resting baseline measurements.

6.3.3.1 Haemodynamics

Heart rate [2 beats·min^{-1} (95% CI, -1, 4)] and mean arterial pressure [3 mmHg (95% CI, 0.2, 5)] were lower following the interventions, although this did not reach statistical significance (*P*=0.07; Table 6.1). There was no main effect of intervention or intervention*time interaction for heart rate and mean arterial pressure. Stroke volume was 3.8 ml (95% CI=0.6, 4.9) higher following the interventions (*P*=0.02), with no main effect of intervention (*P*=0.24) or intervention*time interaction (*P*=0.17). Cardiac output was 0.6 L·min^{-1} (95% CI, 0.1, 1.1) higher following the interventions (*P*=0.07), with no main effect of intervention (*P*=0.26) or intervention*time interaction (*P*=0.51; Table 6.1).

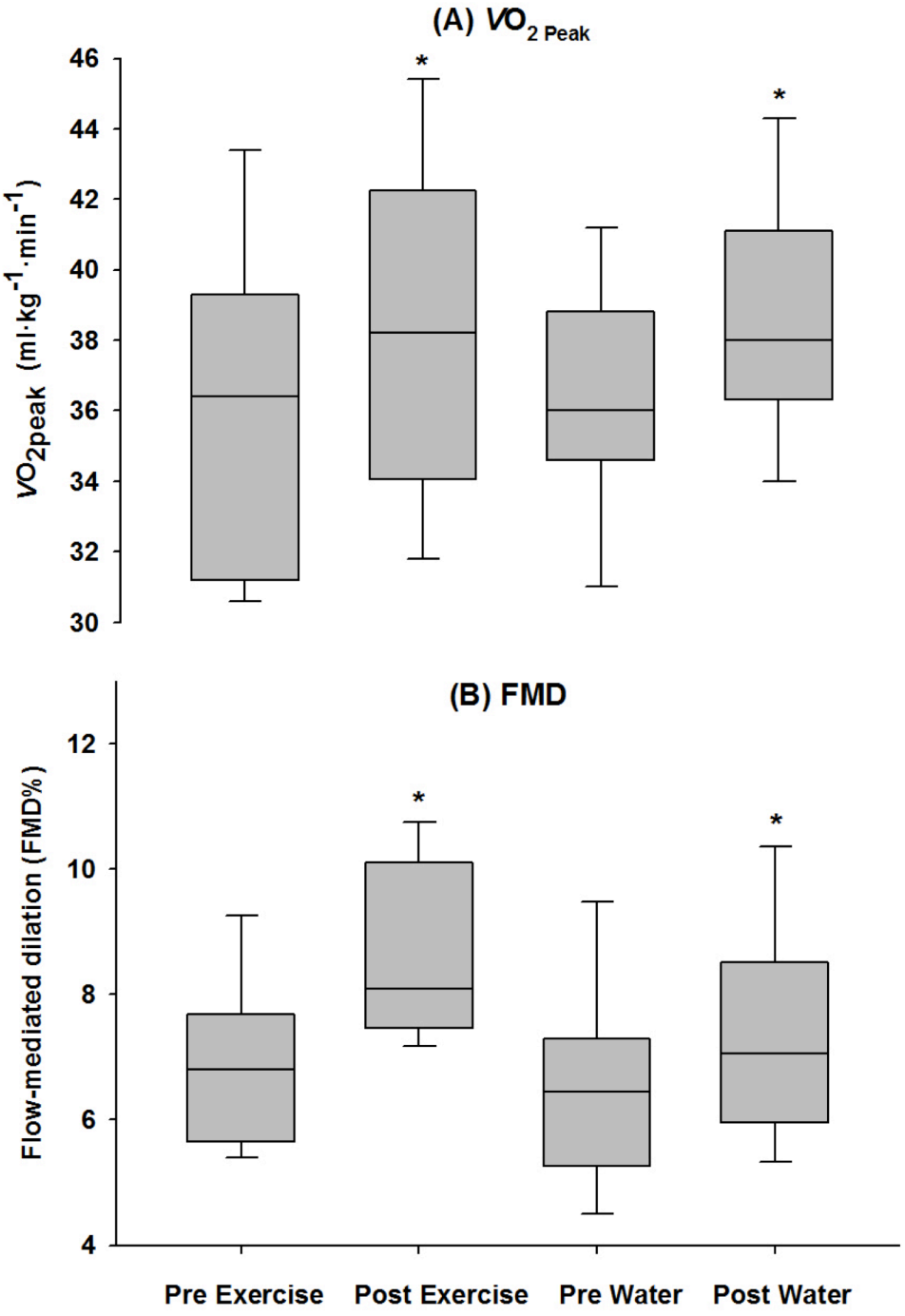


Figure 6.2 Cardiorespiratory fitness (A; VO_{2peak}) and Flow mediated-dilation (B; FMD) before and after exercise and water training interventions. Error bars are SD. *significant difference between pre and post interventions (P<0.05).

Table 6.1. Resting baseline data (mean±SD) before and after exercise (*n*=9) or warm water immersion (*n*=9) training (8-weeks).

Variable	Exercise (n=9)		Water Immersion (n=9)		*P value*		
	Pre	Post	Pre	Post	*Intervention*	*Time*	*Intervention*time*
Heart Rate, (beats·min^{-1})	56±8	54±8	58±7	56±4	0.14	0.07	0.55
MAP (mmHg)	75±6	73±4	76±5	73±5	0.99	0.07	0.84
Stroke Volume, (ml)	87±9	93±14	89±7	94±11	0.24	0.02*	0.17
Cardiac Output, (l·min^{-1})	6.1±0.9	6.3±1	5.4±0.6	6.1±1.2	0.26	0.07	0.51
Core Temperature, (°C)	36.97±0.20	36.88±0.24	37.06±0.25	36.91±0.28	0.74	0.004*	0.70
Skin Temperature, (°C)	32.06±1.57	32.13±1.03	32.58±1.52	32.82±1.58	0.14	0.31	0.29
MCA V_{mean}, (cm/s)	67±4	69±5	68±5	70±6	0.55	0.000*	0.83
CBVC, (cm·s^{-1}·mmHg^{-1})	0.88±0.07	0.90±0.06	0.87±0.06	0.89±0.07	0.73	0.05*	0.89
$P_{ET}CO_2$ (Torr)	42±1	43±3	42±2	43±2	0.12	0.17	0.43
CVC_{chest} (%CVC_{max})	9.6±4.6	11.7±9.0	11.4±6.3	9.8±4.2	0.82	0.88	0.50
CVC_{arm} (%CVC_{max})	5.6±2.5	7.5±2.7	5.8±2.9	8.9±3.2	0.68	0.003*	0.17

*Significant main effect of time (*P*<0.05)

6.3.3.2 Thermoregulatory

Skin temperature was not different between interventions or over time (P>0.05; Table 6.1). Core body temperature was 0.14°C (95% CI, 0.04, 0.23; P=0.004) lower following the interventions, with no main effect of intervention (P=0.74) or intervention*time interaction (P=0.70). There was no main effect of intervention or time for resting skin blood flow at the chest, however skin blood flow at the forearm was 2.2%CVC_{max} (95% CI=0.83, 3.4; P=0.04) higher following the interventions with no main effect of intervention (P=0.22), or intervention*time interaction (P=0.17).

6.3.3.3 Cerebral Blood Flow

Basal MCAv was 2.30 cm/s (95% CI, 1.20, 3.34) higher following the interventions (P<0.001; Table 6.1), with no main effect of intervention (P=0.55) or intervention*time interaction (P=0.83). Basal CBVC was also 0.03 $cm \cdot s^{-1} \cdot mmHg^{-1}$ (95% CI, -0.01 to 0.05; P=0.05; Table 6.1) higher following the interventions. There was no main effect of intervention (P=0.73) or intervention*time interaction (P=0.89). There was no main effect of intervention (P=0.12), time (P=0.17) or intervention*time interaction (P=0.43) for $P_{ET}CO_2$ at rest.

Table 6.2. Cerebrovascular responses to passive heat stress before and after 8 weeks of exercise or water immersion training. *P* values for MCAv (intervention *P*=0.63, time *P*<0.0001, time*temp interaction *P*=0.004, intervention*time*temp interaction *P*=0.76), and $PETCO_2$ (intervention *P*=0.52, time *P*< 0.001, intervention*time interaction *P*=0.39, intervention*time*temp interaction *P*=0.57). Data are mean±SD

Variable	**Exercise**													
				Pre							***Post***			
Tc (°C)	**Rest**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**Rest**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**
MCAv	66±5	61±6	58±7	57±7	56±6	54±9	52±8	69±6	66±7	65±7	63±8	62±9	61±7	60±9
$P_{ET}CO_2$	42±3	42±2	41±3	41±3	40±2	40±3	39±4	42±4	43±2	41±2	41±3	40±2	40±1	39±5
	Water Immersion													
				Pre							***Post***			
Tc (°C)	**Rest**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**Rest**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**
MCAv	67±6	63±7	60±9	58±8	56±9	55±8	54±7	69±6	67±6	66±7	66±8	65±8	63±4	62±7
$P_{ET}CO_2$	42±2	42±3	41±2	40±4	40±2	40±4	39±3	43±2	42±1	42±3	41±2	40±3	41±1	39±3

Tc = core body temperature

6.3.6 Responses during the heat stress challenge

6.3.6.1 Haemodynamics

Changes in heart rate, mean arterial pressure, stroke volume and cardiac output were not different between interventions or over time, whilst there was no interaction between intervention and time (*P*>0.05).

6.3.6.2 Thermoregulatory

Changes in core body temperature and skin temperature were similar between interventions and over time, whilst there was no interaction between intervention and time (P>0.05).

6.3.6.3 Sweat rate

The onset of chest sweating occurred at a 0.10°C (95% CI, -0.14, 0.33, *P*<0.001; Figure 6.3A) lower mean body temperature following the exercise and water interventions. Similarly, the onset of forearm sweating occurred at a 0.19°C (95% CI, 0.12, 0.23, *P*<0.001; Figure 6.3B) lower mean body temperature following the interventions. There was no main effect of intervention at either site (chest, *P*=0.49; forearm, *P*=0.43). There was no intervention*time interaction at the chest (*P*=0.18) or forearm (*P*=0.73).

The rate of sweating at the chest and the forearm, per 1°C increase in mean body temperature, increased following both interventions (P<0.001). At the chest, a significant main effect for intervention was observed (*P*=0.03), alongside a significant intervention*time interaction (P=0.015), where the intervention-mediated change in sweating sensitivity was 1.18 $mg \cdot cm^{2} \cdot min^{-1}$ (95% CI, 0.68, 1.67; *P*<0.001) compared to 0.28 $mg \cdot cm^{2} \cdot min^{-1}$ (95% CI, 0.23, 0.78) higher following the water immersion and exercise

training interventions, respectively (Figure 6.3C). Furthermore, the rate of sweating per 1 ^{o}C increase in mean body temperature was 0.53 mg·cm^{2}·min^{-1} (95% CI, 0.34, 0.74) higher at the forearm following both interventions (*P*<0.001; Figure 6.3D). There was no main effect of intervention (*P*=0.48) or intervention*time interaction (*P*=0.39) at the forearm.

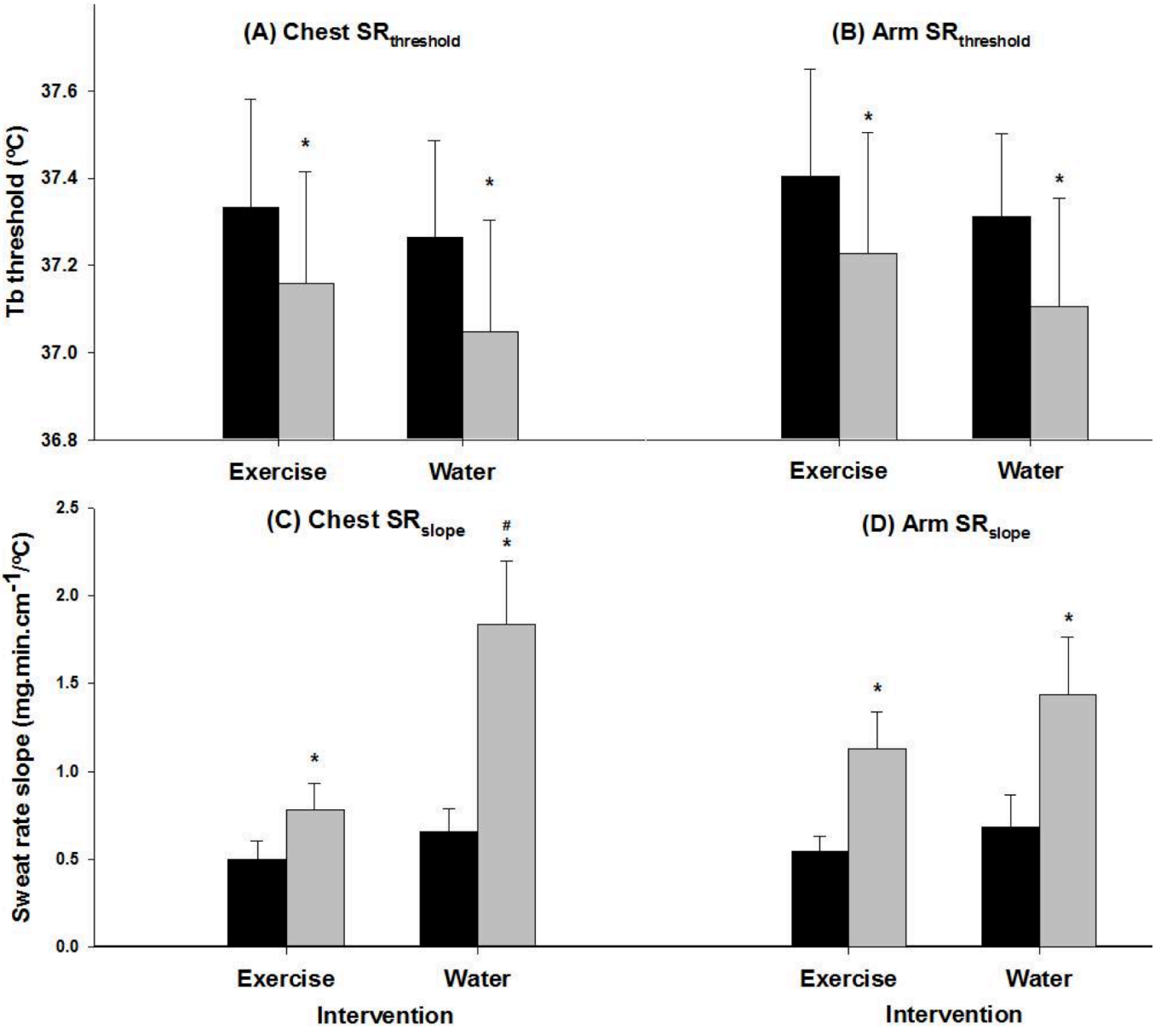


Figure 6.3 Mean body temperature threshold (T_b) for the onset of chest (A) and forearm (B) sweating. Sweat rate sensitivity (slope) at the chest (C) and forearm (D). Error bars are *SD*. * significant difference between pre (black bars) and post (grey bars); # denotes significant interaction between interventions (*P*<0.05).

6.3.6.4 Cutaneous blood flow

The onset of chest cutaneous vasodilation occurred at a 0.20°C (95% CI, 0.14, 0.26, *P*<0.001; Figure 6.4A) lower core body temperature following the interventions. Similarly, the onset of forearm cutaneous vasodilation occurred at 0.19°C (95% CI, 0.12, 0.25, *P*<0.001; Figure 6.4B) lower core body temperature following the interventions. There was no main effect of intervention at the chest (*P*=0.58) or forearm (*P*=0.42), or intervention*time interaction (chest, *P*=0.64; forearm, *P*=0.25). The change in CVC per 1°C change in core body temperature was not significantly different between interventions (chest, *P*=0.92; forearm, *P*=0.71), over time (Figure 6.4C: chest, *P*=0.74; Figure 6.4D: forearm, *P*=0.84) with no interaction (chest, *P*=0.93; forearm, *P*=0.87).

6.3.6.5 Cerebral blood flow

CBF decreased during the heat stress (*P*<0.001). There was a significant time*temperature interaction in MCAv (*P*=0.003), where the reduction in MCAv during heat stress was decreased following the interventions (Table 6.2). MCAv was 6.38 cm/s (95% CI= 5.50, 7.26, *P*<0.001) higher post- compared with pre-intervention. There was no main effect of intervention (P=0.63).

There was a significant intervention*time*temperature interaction in CBVC (P=0.004, Figure 6.5). CBVC was significantly higher post exercise training at 0.1, 0.2, 0.3, 0.5, 0.6 °C increments in core body temperature compared to pre exercise training (*P*=0.008). Mean CBVC (mean for 0.6 °C increase in core body temperature during heat stress) was 0.86 $cm \cdot s^{-1} \cdot mmHg^{-1}$ (95% CI, 0.80, 0.92) post-exercise training compared to 0.80 $cm \cdot s^{-1} \cdot mmHg^{-1}$ (95% CI, 0.73, 0.86) pre-exercise training. CBVC was significantly higher post warm water

immersion at rest and at 0.1, 0.2, 0.3, 0.4, 0.6°C increment in core body temperature (P<0.001). Mean CBVC (mean for 0.6°C in core body temperature during heat stress) was 0.84 $cm \cdot s^{-1} \cdot mmHg^{-1}$ (95% CI, 0.77, 0.90) post-water immersion training compared to 0.81 $cm \cdot s^{-1} \cdot mmHg^{-1}$ (95% CI, 0.75, 0.88) pre warm water immersion.

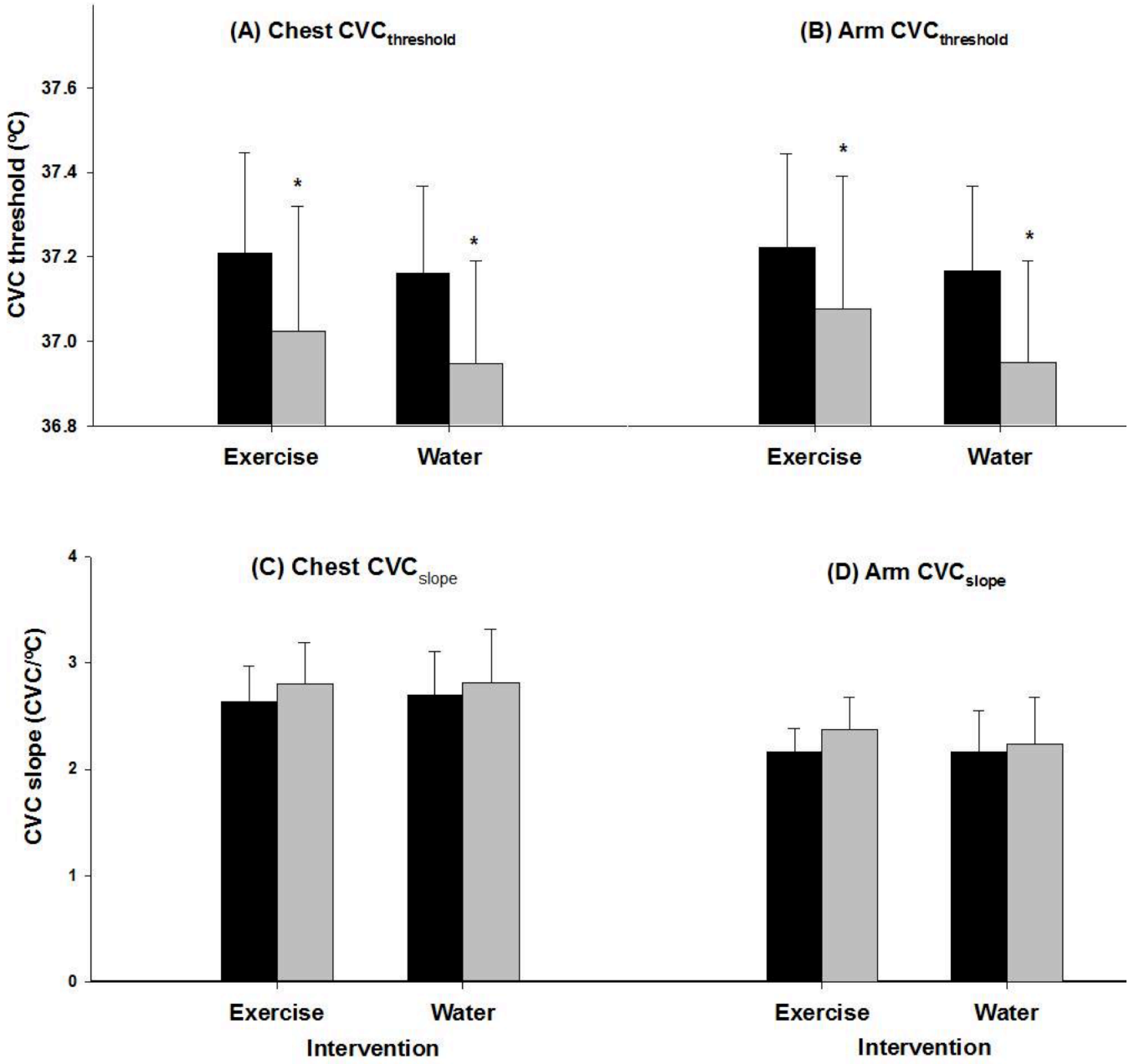


Figure 6.4 Mean core body temperature threshold values for onset of chest (A) and forearm (B) cutaneous vasodilation. CVC sensitivity at the chest (C) and forearm (D). Error bars are *SD*. *significant difference between pre (black bars) and post (grey bars) interventions (P<0.05).

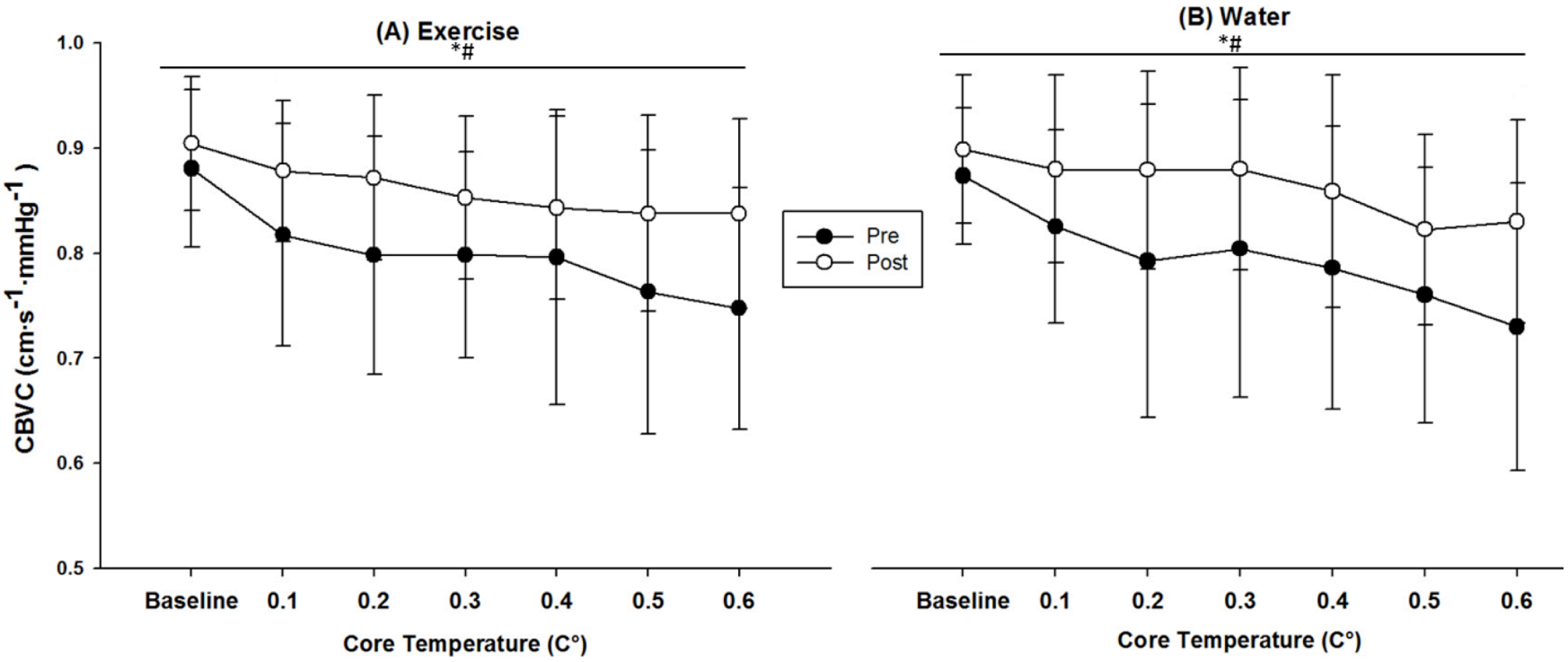


Figure 6.5 Cerebrovascular conductance (CBVC) during passive heating pre (black circles) and post (clear circles) exercise (A) or warm water immersion (B) training. Error bars are SD. *significant reductions from baseline. #significant difference between pre and post intervention

6.4 Discussion

The primary findings of this chapter were that warm water immersion training elicits similar thermoregulatory, conduit- and cerebrovascular improvements to that of exercise training in young females. This is the first study to directly compare an exercise-independent passive heating 'training stimulus' against a traditional endurance exercise training intervention. These findings provide evidence for warm-water immersion training as a useful alternative intervention in improving thermoregulatory, cerebrovascular and vascular function in young healthy individuals.

This study established that 8-weeks of warm water immersion training improved central thermoregulatory function. These enhancements occurred via reduced body and core temperature thresholds for sweating and cutaneous vasodilation, respectively, and were attributed to absolute decreases in resting core body temperature mediated by the training intervention rather than absolute changes in skin temperature (Wingo et al., 2010). Whilst these findings reflect previous studies using short-term (9 days) repeated passive heating (e.g. repeated 30 min leg immersion in warm water (Shido et al., 1999)), and repeated active heating acclimation (Armstrong et al., 2005), this study shows for the first time that these thermoregulatory adjustments are of a similar magnitude as 8-weeks of normothermic cycling training. Enhancements in sweat rate sensitivity with both interventions at the chest and the forearm, with no changes in the sensitivity of cutaneous vasodilation were observed suggesting peripheral sweat gland adaptations. Nevertheless, a greater enhancement in sweat rate sensitivity at the chest was evident with the water immersion intervention compared to the exercise training intervention,

which could suggest that water immersion is more favourable in improving thermoregulatory function.

The exercise training findings in this study are in accordance with previous research work in terms of both central (Yamazaki and Hamasaki, 2003, Patterson et al., 2004, Okazaki et al., 2002, Armstrong et al., 2005, Ichinose et al., 2009) and peripheral (Ichinose et al., 2009, Thomas et al., 1999, Okazaki et al., 2002) adaptations. Enhancements in heat loss responses with exercise training are thought to occur via a decreased temperature threshold for sweating and cutaneous vasodilation (Thomas et al., 1999, Ichinose et al., 2009), followed by a transient change in sweating sensitivity alone (Ichinose et al., 2009, Kuwahara et al., 2005a). The larger improvements in sweat rate sensitivity at the chest following warm water immersion could be related to the chest being submerged in the water during immersion, thereby causing a greater local heat stress (e.g., chest skin temperature) and reducing the surface area for evaporative heat loss. Enhanced sweat rate sensitivity may be due a variety of temperature-induced adaptations mediated by the interventions. These include improved central sudomotor neural activity that results in an enhanced number of sweat expulsions per minute (Ogawa and Sugenoya, 1993), sweat gland hypertrophy (Inoue et al., 1999), increased NO availability (Welch et al., 2009), and/or enhanced sweat gland recruitment at a given internal temperature, or indeed, a combination of the above (Shibasaki et al., 2006). Regardless of the mechanism this study suggests that improved thermoregulatory function with warm-water immersion training occurs in a similar order of magnitude to those observed following exercise training.

Despite no change in the sensitivity of cutaneous vasodilation during the passive heat stress challenge, an increased cutaneous blood flow at rest was observed following both interventions. Reductions in core body temperature in response to heat acclimation or exercise training have been suggested to be due to enhanced cutaneous blood flow and blood volume that enhance heat flow from the core to the body surface (Ichinose et al., 2009). Both exercise training and local (arm) warm water immersion training are known to increase cutaneous blood flow and brachial artery function via up-regulation of nitric oxide (NO) associated with repeated increases in shear stress (Green et al., 2010, Black et al., 2008b, Naylor et al., 2011). Furthermore, enhanced sweat rate sensitivity is also thought to be mediated by shear stress mediated release of NO due to a higher skin blood flow (Lee and Mack, 2006, Wingo et al., 2010). It is probable that both interventions in the current study increased NO bioavailability given that brachial artery endothelial function (FMD) was significantly enhanced. Assessment of conduit artery endothelial function using FMD is a non-invasive assessment of NO dilator function, with at least 50% of the dilator response to FMD NO-mediated in healthy individuals (Green et al., 2014). Enhanced endothelial function with exercise training is thought to be mediated by repeated episodic bouts of increased blood flow and associated shear stress on the artery wall (Tinken et al., 2008) which up-regulates eNOS phosphorylation (Hambrecht et al., 2003). It has recently been demonstrated that increases in brachial FMD with repeated lower limb warm water immersion (and cutaneous function with local warm water immersion) occur via the same shear stress related mechanism A number of central factors that influence vascular adaption may also be possible including the upregulation of circulating hormones or neural outflow, although due to the recent findings of Carter *et al.* (2014) this is unlikely. Taken together, these findings suggest that both whole body exercise and warm water immersion training enhance systemic nitric oxide function.

Another important observation was resting MCAv was higher following both the warm water and exercise training interventions. Recent research studies have suggested that exercise training and fitness are associated with superior cerebrovascular health and cerebrovascular reactivity (Ainslie et al., 2008, Murrell et al., 2013). Yet, this is the first study to demonstrate that repeated passive heating can also enhance basal MCAv. MCAv is extremely sensitive to changes in BP and/or arterial CO_2 (Ainslie and Duffin, 2009), and is also influenced by the distribution of cardiac output (Ogoh and Ainslie, 2009). This study did not find differences in resting $P_{ET}CO_2$ following both interventions; and the higher basal MCAv post-intervention persisted even when normalised for changes in MAP, thereby providing evidence that these variables are unlikely to be the explanation for the increase in basal CBF. Resting cardiac output tended to be higher after both interventions (P=0.07), suggesting that this could have contributed to a higher MCAv following both interventions. One mechanistic explanation for the improved MCAv could be related to improvements in cerebrovascular endothelial function following both interventions via shear stress related mechanisms described above, as cerebrovascular function is partly, nitric oxide dependent (Peebles et al., 2007) and is linked to FMD (Ainslie et al., 2007).

During a bout of exercise (up until very high exercise intensities) CBF increases (Murrell et al., 2013) and thus exercise training causes increases in cerebrovascular shear stress. However, during acute exposure to passive heating a gradual reduction in CBF occurs with increases in core body temperature (Low et al., 2008b, Nelson et al., 2011, Wilson et al., 2006), an observation evident during the heat stress challenge in the current study. This heat stress-induced reduction in CBF is partly attributed to hyperventilatory hypocapnia

leading to cerebral vasoconstriction (Brothers et al., 2009, Wilson et al., 2006). Despite these different responses to CBF during acute exercise and passive heat stress, a greater maintenance of MCAv was observed during the passive heat stress challenge following both interventions suggesting improvements in CBF control that may not be solely explained by changes in vascular function. This study did not observe an attenuated decrease in $P_{ET}CO_2$ during heat stress following the interventions, and therefore the heat stress CBF findings cannot be explained by changes in hyperventilatory hypocapnia (e.g., less hyperventilation or hypocapnia did not occur) (Brothers et al., 2009, Wilson et al., 2006). Although the method of stroke volume and cardiac output estimation used in the present study underestimates the changes in these variables during heat stress (Shibasaki et al., 2011), the changes in stroke volume and cardiac output were similar pre- and post-interventions suggesting that differences in the acute alterations in stroke volume and cardiac output do not explain the better maintained CBF during heat stress post-intervention (Nelson et al., 2011). An alternative explanation may relate to chronic increases in stroke volume, that may have been a function of plasma volume expansion (Nielsen et al., 1993, Schlader et al., 2013) following the interventions. In respect of chronic adaptations, stroke volume is increased following endurance exercise training (Stratton et al., 1994) and along with venous return has been strongly linked to central blood volume (Convertino, 1993, Levine et al., 2002, Convertino et al., 1980). It is well established that endurance training (Sawka et al., 2000, Convertino, 1993) and potentially, repeated heating (Nielsen et al., 1993, Shapiro et al., 1981, Epstein et al., 1975) results in blood volume expansion over a number of weeks. Therefore, whilst this study did not measure blood volume, it is possible that both interventions increased central blood volume, which may have contributed to an attenuated decrease in CBF during heat stress post-intervention. That said, although acute blood volume expansion

during heat stress enables a better maintenance of MAP and attenuates reductions in cerebral perfusion during simulated haemorrhage it does not increase CBF during heat stress alone (Crandall et al., 2008, Keller et al., 2009, Schlader et al., 2013). Similarly, greater increases in plasma volume improvements following exercise relative to passive heating interventions have previously been reported and attributed to exercise-induced non-thermal factors such as increased vasopressin, plasma osmotic and resting plasma protein content (Convertino et al., 1980).

Exercise training is a well-established intervention to improve cardio-respiratory fitness ($\dot{V}O_{2peak}$)(Bassett and Howley, 2000). Whilst not the primary aim, it is interesting, and somewhat surprising, that this study observed for the first time that cardio-respiratory fitness was also improved following the warm water immersion intervention. There are a number of potential reasons for this observation. Central physiological improvements to stroke volume, cardiac output and potentially, blood volume represent several physiological mechanisms that influence maximal exercise performance (Saltin and Calbet, 2006, Sawka et al., 2000). Analogous to exercise training, repeated elevations in cardiac output and stroke volume during warm water immersion (Gabrielsen et al., 1993, Begin et al., 1976, Boussuges, 2006, Weston et al., 1987, Carter et al., 2014b) may induce similar favourable cardiac and haemodynamic adaptations and directly influence blood flow during maximal exercise. Additionally, peripheral improvements in conduit vascular function found in this study may also contribute to improvements in maximal aerobic capacity via enhancements in muscle blood flow and oxygen delivery.

The current study has a number of noteworthy strengths including the inclusion of both central and peripheral thermoregulatory control measurements combined with vascular

function in conduit, cerebral and cutaneous blood vessels to gain insight into the integrative impact of the interventions at a systemic level. Although, this study only included healthy young females who were free of disease, non-smokers and moderately trained, exercise training has previously been shown to enhance both temperature (Wingo et al., 2008) and cerebrovascular regulation (Bailey et al., 2013, Ivey et al., 2011) in older individuals (Bailey *et al.*, 2013) and those with cerebrovascular disease (Ivey *et al.*, 2011). Furthermore, this study illustrates that warm water immersion could have at least similar benefits on both thermal and vascular function which may be useful in patients with chronic conditions (e.g. spinal cord injured), symptomatic post-menopausal females suffering from HFs, or those that have a limited exercise capacity (e.g. heart failure). Despite a relatively small enhancement in MCAv at rest (2.30 cm/s higher following both interventions), it is suggested that these improvements may have clinical significance, and be magnified in populations whereby CBF is already compromised such as following acute stroke (Ivey et al., 2011) or with ageing (Stoquart-ElSankari et al., 2007, Bailey et al., 2013).

In summary, this chapter demonstrates for the first time that short-term warm water immersion training elicits similar thermoregulatory, conduit- and cerebrovascular adaptations to a period of moderate-intensity exercise training in young females. These results may serve as an important indication that warm water immersion can be a useful, alternative intervention to enhance thermoregulatory control and vascular function and its therapeutic use on both healthy and diseased populations warrants further study.

CHAPTER 7: SYNTHESIS OF FINDINGS

7.1 Aims and objectives

The research work described in the current thesis was designed to investigate thermoregulatory and (cerebro)vascular (dys)function in post-menopausal females suffering from HFs, and to establish whether supervised exercise training could ameliorate HFs (subjectively and objectively) via thermoregulatory and cardiovascular adaptation. Furthermore, this thesis investigated the usefulness of warm water immersion training as an alternative to exercise training in targeting thermoregulatory and (cerebro)vascular adaptation.

7.2 Major findings

7.2.1 Exercise training reduces hot flushes in post-menopausal females

Chapter 4 reported that a moderate-intensity exercise training intervention in symptomatic post-menopausal females reduced both the frequency and severity of HFs, and for the first time linked the improvement to enhanced thermoregulatory and (cerebro)vascular function following exercise training compared to a no-exercise control group. Specifically, core body temperature and the temperature thresholds for cutaneous vasodilation and sweating were significantly reduced, alongside improved sweating sensitivity that together improved thermoregulatory efficiency. Furthermore, basal CBF (MCAv and CBVC) was enhanced following exercise training alongside an attenuation of the typical reduction in CBF observed during heat stress. The improved cerebrovascular control, in concert with enhanced brachial artery FMD, suggests a role of enhanced NO bioavailability in the absence of oestrogen following the exercise training in symptomatic post-menopausal females.

7.2.2 Exercise training reduces the physiological response during acute hot flushes

In support of the findings of *chapter 4*, *chapter 5* established that a short-term exercise training intervention reduced the acute thermoregulatory and (cerebro)vascular responses typically observed during a HF in symptomatic post-menopausal females. This is the first study to objectively assess whether exercise training reduces the physiological severity during a HF. Specifically, reductions in sweating and cutaneous vasodilation during a HF were observed following exercise training compared to a no-exercise control group, suggesting a reduced thermoregulatory response during a HF. Attenuation in the acute reduction in CBF during HFs was also observed. The reduction in cutaneous vasodilation and improved CBF during the HF *per se* suggests improved systemic blood flow distribution during a HF. Together, *chapters 4* and *5* confirm that exercise training decreases the subjective/objective frequency and severity of HFs via thermoregulatory and (cerebro)vascular adaptations.

7.2.3 Warm water immersion and exercise training in pre-menopausal females

C*hapters 4* and *5* demonstrated improved thermoregulatory and (cerebro)vascular function that impacted upon the prevalence of HFs, and *chapter 6* investigated if these exercise-mediated thermoregulatory and (cerebro)vascular responses were possible with a warm water (42^{o}C) whole-body immersion training intervention. The findings from *chapter 6* suggest that warm water immersion training elicits similar thermoregulatory and (cerebro)vascular adaptations to exercise training in pre-menopausal females. Both warm water immersion and exercise training significantly reduced resting core body temperature and the temperature thresholds for cutaneous vasodilation and sweating, to a similar degree to post-menopausal females in *chapter 4*. Additionally, the increase in resting CBF following exercise and the attenuation in CBF during heat stress observed in

chapter 4 was also found in pre-menopausal females in *chapter 6*, and again was also evident following warm water immersion. Similarly, this coincided with improved FMD following both interventions, implying a role for improved blood flow distribution via enhanced NO-mediated endothelial function. This suggests warm water immersion training may also be useful in symptomatic post-menopausal females via targeting thermoregulatory and (cerebro)vascular adaptations.

7.3 Implications

In order to provide context, a menopausal HF mimics the physiological responses that occur during a moderate heat stress challenge, but over a much shorter time period. HFs are characterised by large increases in cutaneous vasodilation (~80% change from baseline) that precede rapid surges in sweating (by ~2-3 minutes), that can last ~10 minutes in some females. This corresponds to the thermoregulatory responses that occur to cutaneous vasodilation and sweating observed with changes in core body temperature of ~0.4-0.6^{o}C that are typically apparent during an acute 30 minute exercise bout at 50% $\dot{V}O_{2peak}$ (Ichinose et al., 2009), or during 60 minutes of passive heating (Wingo et al., 2010). After a HF, a drop in core body temperature usually occurs as the heat dissipation response is so pronounced over such a short time-frame. Subjective HF severity (using the HF questionnaire) ranges from a feeling of mild warmth with elevations in skin blood flow to very severe subjective sensations of boiling heat, rolling sweat with a stinging skin sensation, an increase in heart rate, dizziness and nausea. The shear range of severity of HFs that affect >70% of post-menopausal females highlights the need for an effective treatment. Despite the extraordinary amount of individuals affected by HFs, treatment options are severely limited; the causal mechanisms responsible remain ambiguous, and

the basic biological effects of oestrogen on thermoregulatory function are not fully understood.

7.3.1 Implications for hot flush treatment

The current first line treatment for HFs is HRT, yet symptomatic post-menopausal females are avoiding this pathway, or not even reporting symptoms to their G.P. (Watson et al., 2007, Isaacs et al., 2005, Townsend and Nanchahal, 2005), due to the increased risks of cardiovascular disease (Rossouw et al., 2002, Manson et al., 2003) and cancer (Hou et al., 2013, Holmberg et al., 2008). In addition, some females cannot be prescribed this treatment due to their age, history of cardiovascular disease or breast cancer, or other gynaecological interventions (NICE, 2009). As such, symptomatic females are attempting to live with the debilitating symptoms of HFs in the absence of effective alternative treatment. This thesis provides support for exercise training as an effective treatment in reducing the frequency and severity of HFs (Luoto et al., 2012, Mansikkamaki et al., 2012, Moilanen et al., 2012, Lindh-Astrand et al., 2004, Reed et al., 2014), and importantly highlights that these reductions may occur via improvements to thermoregulatory and (cerebro)vascular systems following a short-term moderate intensity exercise training intervention. To prescribe exercise as an effective treatment for symptomatic post-menopausal females the mechanism(s) responsible for improvements in the occurrence of HFs must be established. The current findings in *chapters 4* and *5* are preliminary pilot data, thus before meaningful exercise guidelines can be advocated in the community setting, a RCT that directly investigates the effect of exercise training compared to a no-exercise control group on HFs and thermoregulatory and (cerebro)vascular adaptations should be conducted. Moreover, a high and low intensity exercise group should be incorporated into the research design compared to a no-exercise control, in order to

investigate the level of exercise intensity required to induce the requisite thermoregulatory and (cerebro)vascular adaptations that lead to reductions in HFs.

7.3.2 Implications for cardiovascular risk

The menopause is a time of life associated with increased cardiovascular disease risk (Zaydun et al., 2006, Ossewaarde et al., 2005, van der Schouw et al., 1996), and therefore interventions that also directly impact on the cardiovascular system in a positive way are essential. Furthermore, markers of vascular inflammation (Bechlioulis et al., 2012) and endothelial dysfunction (Bechlioulis et al., 2010) are correlated with severity of HFs in symptomatic post-menopausal females. This thesis reported, for the first time, improved FMD (~2.32%) and reductions in subjective and objective HF severity that supports the notion that enhancing endothelial function with exercise training (in)directly impacts on HFs. FMD is predictive of cardiovascular events, with the risk of a cardiovascular event increased by 21% for every 1 standard deviation (~3.5%) decrease in brachial FMD (Inaba et al., 2010). Improvements in cerebral and cutaneous function were also evident. These findings demonstrate that vascular dysfunction as a consequence of low oestrogen levels with the menopause can be ameliorated in the absence of oestrogen, and is likely due to increases in exercise-induced shear stress and the upregulation of NO (Green et al., 2004b). As higher levels of NO confer anti-atherogenic benefit, the findings of this thesis have potentially important implications for the prevention of atherosclerosis, coronary heart disease and stroke in post-menopausal females, as well as improving quality of life by reducing the negative effects of HFs.

Exercise training as an alternative intervention to HRT would also be beneficial to the NHS. Whilst the costs of HRT per patient is low (~£280 annually), the alternative

treatment of exercise training could be implemented easily at low cost to the NHS. Once recommendations about exercise prescription are established, this information can be communicated via community nurses, G.P.s and other health related professionals and implemented via community G.P. referral schemes. Furthermore, given the potential dual benefits on HFs and cardiovascular disease risk with exercise training, this would have direct impact on decreasing healthcare costs to the NHS via reducing a range of disease morbidities and risk factors (Health, 2004, NICE, 2009).

7.3.3 Implications for understanding the causal mechanisms of hot flushes

It is currently proposed that HFs are induced/triggered by (i) prior elevations (~0.04^{o}C) in core body temperature (Freedman, 2001, Freedman, 2005, Freedman, 2014a, Freedman, 1998), (ii) a narrowing of the thermo-neutral zone (the zone when shivering and sweating do not occur) to ~0.0^{o}C, and (iii) subsequent lower core body temperature thresholds for skin blood flow and sweating (i.e. any slight elevation in core body temperature will trigger dramatic increases in cutaneous vasodilation and sweating); in the absence of oestrogen. *Chapter 5*, however, suggests core body temperature is not elevated in the period that precedes a HF episode and that the increase in core body temperature required to initiate cutaneous vasodilation and sweating (i.e. thresholds) during passive heating in symptomatic females was ~0.22^{o}C (*chapter 4*), which contrasts the 0.0^{o}C null zone hypothesis proposed by Freedman *et al*., (2001). Furthermore, the absolute core body temperature preceding a HF in *chapter 5* (~37.0^{o}C) was less than the core body temperature thresholds for cutaneous vasodilation and sweating observed in *chapter 4* (~37.2^{o}C); thus the data in this thesis suggest HFs are triggered prior to the core body temperature thresholds for cutaneous vasodilation and sweating measured during passive heat stress. Nevertheless, HFs still occurred despite reductions in core body

temperature and associated temperature thresholds with exercise training. This may suggest an alternative theory that a fluctuating core body temperature threshold for cutaneous vasodilation and sweating is responsible for HFs in symptomatic females, and not an increase in core body temperature *per se*.

An alternative explanation for the initiation/trigger of a HF and/or a fluctuating core body temperature threshold, if not an increase in absolute core body temperature, could be linked to absolute increases in sympathetic nerve activity, as it is increased following the menopause (Hart et al., 2011). As oestrogen modulates adrenergic receptors in many tissues (Rettberg et al., 2014), it is possible that hypothalamic alpha-adrenoreceptors are affected by diminished oestrogen during the menopause (e.g., they become up-regulated or 'supersensitive' to changes in oestrogen). Fluctuations, or surges, in sympathetic nerve activity would explain sudden increases in neurally mediated (increases in skin sympathetic nerve activity) cutaneous vasodilation and sweating that are observed during HFs (Low et al., 2010), irrespective of changes in core body temperature, and would also provide an explanation for endothelial dysfunction evident in symptomatic post-menopausal females (Bechlioulis et al., 2010, Bechlioulis et al., 2012) if sympathetic tone is elevated post-menopause (Holowatz et al., 2003, Holowatz and Kenney, 2010). Although not directly measured in this study, one previous study has shown a reduction in sympathetic nerve activity following exercise training in post-menopausal females (Oneda et al., 2014), although symptomatic HF status was undisclosed.

A decline in the number or sensitivity of inhibitory pre-synaptic alpha-adrenoreceptors implicated in the thermoregulatory neural pathway in the absence of oestrogen could also be possible. *Chapter 4* suggested that enhancing thermoregulatory efficiency with

exercise training decreased the occurrence of HFs by improving central core body temperature control and thresholds for cutaneous vasodilation and sweating, and via improved vascular function and blood flow distribution. *Chapter 5* demonstrated attenuation in the acute physiological severity of a HF with exercise training. It is clear from these studies that improving thermoregulatory and vascular (dys)function supports the alleviation in the frequency and severity of HFs. Whether exercise training reduces surges/fluctuations in sympathetic nerve activity, or improves the number or (de)sensitivity of alpha-adrenoreceptors and reverses 'abnormal' hypothalamic function in the absence of oestrogen, which is subsequently responsible for reductions in the frequency and severity of HFs, needs further investigation.

7.3.4 Implications for improving thermoregulatory control in females

This thesis has provided evidence that thermoregulatory function is improved by the same mechanisms (absolute reduction in core body temperature and a subsequent shift in temperature thresholds for cutaneous vasodilation and sweating) in pre- and symptomatic post-menopausal females with either exercise or warm water immersion training. These improvements in thermoregulatory function with exercise training (and warm water immersion) suggests a central adaptation and a change in the set point of core body temperature control, with cutaneous vasodilation and sweating initiated earlier during passive heat stress. In concert with this central adaptation, an increase in sweating sensitivity was also evident, likely due to peripheral sweat gland adaptation, either by increased sweat gland output or the number of activated glands during heat stress (Shibasaki et al., 2006). A change in neural drive may also be responsible for either of these adaptations thus suggesting a central influence on sweat rate sensitivity rather than solely peripheral adaptations. Given that both exercise and warm water training targeted

the thermoregulatory system via similar pathways, it is possible that interventions that target the thermoregulatory system in this way could also be used as an intervention for improving the severity of HFs.

Interestingly, the studies in this thesis and others (Ichinose et al., 2009, Armstrong et al., 2005, Kuwahara et al., 2005b, Kuwahara et al., 2005a, Simmons et al., 2011, Yamazaki and Hamasaki, 2003) report no changes to the sensitivity of cutaneous vasodilation with exercise or warm water immersion (heat) training. This may suggest that the increase in sweating sensitivity is the dominant mediator responsible for improved heat dissipation following thermoregulatory adaptation, and that systemic blood flow distribution including the cutaneous circulation, is only required for delivery of heat from the core to the periphery i.e. for the same amount of cutaneous blood flow a larger increase in sweating occurs following thermoregulatory adaptation. A complete understanding of the mechanisms of reflex cutaneous vasodilation and its relationship with eccrine sweating remains uncertain. Whether cutaneous vasodilation is required for the initiation and duration of sweating in response to heat stress is still unclear. Wingo *et al.* (2010) established that local temperature and skin blood flow independently modify sweat rate, whilst during limb ischemia i.e. near-complete blood flow restriction, sweating is severely attenuated (MacIntyre et al., 1968, Collins et al., 1959), supporting a relationship between skin blood flow and sweating responses. Furthermore, during passive heating in individuals with anhidrotic ectodermal dysplasia (absence of sweat glands) only a slight elevation in forearm blood flow is evident (Brengelmann et al., 1981). It would seem logical to suggest then that with a higher skin blood flow an increase in local sweat rate would occur, however no correlation was reported between regional sweat rate and skin blood flow in response to ACh or whole-body passive heating (Smith et al., 2013b). Given

that during passive heat stress, core body temperature thresholds for cutaneous vasodilation slightly precede those of sweat rate activation, it may be likely that cutaneous vasodilation only initiates sudomotor activity and the sweating response, and that with further increases in core body temperature cutaneous vasodilation and sweating responses then diverge. Additional studies are necessary to explore the functional link between skin blood flow and sweating during heat stress.

Nevertheless, a larger increase in sweating sensitivity was reported at the chest following warm water immersion compared to the forearm (increases were evident at both sites following exercise training) that may have been due to a local temperature stimulus of the warm water on the chest compared to the forearm (which was not immersed in the water). As described earlier, increases in sweating sensitivity is likely a central systemic adaptation to exercise training, that can then be further enhanced with local peripheral responses (i.e. local temperature) as observed with the chest during warm water immersion, as is supported by the findings of Wingo *et al.* (2010) discussed above. Furthermore, a direct haemodynamic response (e.g. hydrostatic pressure) to the water immersion itself cannot be discounted. It has recently been established that lower limb water immersion induces acute increases in haemodynamic responses, specifically mean arterial pressure and cardiac output (Carter et al., 2014b), that may also further enhance sweat gland adaptation, possibly via increased blood flow delivery during water immersion. Whether local temperature or haemodynamic responses influence sweat gland adaptation directly with warm water immersion training, and to what degree they separately improve sweating sensitivity, warrants further investigation.

7.4 Methodological considerations and limitations

This thesis adds to previous findings that subjective HFs decline following a period of exercise training (Luoto et al., 2012, Mansikkamaki et al., 2012, Moilanen et al., 2012, Lindh-Astrand et al., 2004, Reed et al., 2014), and adds a number of novel key strengths. The thesis includes both central and peripheral thermoregulatory control measurements combined with vascular function in conduit, cerebral and cutaneous blood vessels in order to gain insight into the integrative impact of the interventions on HFs at a systemic level. For the first time, this thesis objectively assesses (cerebro)vascular and thermoregulatory dysfunction in symptomatic post-menopausal females prior to and following a supervised, progressive, short-term exercise training intervention. Furthermore, measures of thermoregulatory and (cerebro)vascular function are objective, and cannot be influenced by the participant or the observer when analysed in a blinded fashion. *Chapter 5* is the first study to directly measure physiological HF severity following an exercise training intervention, with previous studies relying on subjective reports only.

The method of stroke volume and cardiac output estimation used in the present study underestimates the changes in these variables during heat stress (Shibasaki et al., 2011). However the data provides insight into the changes in resting stroke volume and cardiac output pre- and post-interventions, whilst the changes observed during passive heat stress were of a similar magnitude. A measure of plasma volume pre and post interventions would strengthen the suggestion of increases in stroke volume (Schlader et al., 2013, Nielsen et al., 1993) following exercise training (and warm water immersion) in *chapters 4* and *6*. This would provide insight into the potential reasons for the findings,

including improved CBF at rest and during heat stress that may be responsible for improved thermoregulatory function via enhanced systemic blood flow distribution.

A measure of skin sympathetic nerve activity during heat stress (*chapters 4 and 6*), and during acute HFs (*chapter 5*) prior to and following the interventions, would provide novel insight into the effect of exercise-induced reductions in sympathetic nerve activity and its potential impact on thermoregulatory, cerebrovascular and conduit function. If HFs are mediated by increased sympathetic nerve impulses as suggested in *chapters 4* and *5*, then a measure of sympathetic nerve activity is vital for future studies.

A limitation to *chapters 4* and *5* is it was not a large RCT. However, for the first time, this thesis provides novel preliminary insight into the potentially positive effect of thermoregulatory and cerebrovascular adaptation on HFs in post-menopausal females that should be further established with a RCT that incorporates a range of exercise intensities and a control group. A limitation to *chapter 5* was that no measures of subjective HF severity were reported during acute HFs in the laboratory prior to and following exercise training. It is important for future research to establish the relationship between subjective and objective HF severity that may further support interventions that target reductions in physiological responses during HFs. Additionally, a larger sample size would also promote the use of a mediation analysis (Kraemer et al., 2002) to determine the relative contribution of causal mediators in the reduction of HFs with exercise training i.e. the causal mediator may be related to sweat rate sensitivity adaptation or improved vascular function that indirectly reduces HFs following exercise training.

7.5 Future direction

7.5.1 Randomised control trial

In light of the discussion in *section 7.3.1*, this thesis provides preliminary evidence for the benefits of exercise training on HFs in post-menopausal females via improvements to thermoregulatory and (cerebro)vascular function. To further establish these positive effects, a RCT that assesses the effects of exercise training compared to a no-exercise control is warranted. Furthermore, this should include an extra arm to the study that compares high- versus low-intensity exercise training so the appropriate exercise intensity can be established and appropriately prescribed. Importantly, a RCT would also allow for a mediation analysis to be performed on the data to highlight the causal pathway(s) for the benefits of exercise training on HFs in this thesis. Moreover, in progression from *chapter 6,* a preliminary study investigating the effect of warm water immersion compared to exercise training on thermoregulatory and (cerebro)vascular function in symptomatic post-menopausal females is warranted.

7.5.2 Trigger of a hot flush

Following on from *section 7.3.3*, it is apparent that there is a current requirement to further understand the trigger of HFs in post-menopausal females. A study designed to investigate whether HFs are mediated via increases in central sympathetic nerve activity is warranted, and leads on from the findings of this thesis. To do this, observing the effect of increases in sympathetic nerve activity (using measures of SSNA and/or MSNA), such as during isometric handgrip exercise (Lalande et al., 2014), on triggering HFs in symptomatic females may be useful. If HFs are truly triggered by rapid surges in sympathetic nerve activity, using isometric handgrip exercise (or other acute

interventions that increase sympathetic nerve activity) may be useful in elucidating the physiological trigger of a HF.

7.5.3 Sweat rate adaptation with exercise training

In response to the implications of *section 7.3.4*, a mechanistic study investigating the effect of cutaneous blood flow on the adaptations of sweating responses to heat stress is warranted. This could be interrogated using a study that investigates the combined influence of skin blood flow (shear rate) and local temperature on thermoregulatory adaptation, and could be accomplished through bilateral forearm interventions using water immersion training (arm 1; increases in temperature and skin blood flow) and handgrip exercise (arm 2; local increases in skin blood flow alongside no increases in temperature). This would establish the effects of increases in skin blood flow alone, and the importance of local temperature, on sweat rate adaptation.

CHAPTER 8: REFERENCES

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CHAPTER 9: APPENDECES

7-DAY HOT FLUSH QUESTIONNAIRE

Name…….

Date started…….. Date completed…….

Hot flush categories are described overleaf. Where possible, please note how long each hot flush lasts.

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
Number of hot flushes (mild, moderate, severe or very severe)	Mild: Moderate: Severe: Very Severe:	Mild: Moderate: Severe: Very Severe:	Mild: Moderate: Severe: Very Severe:	Mild: Moderate: Severe: Very Severe:	Mild: Moderate: Severe: Very Severe:	Mild: Moderate: Severe: Very Severe:	Mild: Moderate: Severe: Very Severe:
Total number of hot flushes today*	flushes	flushes	flushes	flushes	flushes	flushes	flushes

*one day should be considered as a 24h period (i.e. 7am to 7am)

Please also note any of the following symptoms:

	NO	YES		NO	YES
Appetite loss	…..	…..	Constipation	…..	…..
Sleeplessness	…..	…..	Trouble Sleeping	…..	…..
Nausea	…..	…..	Nervousness	…..	…..
Dizziness	…..	…..	Mood Changes	…..	…..
Tiredness	…..	…….			
Dry mouth	…..	…..			
Abnormal sweating	…..	…..			
Other	…..	If YES, please describe……			

……..

FEMALE HOT FLUSH DEFINITIONS

Please refer to these examples of hot flushes that have been given by post-menopausal females in previous studies when describing their hot flush severity. One or more of these descriptions may help to categorise your hot flush as mild, moderate, severe, or very severe.

MILD:

Physical symptoms: Warmth, felt uncomfortable, red face.

Emotional symptoms: Not expected.

Action needed: Usually no action taken

MODERATE:

Physical symptoms: Head, neck, ears, or whole body felt warm; tense, tight muscles; clammy (wet skin); a change in heart rate or rhythm (heart speeds up or changes beat); some sweating; dry mouth.

Emotional symptoms: Felt irritated, felt agitated (restless), felt as though energy had been drained out, felt embarrassed when having a hot flush in front of others, felt tired, felt annoyed.

Action needed: Needed to use a fan, awakened sometimes at night, needed to uncover, took off layers of clothing, drank water, opened the windows even when cold outside, and wore lighter clothing.

SEVERE:

Physical symptoms: Warmth, sometimes described as a raging furnace or burning up; a change in heart rate or rhythm (heart speeds up or changes beat); felt faint; headache; severe sweating; weakness; a prickling, stinging sensation over skin; chest heaviness.

Emotional symptoms: Embarrassment, anxiety, feelings of having a panic attack.

Action needed: Needed to stop what was being done at the time, usually awakened at night and removed the covers; needed to remove clothes; opened windows; kept the house a cooler temperature, frequently used fans.

VERY SEVERE:

Physical symptoms: Boiling heat, rolling sweat, difficulty breathing, felt faint, dizzy, feet and/or legs cramping, a change in the heart rate or rhythm (heart speeds up or changes beat), felt slightly sick to stomach.

Emotional symptoms: Felt distressed, had the urge to escape, had difficulty functioning.

Action needed: Awakened frequently at night, needed to change sheets and pyjamas, need to take a cold shower, cold compress on skin.