THE INTERACTION OF ENVIRONMENT AND EXERCISE ON SYSTEMIC VASCULAR FUNCTION

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Abstract

The incidences of cerebrovascular events, such as stroke, appear to have seasonal variation, increasing in the winter months, particularly in older adults. Whilst, the cerebrovascular mechanisms underlying the winter increase in stroke with cold has not been investigated. There is some evidence that a cold stress such as skin surface cooling causes an increase in cerebral blood flow (CBFv) which could contribute to an increase risk of stroke. Conversely, an increase in CBFv may be useful in instances where CBFv has declined (e.g. with age) or where increases in CBFv are beneficial (e.g. during exercise) to cerebrovascular health. The overarching aim of this thesis was to investigate whether the responses to an acute cold stress differ in older individuals and whether the combination of exercising in a cold environment could alter cerebral, conduit and micro-vascular function in young healthy individuals.

In study 1, 12 young (25±5 years) and 9 older healthy individuals (62±6 years) were recruited. CBFv was quantified in the middle cerebral artery using transcranial Doppler, and function assessed via cerebrovascular reactivity to CO_2 (CVR_{CO2}; rebreathing gas concentration of 5% CO_2), dynamic cerebral autoregulation (dCA; manipulations of BP with squat-stand manoeuvres at 0.1Hz.min^{-1}). Conduit endothelial function was assessed via flow mediated dilation (FMD) whilst participants wore a tube-lined suit perfused with 34^{o}C water (thermoneutral condition). Subsequently, the suit was perfused with 12^{o}C water for 30 mins. CBFv, temperature and haemodynamics were monitored during cooling and were followed by repeat measurements of cerebrovascular and endothelial function (cold condition). Cerebrovascular reactivity and autoregulation was not altered by the cold and this was similar in both age groups. FMD reduced by 2.6% (-5.1, -0.2, P=0.05), peak artery diameter was reduced by 0.02 cm (-0.03, 0.00, P=0.05), and SRAUC was reduced by 10 (-19, -2, P=0.02) following cooling. During cooling skin temperature reduced by 6.2^{o}C (-6.9, -5.4, P<0.001) but CBFv did not significantly increase and was similar in both the young and older individuals.

Study 2 employed a randomised control design where 21 (16 males, 22±5 years) individuals were randomly allocated to either exercise training in a cold (5º) or thermoneutral (15º) environment. Exercise consisted of 50-minutes cycling at 70% heart rate max (HR_{max}), 3 times per week for 8 weeks. CBFv, cerebrovascular reactivity and autoregulation were assessed prior to and following the exercise intervention. FMD, skin microvascular function (gradual local heating) and cardiorespiratory fitness (VO_{2peak}) were also assessed. During one session at the midpoint of the training intervention CBFv, temperature and haemodynamics were measured continuously during an exercise bout. Cardiorespiratory fitness improved (2.9 $ml.min.kg^{-1}$, 95%CI 0.5, 5.3; P=0.02), regardless of environment. Neither exercise intervention had an impact on CBFv, cerebrovascular reactivity, FMD or skin microvascular function (P>0.05). There was a significant time*condition interaction for normalised gain (marker of autoregulation), with evidence of a 0.192 $\%cm.s^{-1}.\%mmHg^{-1}$ (95%CI -0.318, -0.065) decrease following training in the cold. There was also evidence of an increase in dCA phase by 0.072 radians (95%CI -0.007, 0.152) following training in the cold (P=0.02). Despite lower T_{sk} during the acute exercise bout in the cold (P<0.001), CBFv, blood pressure, skin temperature or skin blood flow were not different between environmental conditions (P>0.05).

These novel findings indicate that (1) skin surface cooling does not influence cerebrovascular haemodynamics and function, although causes a reduction in peripheral vascular function in both young and older individuals and (2) exercise training in a cold environment may improve cerebral autoregulation more than training in a thermoneutral environment.

Declaration

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

Submitted manuscripts directly based on the work described in this thesis.

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Now, let’s get planning.

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List of abbreviations

Abbreviation	Title
%CVC_{max}	CVC normalised to the maximal flux achieved during 44°C
ACA	Anterior Cerebral Artery
ACAs	Anterior Cerebral Arteries
ACoAs	Anterior Communicating Arteries
ANS	Autonomic nervous system
BA	Basillar Artery
BP	Blood Pressure
CA	Cerebral Autoregulation
CARNet	Cerebral Autoregulation Research Network Group
CBF	Cerebral Blood Flow
CBFv	Cerebral Blood Flow velocity
CBVC	Cerebrovascular conductance
CBVR	Cerebrovascular Resistance
CCA	Carotid Coronary Artery
CO	Cardiac Output
CO_2	Carbon Dioxide
CV	Coefficient of Variation
CVC	Cutaneous Vascular Conductance
CVD	Cardiovascular Disease
CVR_{CO2}	Cerebrovascular Reactivity to CO_2
CVR_{CO2MAP}	Cerebrovascular reactivity to CO_2 (normalised to MAP)
DBP	Diastolic Blood Pressure
dCA	Dynamic cerebral autoregulation
eNOS	Endothelial nitric oxide synthase
FMD	Flow mediated dilatation
H_2	Hydrogen
HF	High Frequency
HIIT	High Intensity Interval Training
HR	Heart Rate
HR_{max}	Maximum Heart Rate
ICAs	Internal Carotid Arteries
K^+	Potassium
LDF	Laser Doppler Flowmetry
LF	Low Frequency
MAP	Mean arterial pressure
MCA	Middle Cerebral Artery
MCAs	Middle Cerebral Arteries
MCAv	Middle Cerebral Artery velocity
MRI	Magnetic Resonance Imaging
nGain	Normalised Gain
NO	Nitric Oxide
O_2	Oxygen
OCT	Optical Coherence Tonography
P_aCO_2	Arterial partial pressure of carbon dioxide
P_aO_2	Arterial partial pressure of oxygen
PCA	Posterior Cerebral Artery
PCAs	Posterior Cerebral Arteries
PCAv	Posterior Cerebral Artery velocity
PCoAs	Posterior Communicating Arteries
$P_{ET}CO_2$	Partial pressure of carbon dioxide
pH	Power of Hydrogen

RPE	Rating of Perceived Exertion
SBP	Systolic Blood Pressure
SkBF	Skin Blood Flow
SNS	Sympathetic nervous system
SR_{AUC}	Shear Rate Area Under the Curve
SV	Stroke Volume
T2D	Type 2 diabetes
$T_{arm,}$	Arm Temperature
T_c	Core temperature
T_{calf}	Calf Temperature
TCD	Transcranial Doppler
T_{chest}	Chest Temperature
TFA	Transfer Function Analysis
TPR	Total Peripheral Resistance
T_{sk}	Skin Temperature
T_{thigh}	Thigh temperature
TTP	Time to Peak
v	Velocity
VAs	Vertebral Arteries
VLF	Very Low Frequency
VO_{2max}	Maximal oxygen uptake
VO_{2peak}	Cardiorespiratory fitness
VOP	Venous Occlusion Plethysmography

Definitions of key (in)dependent parameters

Parameter	Definition
Cerebral Blood Flow	Adequate cerebral blood flow is imperative for cerebrovascular health. Chronic reductions in cerebral blood flow (CBF) and cerebrovascular function are strongly associated with clinical conditions, including stroke (Markus et al., 2004), cognitive impairment (Benedictus et al., 2017), dementia and Alzheimer's disease (Kisler et al., 2017).
Cerebrovascular Function	The regulation of CBF is multi-factorial and largely influenced by; arterial blood gases, blood pressure, cerebral metabolism and the autonomic nervous system (Ainslie and Bailey, 2013; Willie et al., 2014).
Cerebrovascular reactivity to CO_2	A measure of cerebrovascular function assessing the response of cerebral blood flow to local changes in carbon dioxide concentrations.
Cerebral Autoregulation	A measureof cerebrovascular function assessing the ability of the brain to control cerebral blood flow locally/independently of changes in blood pressure.
Cerebrovascular Health	The state of being free from injury or ill health within the cerebrovasculature. Able to maintain adequate cerebral blood flow for normal functioning.

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Chapter 1

General Introduction

Cardiovascular disease (CVD), including cerebrovascular disease, is the leading cause of morbidity (Yang et al., 2017) and all-cause mortality (Townsend et al., 2016) globally. Chronic reductions in cerebral blood flow (CBF) and cerebrovascular function are strongly associated with clinical conditions, including stroke (Markus et al., 2004), cognitive impairment (Benedictus et al., 2017), dementia and Alzheimer's disease (Kisler et al., 2017). The human brain receives approximately 15% of total blood flow and ~20% of resting oxygen consumption (Kety and Schmidt, 1948; Bain et al., 2013), therefore, the precise regulation of CBF is critical to maintain optimal cognitive function and reduce cerebrovascular disease risk (Willie et al., 2014). CBF is controlled by various mechanisms, including, perfusion pressure (e.g., blood pressure) and cerebral autoregulation (the ability of the brain to control its flow locally/independently of changes in blood pressure) and local changes in carbon dioxide concentrations (CO_2 reactivity) (Willie et al., 2012). The current research literature suggests global CBF declines with age (Stoquart-ElSankari et al., 2007) with some evidence of a decline in cerebrovascular function (Bailey et al., 2013b; Barnes et al., 2013), thus, older individuals are at greater risk of cerebrovascular disease (Tang et al., 2014) but more insight into how cerebrovascular function changes over the lifespan is needed. Ageing is also associated with an increase in arterial blood pressure (Bots, Grobbee and Hofman, 1991) and an impaired response to regulate body temperature (Holowatz and Kenney, 2010). Age related reductions in CBF, increases in blood pressure (BP) and an impaired thermoregulatory response to the cold may be important given that there is some evidence of a higher occurrence of cerebrovascular morbidity and mortality in periods of low ambient temperatures (Bunker et al., 2016; Ryti, Guo and Jaakkola, 2016; Song et al., 2017; Ikaheimo, 2018), including an increased number of stroke hospitalisations (Lichtman et al., 2016). Nevertheless, research studies examining cerebrovascular responses during cold exposure are limited with no studies investigating if cerebrovascular responses to cold differ with ageing.

Intriguingly, CBF increases and vascular resistance decreases in response to acute cold exposure (Wilson and Metzler-Wilson, 2018), this is in contrast to other vascular beds including the cutaneous vessels that actively vasoconstrict in response to cold stress (Wilson et al., 2007a). A cold- induced increase in CBF may be useful in situations where hypoperfusion occurs (e.g., orthostatic syncope) and also in conditions where CBF may be reduced (e.g., ageing). Research evidence suggests that lifelong exercise and/or a period of exercise training can maintain or increase CBF velocity (v) (Ainslie et al., 2008; Murrell et al., 2013). During an acute bout of sub-maximal exercise in thermoneutral conditions, CBFv increases linearly with exercise intensity from rest up to ~60-70% of maximal oxygen uptake followed by a plateau/decrease with higher exercise intensities (Moraine et al., 1993; Smith et al., 2014). Face cooling also amplifies the increase in CBF during exercise (Kjeld, Pott and Secher, 2009). Adding exercise and a cooling stimulus together could potentiate CBF responses in a positive way to enhance the acute exercise response as well as the chronic training adaptations but, to date, this has never been examined.

A small number of studies have examined the impact of exercise training on CBF and cerebrovascular function in young, old and those with cerebrovascular disease (Ivey et al., 2011; Murrell et al., 2013; Drapeau et al., 2019b; Klein et al., 2019a; Lewis et al., 2019a). Although some studies have conflicting findings on the impact of exercise training on cerebrovascular health, there is evidence of beneficial effects of exercise training on cerebrovascular function (Ivey et al., 2011; Murrell et al., 2013). Being able to identify exercise that induces the greatest increases in MCAv could optimise the effects of exercise training (Lucas et al., 2015). Therefore, incorporating an exercise-training programme, alongside a stimulus of cold exposure may improve cerebrovascular function to a greater extent than exercise in a thermoneutral environment. The overarching aim of the current thesis is to examine the impact of cold stress on cerebrovascular health.

1.1 Aims

The specific aims of this thesis are:

1. To examine the effects of cold stress on cerebral and peripheral vascular function in young and older individuals (chapter 3).
2. To examine cerebral, conduit and skin micro-vascular responses during an acute cold stress in younger and older individuals (chapter 3).
3. To assess the impact of 8 weeks of exercise training in a cold environment on middle cerebral artery velocity (MCAv), cerebrovascular, peripheral vascular and microvascular function compared to exercise training in a thermoneutral environment in young individuals (chapter 4).
4. To examine the acute effects of a single exercise bout in thermoneutral vs cold environmental conditions on cerebrovascular haemodynamics (chapter 4).

1.2 Objectives

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

In line with **Aim 1:**

1. Utilising transcranial Doppler ultrasound, to assess MCAv, cerebral autoregulation and cerebrovascular CO_2 reactivity following an acute bout of cold stress in healthy young and older individuals.
2. Using vascular ultrasound to measure brachial artery vascular function prior to and following an acute bout of cold stress in healthy young and older individuals.

In line with **Aim 2**:

1. Utilise a tube-lined water perfused suit to simulate thermoneutral conditions (water perfused at 34^{o}C) and whole body cold exposure (water perfused at 12^{o}C).

2. Assess the responses of core and skin temperature, skin blood flow, blood pressure and cerebrovascular haemodynamics during 30 minutes of cooling in young and older individuals.

In line with **Aim 3**:

1. Engage young healthy individuals in an 8-week supervised moderate intensity aerobic exercise programme for 50 minutes x 3 times per week in a temperature controlled environmental chamber simulating either thermoneutral (15^{o}C) or cold (5^{o}C).
2. Utilising transcranial Doppler ultrasound, assess MCAv, cerebral autoregulation and cerebrovascular CO_2 reactivity prior to and following an exercise intervention in healthy young individuals.
3. Using vascular ultrasound, measure brachial artery vascular function prior to and following an exercise intervention in healthy young individuals.
4. Using laser Doppler flowmetry, assess microvascular reactivity to gradual local heating prior to and following an exercise intervention in healthy young individuals.

In line with **Aim 4**:

1. At the midpoint of the 8 week exercise intervention (mentioned above), assess the responses of MCAv, blood pressure, skin and core temperature and skin blood flow throughout an acute bout of exercise in either a thermoneutral (15^{o}C) or cold environment (5^{o}C).

Chapter 2

Literature Review

The purpose of the following literature review is twofold; firstly to outline the cerebrovascular system and how to assess cerebrovascular function to monitor potential changes with interventions such as exercise training. Secondly, to introduce “cold stress”, highlighting the response of the cerebral vasculature to an acute cold stress and describing the potential impact of using this stressor as an intervention to alter cerebrovascular function.

Cerebrovascular diseases including cognitive impairment, dementia and cerebrovascular events including stroke are complex debilitating conditions that are vascular in nature and usually occur as a consequence of age and/or cardiovascular disease risk factors. Cerebrovascular diseases and events cost millions to treat and are among the leading causes of death globally. Understanding how the brain ages and changes in responses to risk factors, disease and cerebrovascular events is important to enable treatment and interventions aimed at enhancing and/or preventing decline in cerebrovascular health. In order to understand cerebrovascular health, knowledge of the cerebral vasculature together with measurement techniques to assess the responsiveness of the cerebral vessels is essential.

2.1 The Cerebral vasculature and Cerebrovascular Function

2.1.1 Anatomy of the Cerebral Vasculature

The brain only accounts for approximately 2-3% of body weight, but CBF accounts for approximately ~15% of total blood flow and ~20% of resting oxygen consumption (Kety and Schmidt, 1948; Bain et al., 2013). Despite this high metabolic demand, the brain has a limited substrate storage capacity, emphasising the importance of a precise CBF regulation for maintenance in the supply of nutrients and oxygen (Payne, 2016). A sufficient supply of CBF is imperative to maintain function. A reduction in CBF (hypoperfusion) can lead to unconsciousness (van Leishout et al., 2003) and if sustained, can lead to brain damage from ischemic injury and death (Willie et al., 2013). Likewise, excessive CBF

(hyperperfusion) can lead to headaches, seizures, and possible ischemic and haemorrhagic stroke (Fantini et al., 2016).

Blood is supplied to the brain by four extra-cranial arteries; bilateral internal carotid arteries (ICAs) and vertebral arteries (VAs). Approximately 73-82% of CBF is delivered through the right and left ICAs and branch within the Circle of Willis to supply the anterior cerebral arteries (ACAs) and the middle cerebral arteries (MCAs). These vessels supply blood to the forebrain (frontal and parietal lobes) and midbrain (temporal lobes), which comprise the anterior circulation. The remaining global CBF is supplied via the VA's that join together to form the Basillar artery (BA), connecting to the posterior portion of the Circle of Willis and bifurcating to supply the posterior cerebral arteries (PCAs). These vessels supply the hindbrain (e.g. occipital, cerebellum and medulla oblongata) (Bradac, 2011). The vascular structure mentioned herein, the Circle of Willis, is where the cerebral circulation is joined. The anterior communicating arteries join the two ACA's, allowing blood supply to both hemispheres and the posterior communicating arteries connect the anterior and posterior circulations at the base of the brain.

2.1.2 Techniques to measure Cerebral Blood Flow

Cerebral blood flow was first quantified in humans in 1945 using an inert gas method (Kety and Schmidt, 1945). This was based on the Fick principle and the concept that the rate at which the content of an inert gas in the cerebral venous blood approaches that in the arterial blood is dependent on the volume of blood in the cerebrovasculature. Following on from this, other methodologies relied on the same principles of tracking blood through the use of a tracer. Gibbs and colleagues performed CBF measurements using the indicator dilution method that measures the venous dilution of an intra-arterially injected indicator (Gibbs, Maxwell and Gibbs, 1946; Maxwell and Gibbs, 1947). From the aforementioned and subsequent research, it was established that global CBF in a "normal" man was estimated to be 54 ± 12 ml/100g/min (Kety and Schmidt, 1948). Lassen advanced the nitrous oxide

method in the assessment of CBF through inhalation of an inert gas containing the radioactive isotope ^{85}krypton (Lassen and Munck, 1955). This utilised a similar methodology as described by Kety & Schmidt (Kety and Schmidt, 1945; Kety and Schmidt, 1948) and determined global CBF through the Fick method (Lassen, 1959), supporting previous findings that global CBF varied from 33-67 ml/100g/min. Then in the 1960's Lassen and colleagues established a method for measuring regional CBF measurements by modifying the methods of Gibbs, Maxwell and Gibbs (Gibbs, Maxwell and Gibbs, 1946; Maxwell and Gibbs, 1947). In this study, they injected radioactive isotopes (^{85}Krypton and ^{133}Xenon) dissolved into saline into the internal carotid and vertebral arteries (Lassen and Hoedt-Rasmussen, 1966). The accumulation and clearance of these isotopes were monitored for 15 minutes by collimated scintillation detectors. This method established the first regional measurement of CBF in the middle frontal gyrus (Ingvar and Lassen, 1961) in man and in subsequent studies further regional regions were identified (Ingvar et al., 1995). The difficulty that this measure imposed included the time taken to assess the measurements (taken over 10-20 minutes per measure), inhibiting the evaluation of any dynamic changes in CBF. Furthermore, the internal jugular and peripheral arterial lines are quite invasive (Willie et al., 2011). Aaslid et al (1982) then published a landmark study demonstrating the use of transcranial Doppler ultrasound (TCD) for a real time measurement of cerebral artery velocity in large cerebral arteries that has shaped contemporary understanding over cerebrovascular physiology.

2.1.3 Measurement of Cerebral Blood Flow velocity using TCD

The ability to measure CBF and the cerebral vasculature has enhanced researchers understanding of the functional status of blood vessels supplying the brain and the regulatory control of CBF. TCD allows the measurement of cerebral blood flow velocities (CBFv) in large cerebral arteries (e.g, MCA) through a non-invasive, high temporal resolution Doppler ultrasound probe. TCD has been imperative in furthering the

understanding and knowledge of the CBF response to many physiological stressors (e.g. autoregulation, reactivity, neurovascular coupling and exercise) (Willie et al., 2011). The principle of TCD is that a 2 MHz Doppler probe is placed over the acoustic windows at the thin bones of the temporal region of the skull. The Doppler probe emits ultrasound waves that are reflected off the moving red blood cells and are reflected back to the transducer, to observe the Doppler shift of the red blood cells as they pass through the large intracranial vessel of interest (Willie et al., 2011). The Doppler shift is used to determine the velocity of the red blood cells and can be explained as the difference between the transmitted signal and the received signal (Aaslid, 1986), with higher velocities determined from faster red blood cell movement. TCD is most commonly used to assess CBFv in the MCA, ACA and PCA but also the basilar artery and vertebral artery. Having knowledge of the insonation depths and flow directions of the vessel of interest is crucial in order to maintain reliable, valid and repeatable signals (Table 2.1). Vessel identification can also be confirmed by applying further stimuli. Carotid artery compression results in a reduction in MCAv, whereas PCAv will display negligible or no change. Additionally, with activation of the occipital lobe (eyes open to eyes closed), PCAv will increase by approximately 15-20% whereas MCAv will have a small response (<5%). TCD is employed as a measurement technique within this thesis, MCAv is used as a marker of CBF as it accounts for approximately 80% of global CBF and is easiest to measure through its proximity to the temporal window (Skow et al., 2013).

Table 2.1. Typical criteria for identification of cerebral arteries using Transcranial Doppler ultrasound (Willie et al., 2011)

Artery	Insonation Window	Probe Direction	Depth (mm)	Flow Direction	Mean Flow velocity ($cm.s^{-1}$)
ACA	Posterior	Anterior	60-70	Away	50 ± 11
MCA	Anterior	Perpendicular	25-50	Toward	50 ± 12
PCA	Anterior	Posterior	60-70	Toward	40 ± 10

Abbreviations; Anterior cerebral artery, ACA; Middle cerebral artery, MCA; Posterior cerebral artery, PCA.

TCD relies on the concept that changes in MCAv accurately reflect the changes in CBF, however this is largely dependent of the assumption that the MCA diameter is unchanged at the point of insonation across time and experimental conditions (Hoiland and Ainslie, 2016). Magnetic resonance imaging (MRI) is a technique that has been used to address the validity of TCD in the assessment of CBFv. Early studies using MRI found there to be no changes to MCA diameter during hypercapnia (Serrador et al., 2000a) and hypocapnia (Valdueza et al., 1997; Serrador et al., 2000a). However, more recent studies have found that greater changes from eucapnia (e.g +10-15 mm Hg) can display an increase in MCA diameter to a hypercapnic stimulus and decrease the diameter during a hypocapnic stimulus (Coverdale et al., 2014; Verbree et al., 2014). Furthermore, evidence suggests that the MCA cross-sectional area observed a 2% decrease using MRI following rhythmic handgrip exercise, leading to an overestimation when using TCD to assess CBF changes during exercise (Verbree et al., 2014). On the basis of the previous studies, it is assumed that there is a sigmoidal relationship of MCA diameter to changes in carbon dioxide (CO_2) (Ainslie and Hoiland, 2014) and that vasoconstriction occurs in the MCA during exercise induced sympathetic activation (Verbree et al., 2014), therefore data collected using CBFv as a surrogate for CBF must be interpreted with caution (Coverdale et al., 2014). Despite MRI being an advanced measurement technique, there are many difficulties in its utilisation for widespread use including expertise, cost, difficulties in repeated method comparisons and complications with performing non-stationary research such as exercise (Ainslie and Hoiland, 2014). In the absence of MRI and in combination with TCD, research studies have utilised vascular ultrasound to assess diameter change in the large extracranial vessels of the neck during CO_2 changes (Willie et al., 2012; Carter et al., 2016; Hoiland et al., 2017) in order to try to understand the possible changes to the intracranial cerebral vessels. This is discussed further in *Section 2.1.4 Arterial Blood Gases*.

The regulation of CBF is multi-factorial and largely influenced by; arterial blood gases, blood pressure, cerebral metabolism and the autonomic nervous system (Ainslie and Bailey, 2013;

Willie et al., 2014). Regulation and changes in arterial blood gases and blood pressure are explored within this current thesis. The reader is referred to Phillips et al. (2016) for a detailed description of assessment and changes in cerebral metabolism and the autonomic nervous system.

2.1.4 Arterial blood gases

Carbon Dioxide

The brain is extremely susceptible to changes in partial pressure of arterial carbon dioxide (P_aCO_2) and hypoxia (Kety and Schmidt, 1948; Ainslie and Duffin, 2009), with changes in these factors controlling the delivery of CBF. Investigators have assessed the reactivity of the cerebral vasculature to hypercapnia (increases in P_aCO_2) and hypocapnia (decreases in P_aCO_2), termed cerebrovascular reactivity (CVR_{CO2}) that is most often portrayed as the change in CBFv, per change in vasoactive stimulus (Fierstra et al., 2013). Hypercapnia promotes vasodilation of the cerebral arterioles and a subsequent increase in CBF, whereas hypocapnia induces a decrease in CBF and is a key homeostatic function to maintain central power of hydrogen (pH) (Ainslie and Duffin, 2009). It was initially suggested that changes of cerebrovascular resistance (change in blood flow) only occurred in the pial vessels, supported by no change in the MCA diameter during hypercapnia (Serrador et al., 2000a) and hypocapnia (Valdueza et al., 1997; Serrador et al., 2000a) and within minor changes to P_aCO_2 (±8 mm Hg from eucapnia) using MRI (Ainslie and Hoiland, 2014; Coverdale et al., 2014; Verbree et al., 2014). Although changes greater than 8 mm Hg from eucapnia resulted in diameter increases during hypercapnia (Coverdale et al., 2014; Verbree et al., 2014) and diameter reductions during hypocapnia (Coverdale et al., 2014). This suggests that there appears to be a physiological range in which end tidal volume of CO_2 ($P_{ET}CO_2$) has a negligible effect on the discrepancy between cerebral blood flow velocity and cerebral blood flow as indexed by transcranial Doppler (Ainslie and Hoiland, 2014).

Supporting the notion that cerebral vessel diameter changes are greater with larger hypocapnic and hypercapnic alterations to P_aCO_2, there is also evidence of this effect on CBF. The typical increase in CBF is approximately 3-6% and the decrease is 1-3% in flow per mm Hg change in CO_2 from rest (Willie et al., 2014). The cerebrovasculature is deemed more sensitive to increases in P_aCO_2, such as in circumstances like exercise, postural change and syncope (Ainslie and Duffin, 2009). CVR_{CO2} has been used extensively to assess cerebrovascular regulation in young healthy individuals and patients with various forms of cerebrovascular disease (Ainslie and Duffin, 2009), with a lower reactivity to a CO_2 stimulus being associated with higher all-cause mortality (Vernieri et al., 2004; Silvestrini et al., 2006; Portegies et al., 2014) and increased risk of CVD (Carter et al., 2016). In addition, lower responses to a CO_2 stimulus have been observed in patients with Alzheimers (den Abeelen et al., 2014) and heart failure (Georgiadis et al., 2000).

Although not fully understood, the mechanism of CVR_{CO2} involves an increase or decrease in CO_2 levels responsible for a change in plasma pH. This change induces activation of potassium (K^+) channels in the vascular smooth muscle, in turn dilating (relaxing) the cerebral vessels (Jackson, 2005). Alternatively or in combination, changes in CBF may be induced via shear stress-mediated releases of vasodilatory agents such as nitric oxide (NO) and prostaglandins that coincide with increased flow associated with hypercapnia (Ainslie and Duffin, 2009), emphasising that individual responses are likely associated with the integrity of the vascular endothelium (Ainslie et al., 2007a). In support of this, previous work has also identified that the cerebral vessels dilatory responses are attenuated when a NO synthase inhibitor is infused (Smith et al., 1997) and shear stress is reduced if not absent during hypocapnia (Willie et al., 2012). Research has also identified that the extracranial arteries in the neck also respond to alterations in P_aCO_2 (Willie et al., 2012). Similarly to the cerebral vessels, using vascular ultrasound, the large arteries do not visibly change in diameter to modest changes in P_aCO_2 from eucapnia (Willie et al., 2014). However, alterations in P_aCO_2 of ±25 mm Hg lead to an increase in diameter in response to

hypercapnia (11.5%) and a decrease in diameter in response to hypocapnia (6.5%) (Willie et al., 2014). More recently, studies have identified the role of shear stress mediated vasodilation in the internal and common carotid artery as a result of increased CO_2 (hypercapnia) by measuring these arteries using vascular ultrasound simultaneously with the measurement of CBFv via TCD, suggesting the use of such measurements to quantify cerebral endothelial function and a potential avenue to assess cerebrovascular health and risk (Carter et al., 2016; Hoiland et al., 2017)

To assess the cerebrovascular response to changes in CO_2 concentration, previous studies have developed three key methodologies including breath holding, to prevent CO_2 removal and induce a progressive increase in P_aCO_2 (Fierstra et al., 2013), rebreathing of exhaled gas to measure a sigmoidal increase of P_aCO_2 (Claassen et al., 2007). Thirdly, a method used to increase P_aCO_2 is by administering a fixed inspired fractional concentration of CO_2, most typically 2-7% CO_2 via a non-rebreathing face mask to induce a standard hypercapnic stimulus (Vernieri et al., 2004). Using this latter method, $P_{ET}CO_2$ is deemed a suitable surrogate for P_aCO_2 and can be used as the stimulus for CBF (Mark et al., 2010; Fierstra et al., 2013). Of the three methodologies mentioned previously, inhalation of a fixed concentration of CO_2 is the most favourable as it has the least confounding factors and can produce a standardised hypercapnic stimulus (Vernieri et al., 2004).

Oxygen

The cerebrovasculature is not only sensitive to changes in CO_2, but oxygen (O_2) also plays a role in vascular tone. Hyperoxia (increase of arterial pressure of O_2; P_aO_2) has little effect on CBF, whereas hyperoxemia (hypoxia) effects the cerebrovasculature, but only when PaO_2 levels drop below ~50 mm Hg causing a substantial increase in CBF to maintain oxygen delivery (Willie et al., 2012; Willie et al., 2014). The response to hypoxia however is still affected by P_aCO_2 given that studies have shown it is the ventilatory response to the hypoxia which ultimiately determines the CBF response, as hypercapnia increases and

hypocapnia decreases the sensitivty of the cerebrovasculature to hypoxia (Mardimae et al., 2012; Willie et al., 2014).

2.1.5 Blood Pressure (Cerebral Autoregulation)

Cerebral autoregulation (CA) ensures sufficient blood flow is maintained to active regions of the brain despite changes in arterial blood pressure (Lucas et al., 2010). Due to the brains dependency on a continuous supply of oxygenated blood, an intact cerebral autoregulation is imperative to protect against hypo- and hyperfusion (Claassen et al., 2016). Bayliss and Hill (1895) originally proposed that increases to mean arterial pressure would result in an increase in CBF and a reduction in arterial pressure would reduce CBF. This notion prevailed until Lassen first proposed the concept of “autoregulation” in 1959 and since then the research literature has been in agreement that steady state CBF is known to remain stable across a wide range of blood pressures (e.g. 60-150 mm Hg) as a result of adjustments to cerebrovascular resistance (Willie et al., 2014). More recently, data indicates that the CBF-mean arterial pressure (MAP) relationship is not flat through a wide range of MAP and that the plateau phase is understood to exist within a much smaller range (± 10 mm Hg change) (Tan, 2012; Willie et al., 2014) (Figure 2.1), implying that cerebral blood vessels have an intrinsic ability to maintain CBF over a range of BP levels, by myogenic, neural or metabolic mechanisms (van Beek et al., 2008). This implies that the cerebrovasculature does have autoregulatory capacity but depends on the severity and duration of perfusion pressure. Cerebral perfusion pressure is the primary driver for CA; calculated as MAP-intracranial pressure. Following a variation in perfusion pressure, an adaptation in cerebrovascular resistance will cause CBF to return to ‘baseline’ (Panerai, 1998). In order to ensure that the brain is supplied with sufficient blood flow, vasodilation occurs in response to decreases in BP and vasoconstriction in response to increases in BP within the upper and lower limits outside of the autoregulatory “plateau” phase (Pires et al., 2013). It has been highlighted that the cerebrovasculature is better able to buffer transient increases in perfusion pressure, indicating that the brain has a greater protective response

to over perfusion, however this difference is lost when studies corrected for changes in P_aCO_2 (Numan et al., 2014).

CA can be categorised into both static cerebral autoregulation that refers to CBF regulation to steady state changes in blood pressure (e.g. over a period of minutes) and dynamic cerebral autoregulation (dCA) that refers to rapid changes in blood pressure (seconds; e.g. postural change) (Zhang, 2002; Tzeng et al., 2010).

Figure 2.1 removed due to third party copyright.

Figure 2.1. Representation of the classical (left) and modern (right) interpretation of the relationship between cerebral blood flow and mean arterial pressure; cerebral autoregulation. Red dotted lines represent the original "autoregulatory plateau" (left) put forward by Lassen et al (1959) and the modern (right) interpretation put forward by Tan et al (2012). Blue dotted lines represent cerebrovascular resistance; black dotted line represents stable cerebral blood flow; black solid lines represents cerebral blood flow changes. Adapted from (Willie et al, 2014).

Early literature focused on the assessment of steady-state measurements (static cerebral autoregulation, >5minutes) of cerebral blood flow and blood pressure (Bayliss and Hill, 1895; Lassen, 1959; Numan et al., 2014). The monitoring and assessment of dCA has arisen from the ability to non-invasively monitor both beat-to-beat blood pressure through finger photoplethysmography and beat-to-beat velocity of typically the MCA via TCD (Willie et al., 2014). The assessment of dCA has found that autoregulation is impaired in a number of individuals with clinical conditions such as type-2 diabetes (T2D) (Vianna et al., 2015), Alzheimers disease (Meel-van den Abeelen et al., 2014) and acute ischemic stroke (Eames et al., 2002). This impairment may deem a person susceptible to large changes in BP and a failure in autoregulatory response.

The dynamic relationship between CBF and BP is assumed that information from a time-domain can be converted into the frequency-domain and has the characteristics of a high

pass filter. For example, if someone has a respiratory rate of 15 breaths per minute, each breath takes ~4 seconds; therefore ¼ of a breath happens every second which equates to 0.25 Hertz (Hz = cycles per second). With a high pass filter, higher frequency oscillations pass through unimpeded, whereas slower oscillations are filtered. It is believed that the cerebral arterioles adapt in response to changes in perfusion pressure but is not fast enough to counteract higher frequency oscillations (>0.20Hz; e.g. resting heart rate ~1 second), which pass unimpeded into oscillations in CBF. Slower frequency oscillations (<0.20Hz; less than the respiratory rate) can be dampened by the cerebral arterioles (Diehl et al., 1998). The frequency bands of interest for the relationship between CBF and BP are thought to be the very low frequency (VLF: 0.02-0.07 Hz), that is thought to be under the influence of myogenic properties as shown by studies using cholinergic blockades (Hamner et al., 2012; Tan, Hamner and Taylor, 2013b) and ganglion blockade (Zhang et al., 2002). The low frequency range (LF; 0.07-0.20 Hz) that is controlled by more sympathetic influences, as demonstrated by sympathetic agonist drugs midazolam (Ogawa et al., 2010) and antagonist drugs prazosin (Purkayastha et al., 2013). Also, the highest frequency range (HF: >0.20 Hz), the range that CBF is most poorly regulated, is largely under the control of normal respiration rate (Reinhard et al., 2003).

After the assessments of proposed driven methodologies to assess CA, repeated squat-stand manoeuvres elicit the greatest oscillations in blood pressure within the high frequency (HF) range (<0.20Hz) (Claassen et al., 2016) and represent a physiological challenge experienced in everyday life that induces a depressor change in BP and CBFv. If the squat-to-stand challenge is periodically repeated at a specific frequency, oscillations in BP will be produced, with CBFv following these induced oscillations (van Beek et al., 2008). The pressure-flow response of CBF to changes in BP can be described using transfer function analysis (TFA) (Panerai, 1998). Transfer function analysis quantifies CA in three parametrers: gain (the amplitude change in the signals), phase shift (the displacement of the waveform relative to the other) and most importantly, coherence (the linearity of the

input (BP) and the output (BP)). If coherence is close to 1.0, this indicates that the system is linear and that changes to gain and phase are interpretable. However if coherence is <0.5, this could be as a result of a range of factors and must be interpreted with caution (Tzeng et al., 2012; Tan, Hamner and Taylor, 2013a). Cerebral Autoregulation Research Network (CARNet) group (Claassen et al., 2016) outlined specific guidance in the use of transfer function analysis (TFA) that standardises the analysis technique to allow comparisons within the literature.

2.1.5.1 Autonomic Control

Despite the mechanisms being poorly understood and somewhat controversial, neurogenic control is thought to independently impact autoregulation of the cerebral blood vessels. Cerebral blood vessels are innervated by both adrenergic and cholinergic fibres of extrinsic (present outside of the parenchyma) and intrinsic origin (present within the parenchyma) and based on this anatomy would seem logical that there is a neural role in cerebral blood flow regulation (Peterson, Wang and Britz, 2011; Tan, Hamner and Taylor, 2013a; Willie et al., 2014). Willie and Colleagues (2014) summarised a series of studies identifying that unilateral and bilateral cervical ganglion excision on diseased individuals in humans resulted in an increase in CBF, suggesting that there is evidence that the sympathetic nervous system is involved in cerebral vessel tone and CBF regulation. It has also been speculated through animal studies that the sympathetic nervous system (SNS) is particularly important in buffering surges in perfusion pressure and largely involves the larger arteries (Peterson, Wang and Britz, 2011; Willie et al., 2014). Furthermore, studies employing sympathetic nervous system blockade indicated an impaired cerebral autoregulation depicted as an increased gain and decreased phase lead at low frequencies (Zhang, 2002; Hamner et al., 2010). Although possible confounded systemic effects from the SNS blockade should have been taken into consideration (Willie et al., 2014). Taken

together, the literature supports the notion that cerebrovascular regulation is imperative but occurs through a highly complex integration of different mechanisms.

2.1.6 The impact of age and fitness on cerebral blood flow and cerebrovascular function

2.1.6.1 The effect of ageing on cerebral blood flow and cerebrovascular function

Cross sectional studies have established that CBF gradually declines with ageing by approximately 25-30% (Stoquart-ElSankari et al., 2007; Liu et al., 2012; Murrell et al., 2013) or as displayed in CBFv as 0.76 ± 0.04 $cm.s^{-1}.year^{-1}$ between 20 and 80 years (Ainslie et al., 2008). Furthermore, cross sectional data in young and old recreationally active and sedentary individuals identified that there is also a longitudinal decline in an aged individuals cerebrovascular reactivity to CO_2 (Bailey et al., 2013b; Barnes et al., 2013), although dCA appears to be maintained with age (Carey et al., 2000). Importantly, reductions in CBFv and cerebrovascular function are risk factors and/or a consequence of cerebrovascular disease including stroke (Markus et al., 2004), cognitive impairment (Benedictus et al., 2017), dementia and Alzheimer's disease (Kisler et al., 2017).

2.1.6.2 The effect of cardiorespiratory fitness on cerebral blood flow and cerebrovascular function

The age-related decline in CBFv can be attenuated by high cardiorespiratory fitness with evidence of higher CBFv in lifelong exercisers compared to their sedentary matched controls (Ainslie et al., 2008). Cerebrovascular reactivity has also been positively associated with aerobic fitness (Bailey et al., 2013b; Barnes et al., 2013), whereas the impact of cardiorespiratory fitness on dCA, remains equivocal with some studies displaying no difference due to fitness (Aengevaeren et al., 2013; Ichikawa et al., 2013) and some have identified attenuated dCA with elevated cardiorespiratory fitness (Lind-Holst et al.,

2011; Labrecque et al., 2017; Labrecque et al., 2019). Taken together, improving fitness with exercise training could be a useful non-pharmacological intervention for maintaining cerebrovascular health and reducing the risk of associated diseases.

2.1.7 Exercise training interventions on cerebrovascular function

A small number of research studies have employed structured exercise interventions in an attempt to improve CBFv and cerebrovascular function or attenuate the age-related decline in CBFv. Nevertheless, CBFv did not change following 8 -12 weeks of moderate intensity aerobic exercise in healthy individuals (Murrell et al., 2013; Lewis et al., 2019a) nor 6 weeks of high intensity interval training (HIIT) in endurance trained individuals (Drapeau et al., 2019b). However, 8 weeks of moderate intensity exercise elicited an increase in CBFv (2.3 $cm.s^{-1}$) in young healthy females (Bailey et al., 2016b) and menopausal women (Akazawa et al., 2012). In terms of cerebrovascular function, hypercapnic cerebrovascular reactivity was improved from 1.4 ± 0.6 $cm.s^{-1}/mmHg^{-1}$ to 1.9 ± 0.7 $cm.s^{-1}/mmHg^{-1}$ following both 12 weeks of moderate intensity cycling in young and old individuals (Murrell et al., 2013) and also cerebral vasomotor reactivity increased by 27-28% following 6 months of aerobic treadmill exercise in stroke survivors (Ivey et al., 2011). Cerebral autoregulation, has been shown to be attenuated in trained individuals after 6 weeks of HIIT, evidenced by an increased TFA phase during repeated squat stands (Drapeau et al., 2019b), but has also been shown to be unchanged following 8 weeks of exercise training in healthy and individuals with chronic pulmonary obstructive disorder (Lewis et al., 2019b). Collectively, these data suggest that exercise training has some promise in positively impacting CBFv and some aspects of cerebrovascular function in healthy individuals and those with cerebrovascular disease but the training stimulus (including intensity of exercise and duration of the training) may impact on the outcome of the exercise intervention on CBFv and cerebrovascular function. Recent research has aimed to determine the most potent exercise stimulus such as high intensity exercise (Drapeau et al., 2019b) and interval

exercise (Klein et al., 2019a) and have identified that interval training may provide a higher accumulated change in MCAv during exercise and recovery than when compared to continuous exercise (Klein et al., 2019a). This suggests a need for further research to understand different types of exercise training, how the exercise stimulus may be increased and what the impacts of this training are on CBFv and cerebrovascular function.

Another important factor to consider when examining responses to exercise training is what happens to CBF during an acute bout of exercise. Research studies suggest that during a bout of exercise, blood pressure progressively increases up to maximal intensities by ~20-30% (Smith and Ainslie, 2017), equally CBFv increases linearly with exercise intensity only up to approximately 60-70% maximal oxygen uptake (VO_{2max}) (Smith and Ainslie, 2017) or below the anaerobic threshold (Moraine et al., 1993). Importantly, the initial rise in CBFv is elevated in active individuals compared with sedentary controls (Brugniaux et al., 2014) and is also greater in young trained individuals compared with older individuals (Marsden et al., 2012). Despite the large number of factors that influence CBFv, P_aCO_2 is the primary factor and mediator of CBFv in response to exercise (Rasmussen et al., 2006; Ogoh and Ainslie, 2009b). During exercise, P_aCO_2 increases linearly until the exercise intensity increases above this threshold (<70-80%VO_2max). This then induces hyperventilatory-induced hypocapnia that causes a reduction in P_aCO_2 and subsequently CBFv to plateau or decrease towards resting values (Smith et al., 2014). The response of CBF occurs despite continued elevations of arterial blood pressure, cardiac output and increasing oxygen consumption demands of the brain (Moraine et al., 1993). Furthermore, cerebral CO_2 reactivity is increased during exercise, particularly in hypercapnia in order to maintain CO_2 homeostasis in the brain (Ogoh et al., 2008).

Despite the known importance of P_aCO_2, blood pressure may also have an influence on the CBF response due to rapid changes in systemic blood pressure that occur during exercise preventing a substantial challenge to the cerebrovasculature (Marsden et al., 2012) and

emphasising the need for an intact cerebral autoregulation. CA is maintained during mild to moderate exercise in young and old adults (Ogoh et al., 2005b; Fisher et al., 2008) but is impaired following maximal exercise (Ogoh et al., 2005a). It is believed that CA is more proficient in counteracting brief hypertension than hypotension, possibly due to the dangers associated with increased blood pressure (Marsden et al., 2012; Brassard et al., 2017). Recent work has supported this notion with evidence of the brain limiting CBF to avoid elevated perfusion pressure, as seen in sprinting (Curtelin et al., 2018). Taken together, the data suggests the increases in CBFv observed during exercise likely contribute to the positive cerebrovascular adaptations (Smith and Ainslie, 2017). Furthermore, the form of exercise that increases CBFv to a greater extent may optimise the potential for cerebrovascular adaptations (Lucas et al., 2015). Research studies have been performed investigating HIIT exercise but due to most HIIT prescription requiring individuals to exercise above ~70% VO_{2max}, this currently seems contradictory (Lucas et al., 2015; Klein et al., 2019a). Nevertheless, intensity and work matched interval and continuous exercise displayed that the initial rise in CBFv in the MCA was not different in older men (Klein et al., 2019a) and therefore interval training could be used for populations that could not sustain continuous exercise. Thus increasing or modifying the exercise stimulus to mediate greater CBFv during exercise may cause a greater or practical training response and will be examined within the present thesis.

2.1.8 Systemic vascular function

Alongside understanding and assessing cerebrovascular function, understanding the potential mechanisms for change and the relationship between cerebral and other vessels is important. Previous research has demonstrated a link between vascular health (endothelial function) in the peripheral arteries and cerebrovascular function (Lavi et al., 2006; Ainslie et al., 2007a; Hoth et al., 2007) and more recently research has focused on examining the extra-cranial arteries such as the ICA using vascular ultrasound to assess cerebral endothelial function or dysfunction and its potential role in predicting

cerebrovascular disease risk (Carter et al., 2016; Hoiland et al., 2017). There is evidence to suggest that the hypercapnic-induced vasodilation that occurs as a result of transient exposure to a CO_2 stimulus produces ICA dilation following increases in intravascular shear stress similar to that of which is observed in a peripheral vascular function test called flow mediated dilation (FMD) (Hoiland et al., 2017). Furthermore, there is also evidence that aging can attenuate shear-mediated dilation of the ICA in response to high levels of hypercapnia (Iwamoto, Bock and Casey, 2018). As these studies highlighted shear stress as an important stimulus for vasodilation of the ICA, matched increases of shear were induced from exercise and hypercapnia to assess if this would stimulate similar vasodilation of the large extracranial arteries (Smith et al., 2019b). It was highlighted that irrespective of the physiological stimuli, matched levels of shear induce extra-cranial artery dilation (Smith et al., 2019b). This evidence suggests a role for the endothelium in regulating cardiovascular function. The entire of the circulatory system is lined by the endothelium; a single layer of cells located between the circulating blood and the vascular walls and is vital in responding and mediating regulatory mechanisms for vascular health (Green et al., 2004). Within the present thesis, conduit vessels (macro) and microvessels of the skin will be assessed alongside cerebral vessels to provide insight into systemic vascular health.

2.2 The Peripheral Vasculature

2.2.1 Conduit arteries

The circulatory system can be simply explained as a pump (the heart) and tubes (conduits) that circulate the blood. A conduit artery is an artery with elastin and collagen filaments in the tunica media giving it an elastic capability to stretch in response to each pulse (Shadwick, 1999). Conduit artery function depends on the integrity of the endothelium to respond to changes in its physical, chemical and humoral environment and modify the regulation of vascular tone by secreting relaxing or constricting factors in response to physiological or pathological stimuli (Sima, Stancu and Simionescu, 2009). The

endothelium is highly susceptible to detect numerous hemodynamic stimuli such as shear stress (the frictional drag force exerted by blood as it flows across the arterial wall), to modify the regulation of vascular tone, growth, adhesion and coagulation in an endocrine-paracrine manner (Thijssen et al., 2019a). Additionally, there is indirect evidence that the endothelium has a common relationship with the sympathetic neural control of conduit vessels, often displayed by cardiovascular diseases categorised by increased sympathetic outflow and reduced endothelial function (Bruno et al., 2012).

Major cardiovascular diseases are associated with pathophysiological alterations in endothelial cell structure and function (Rubanyi, 1993). The endothelium derived relaxation factor, nitric oxide (NO), maintains systemic basal blood flows at rest and in response to pharmacological stimulation (Vallance, Collier and Moncada, 1989). NO is a potent anti-atherogenic molecule, inhibiting platelet and leukocyte adhesion and elicits vascular smooth muscle relaxation (Green et al., 2011). The physiological stimulus to release endothelium-derived NO is known to be a rise in intimal shear stress (Pohl et al., 1986), which in turn induces vasodilation. Conduit artery endothelial function demonstrates a gradual decline with age and is a sentinel event in the atherosclerotic disease process (Celermajer et al., 1994; Thijssen et al., 2019a).

2.2.2 Measurement of conduit artery function

In the peripheral conduit vessels of the arms and legs, research studies use a non-invasive technique called FMD as a measurement of conduit artery function (Parker, Ridout and Proctor, 2006; Black et al., 2009; Thijssen et al., 2019a) and as a surrogate marker of cardiovascular disease (Green et al., 2011). Originally described by Celermajer et al (1992), this involves an assessment of endothelium-dependent dilation following a period of distal limb ischemia (Thijssen et al., 2011; Thijssen et al., 2019a). The FMD is a largely NO-dependent test of endothelial function, which has been established by the attenuation in dilation from using NO blockades (Kooijman et al., 2008; Green et al., 2014). A large number of studies have examined the validity of the technique as an assessment of

endothelium-dependent NO specific index of endothelial function and guidelines on how to perform the technique have be published and recently updated (Thijssen et al., 2019a). FMD is typically examined in the brachial artery (Thijssen et al., 2019a) and assessment of the brachial artery will be employed within this thesis. Impairment in FMD has been shown with advancing age (DeSouza et al., 2000; Taddei et al., 2000; Thijssen et al., 2006) and a reduction in FMD has been observed in populations with various clinical conditions such as atherosclerosis and cardiovascular disease (Celermajer et al., 1994; Versari et al., 2009; Charakida et al., 2010).

2.3 Cutaneous Microvasculature

The skin is a barrier between the external and internal environments and is critical to maintain thermoregulation and the homeostatic control of core temperature (T_c) (Rowell, 1974). It is well established that the central control of thermoregulation is located in the preoptic/ anterior hypothalamus of the brain (Boulant, 2000; Boulant, 2006) which contains temperature-sensitive and temperature-insensitive neurons that interact to control core body temperature (Charkoudian, 2003; Boulant, 2006). Afferent information from the skin surface, visceral and spinal thermoreceptors are important for the regulation of core body temperature in response to alterations in internal and external environments. Therefore, afferent skin temperature information will stimulate heat dissipation responses under increases in temperature and increase heat generation in response to body cooling (Charkoudian and Stachenfeld, 2014).

The regulation of the skin is highly innervated and involves the integration of both neural and local or intrinsic mechanisms. In normothermic conditions, skin blood flow (SkBF) utilises approximately 5% of cardiac output, however, the skin can receive up to almost 60% of cardiac output dependent on the physiological (e.g., exercise) or environmental stressors (Johnson, Minson and Kellogg Jr, 2011). In non-glaborous regions (hairy; e.g., limbs, head and trunk), SkBF is controlled by two distinct branches of the sympathetic nervous system;

a) the cutaneous adrenergic vasoconstrictor nerves, acting through α_1 and α_2 receptors, that elicit vasoconstriction (reduction in SkBF), and b) active vasodilatory nerves, acting through sympathetic cholinergic nerves which elicit vasodilation (increased SkBF) (Kellogg, 2006).

Upon exposure to (whole-body) cold stress the adrenergic vasoconstrictor nerves are engaged and elicit vasoconstriction. During whole-body heat stress (passive exposure and or exercise) the skin blood flow response is multi-faceted. Initially, with minimal changes in core temperature (T_c), extant vasoconstrictor tone is reduced and skin blood flow may increase slightly. With continued heating and increases in T_c, robust and continued elevations in SkBF occur via the active vasodilatory nerves after T_c has surpassed a vasodilatory threshold essential for heat (Charkoudian, 2010; Johnson and Kellogg, 2010). Upon the commencement of exercise, SkBF reduces (Kellogg et al, 1991) which is thought to be as a result of increased systemic sympathetic vasoconstrictive outflow to redirect blood flow to active vascular beds such as the working muscles. If exercise persists and the intensity and/or duration increase causing T_c to rise, the withdrawal of vasoconstrictive tone causes for an initial increase in SkBF and cutaneous vasodilation. Subsequently, once a T_c threshold is reached, activation of cholinergic vasodilator nerves triggers significant cutaneous vasodilation (Kellogg, 2006). SkBF increases linearly with T_c until a point that it reaches a plateau, to help maintain central blood volume and blood pressure (Nadel et al., 1979).

Local changes in skin temperate also directly induce local regulation of SkBF. A decrease in local skin temperature (T_{sk}) triggers a local reflex-mediated increase in arteriolar vasoconstrictor tone that enables arteriole vasoconstriction, reducing SkBF to minimise heat loss. Similarly, if local skin temperature increases, local skin blood flow will increase in a bi-phasic pattern and the hyperaemic vasodilatory response to non-painful local heating has been characterized by assessing the skin blood flow responses to local heater discs

(Minson et al., 2002). Upon the application of heat, SkBF peaks rapidly (e.g. axon reflex) mediated by neural factors, followed by a brief nadir, after which the cutaneous microvessels undergo a secondary prolonged vasodilation to a plateau that is predominantly mediated by locally released substances (e.g.NO) (Minson et al., 2002; Johnson, Minson and Kellogg, 2014). The speed at which the skin is heated modifies the contribution of NO to the heating response. Rapidly heating the skin by 0.5^{o}C per 5 seconds causes an axon-reflex mediated vasodilation, followed by a secondary vasodilation that is ~60-70% NO-mediated (Minson et al., 2002; Black, Green and Cable, 2008b). However, slowly heating the skin at a rate of 0.5^{o}C per 5 minutes avoids eliciting an axon reflex-mediated vasodilation and is primarily a NO-mediated vasodilation (Black, Green and Cable, 2008b). Maximal cutaneous vasodilation is achieved when locally heating the skin to 42-44°C and allows SkBF to be normalised for comparison between measurement sites or subject groups in healthy individuals (Kellogg et al., 1999; McCord and Minson, 2005; Minson, 2010; Johnson, Minson and Kellogg, 2014).

The skin microvasculature is considered as an insight into vascular health and has been noted to detect an early change in endothelial dysfunction (Holowatz, Thompson-Torgerson and Kenney, 2008; Minson, 2010). There is evidence of an age related impairment in the microvascular dilatation response to local heating (Black, Green and Cable, 2008b), reflecting an impaired NO signaling, consistent with endothelial dysfunction (Minson et al., 2002; Holowatz, Thompson-Torgerson and Kenney, 2007). Microvascular endothelial dysfunction has been associated with cardiovascular risk factors such as hypertension (Brunner et al., 2005; Virdis et al., 2013), rheumatoid arthritis (Bordy et al., 2018) and T2D (Jonasson et al., 2017) and is suggested that it can precede and predict conduit artery atherosclerosis (Bordy et al., 2018). Even in the absence of pathology, the control mechanisms of vasodilation and vasoconstriction are diminished (Holowatz and Kenney, 2010), leaving aged individuals more susceptible to cold or heat related illnesses (Hajat, Kovats and Lachowycz, 2007).

2.3.1 Measurement of cutaneous microvascular function

Numerous techniques have been historically used to assess skin blood flow, thermoregulatory control of skin blood flow and in clinical assessments of global vascular function (Figure 2.2) (Low et al., 2020). From these techniques, venous occlusion plethysmography (VOP) and laser Doppler flowmetry (LDF) have been primarily utilised to assess skin blood flow (Charkoudian, 2003), with recent technological advancements identifying optical coherence tomography (OCT) as a novel technique to enable a comprehensive assessment of cutaneous microvascular structure and function in humans (Smith et al., 2019a). Venous occlusion plethysmography can be used to measure the blood flow of an extremity (e.g. lower leg or mostly forearm) via placing a cuff around the distal portion of a limb and inflated to a pressure greater than venous pressure but less than arterial pressure, so that limb volume change by displacement (and limb circumference) increases at the rate of arterial inflow into the limb (Johnson, Minson and Kellogg, 2014; Low et al., 2020). The forearm is most typically used to assess skin blood flow responses and in order to eliminate uncertainty around the contribution from the hand it is excluded from the circulation by placing an occlusive cuff around the wrist and inflated well above systolic pressures (Wilkinson and Webb, 2001). VOP has been deemed to be an easily performed and reproducible measurement (Roberts, Tsao and Breckenridge, 1986), although is limited in its ability to only provide an infrequent, indirect measurement of limb blood flow and does not distinguish between skin and muscle blood flow (Low et al., 2020). Furthermore, the methodology requires the participant to remain still during recordings, preventing the ability to assess blood flow recordings during dynamic situations such as exercise (Low et al., 2020).

The development of Laser Doppler Flowmetry and its high temporal and spatial resolution beyond that of VOP (Charkoudian and Stachenfeld, 2014) has made it possible to detect relative changes of an index of skin blood flow in response to a stimulus (e.g. heat or cold stress) or an intervention (e.g. exercise), independent of muscle blood flow. LDF measures the Doppler shift induced by the movement of red blood cells through the skin vasculature

(Low et al., 2020) and is quantified as the product of average red blood cell velocity and concentration (Roustit and Cracowski, 2013). The measurement output does not directly measure flow and therefore is referred to as flux, which has a linear relationship (r=0.9) with flow (Ahn et al., 1987). Current laser Doppler flowmeters measure a very small area of blood flow measurement estimated as ~1 mm^3, providing the ability to measure SkBF from any area of skin (Johnson, Minson and Kellogg, 2014) and under various local and whole body perturbations (Low et al., 2020). Despite the advantages of LDF, there are a number of limitations. Due to the small area and volume of measurement under the laser Doppler probe and the inherent heterogeneity of the skin microvasculature, direct comparisons of the raw laser Doppler flux signals are highly variable (Roberts et al., 2017) and global levels of blood flow cannot be estimated from small areas (Johnson, Minson and Kellogg, 2014). One approach to resolve the issue for anatomical differences is to utilize a normalisation process. Low et al. (2020) summarise the normalisation procedures of LDF as converting the raw data to either i) a percentage change from a physiological zero induced by arterial occlusion, ii) a percentage change from a thermoneutral baseline (33-34^oC), or a calculated percentage of iii) a maximum vasodilation induced by either local heating (Kellogg Jr et al., 1993; Johnson, Minson and Kellogg, 2014) or perfusion of an endothelium dependent vasodilator to directly act on the smooth muscle (Kellogg et al., 1999; Minson, Berry and Joyner, 2001; Abularrage et al., 2005) or both. Normalisation to maximum vasodilation is deemed to produce the least amount of variability and can be compared among sites (Johnson, Minson and Kellogg, 2014; Roberts et al., 2017) but caution is required to choose the normalisation method sufficient for the experiment and hypothesis (Johnson, Minson and Kellogg, 2014; Low et al., 2020). Therefore LDF is seen to be a simple technique that when performed within an appropriate study design is reliable (Low et al., 2020) and has proven valuable in the current understanding of neurovascular and local mechanisms (Johnson, Minson and Kellogg, 2014).

Figure 2.2 removed due to third party copyright

Figure 2.2. Timeline for the use of different methods for skin blood flow assessment in humans from 1930's to current era. Adapted from (Low et al, 2020).

2.4 Exercise training interventions

2.4.1. Exercise training interventions on peripheral artery function

There are a wealth of studies suggesting endothelial function; measured using FMD can be enhanced with exercise training (as reviewed in (Green et al., 2017)). The time course of changes in endothelial function is longer in individuals with CVD risk factors or *a priori* endothelial dysfunction. In healthy individuals, studies that have measured FMD at 2-week intervals across an 8-week exercise intervention have shown that 2 weeks of exercise training is sufficient to elicit an improvement in FMD (Tinken et al., 2008; Tinken et al., 2009; Birk et al., 2012; Schreuder et al., 2015b), eventually normalising after 6-8 weeks due to structural adaptations and arterial remodelling (e.g. increased artery diameter) (Green et al., 2012; Schreuder et al., 2015b; Green et al., 2017). In individuals with endothelial dysfunction such as those who are older, with CVD risk or disease, this time course for functional and structural adaptation are delayed (Schreuder et al., 2015b). For example, in exercise training menopausal women, FMD is improved after 8 weeks (Bailey et al., 2016b) and in patients with type-2 diabetes, the functional changes occur after 2 weeks and are preserved after 8-12 weeks of exercise training (Okada et al., 2010; Schreuder et al., 2015b).

The improvement in endothelial function with exercise training is thought to be as a result of repeated increases in blood flow and shear stress during each exercise bout, upregulating endothelial nitric oxide synthase (eNOS) expression and NO thereby improving NO mediated endothelium-dependent vasodilation (Maiorana et al., 2001; Hambrecht et al., 2003). Increases in blood flow and shear stress are important in

physiological stimuli to evoke modulation of endothelial function and NO bioavailability. During exercise, the increased demand of the active musculature cause vasodilation and an increase in blood flow (Padilla et al., 2011). Research has demonstrated that shear stress mediated improvements in vascular endothelial function that occurred as a result of exercise training were eradicated when conduit artery shear stress was experimentally removed using bilateral cuff placement to attenuate blood flow (Tinken et al., 2009; Tinken et al., 2010).

2.4.2 Exercise training interventions on cutaneous microvascular function

Exercise training elicits improvements in the NO-mediated cutaneous microvascular function in healthy young and older individuals (Black, Green and Cable, 2008b; Simmons et al., 2011). It has been proposed that the means of adaptation to exercise training may differ along the arterial tree (Atkinson et al., 2018) as larger arteries respond primarily to shear stress and haemodynamic stimuli via arterial enlargement (Rowley et al., 2011), whereas micro vessels respond to these stimuli via sprouting or budding (Brown, 2003; Cocks et al., 2013). Further evidence also suggests that adaptation of the cutaneous microvasculature may be dependent on the combination of changes in local skin temperature in addition to an increase in shear stress and blood flow from an increased core temperature with exercise (Carter et al., 2014; Atkinson et al., 2018). Episodic increases in skin blood flow are known to be essential for enhanced responsiveness to local heating testing and cutaneous vascular function (Green et al., 2010; Carter et al., 2014; Atkinson et al., 2018). In support of this, a previous study found that by utilising unilateral cuff inflation to attenuate the increases in skin blood flow and shear rate responses in a contralateral limb found no adaptation in the skin microcirculation in comparison to the uncuffed arm (Atkinson et al., 2018).

In summary, the maintenance of systemic vascular health is imperative to maintain normal vascular function and reduce disease risk and complications. Exercise training has been shown to have a beneficial effect on both peripheral vascular function, measured using FMD, and micro-vascular function, measured using LDF combined with local heating and can have a significant role to play in the prevention and treatment of disease. However, the effect of exercise training is unclear in its effect on cerebrovascular function. Therefore, alternative or additional exercise interventions might provide a larger stimulus to improve cerebrovascular function. One potential way to do this is to combine exercise training with an additional thermal stimulus.

2.5 Cold stress

The second purpose of this literature review is to introduce “cold stress”, to highlight the response of the cerebral vasculature to an acute cold stress and describe the potential impact of using this stressor as an intervention to change measurements of cerebrovascular function. Cold exposure is defined as any environmental condition where there is potential to lose a significant amount of heat from any region of the body (Doubt, 1991). Humans are subjected to different types of cold exposures including; cold air, with or without wind, contact with cold objects and immersion in cold water (Ikaheimo, 2018). There are also many jobs that can require individuals to work and partake in physical tasks in cold environments (e.g. factory work, farming) (Makinen and Hassi, 2009) and many sporting events that involve both physical exertion and cold exposure (Muller et al., 2012).

2.5.1 Increased CVD incidence in cold weather

Epidemiological data taken from systematic reviews suggest that during the winter seasons and prolonged periods of low environmental temperatures there is evidence of a higher occurrence of cardiovascular and cerebrovascular morbidity and mortality (Ryti, Guo and Jaakkola, 2016; Ryti et al., 2017; Song et al., 2017; Ikaheimo, 2018). Evidence from a case crossover study suggested that decreasing environmental temperatures demonstrated

higher stroke onset and occurrences (Hong et al., 2003). Summary data from a systematic review and meta-analysis have identified positive associations between low environmental temperature and an increased number of deaths related to cardiovascular disease (Rate ratio = 1.11; CI: 1.04 - 1.17) (Ryti et al., 2017). Exposure to low environmental temperature or cold is thought to increase cardiovascular stress in healthy individuals through physiological responses such as blood pressure, vasoconstriction, blood viscosity and inflammatory responses (Keatinge et al., 1984; Woodhouse et al., 1994; Liu, Yavar and Sun, 2015) and could contribute to an increased risk in persons with existing cardiovascular disease (Kuniyoshi et al., 2003; Park, Middlekauff and Campese, 2012; Ikaheimo, 2018). A study by Zhang et al. (2014) investigated the effects of moderate intensity cold air (reduction in outdoor temperature of 6-8^{o}C) on blood pressure and biochemical indicators in cardiovascular and cerebrovascular disease patients including cerebral thrombosis or haemorrhage, coronary heart disease and high blood pressure. They evidenced that the effect of cold air on cardiovascular and cerebrovascular patients was greater than that of healthy people (Zhang et al., 2014). Despite less research studies examining cold-related mortality compared to heat related mortality, the cold-related mortality data suggest more harmful effects than heat (Gasparrini et al., 2015; Yang et al., 2015).

The association between increased cerebrovascular and cardiovascular mortality and morbidity during lower environmental temperatures may involve the acute and chronic (seasonal) changes in cardiovascular system function (Hess et al., 2009). The changes that lower annual temperatures could provoke on the cardiovascular system could include the non-favourable effects on endothelial function, with FMD lowest during the coldest outdoor temperatures (Widlansky et al., 2007), and higher BP associated with lower outdoor temperatures evident in winter than in summer; responses which are greater in older compared to younger individuals (Brennan et al., 1982; Goodiwn et al., 2001; Hayashi et al., 2008). Acute cold exposure could elicit physiological responses that could enhance the possibility of a cardiovascular event, for example an increase in blood pressure could

explain the subsequent increase in the symptoms and severity of angina (Bunker et al., 2016).

2.5.2 Thermoregulatory responses to cold stress

Upon exposure to a cold environment or when an ambient temperature drops, an individual experiences a decrease in skin temperature that is detected via thermosensation and allows the body to provide autonomic responses to initiate an increase in thermogenesis and vasoconstriction. This represents the primary cold-effector response to maintain the body at an optimal working temperature (Stocks et al., 2004). Peripheral thermoreceptors in the skin feed forward the afferent information to the anterior hypothalamic pre-optic area, the thermoregulatory centre in the brain, to initiate defensive thermogenic responses (Morrison, Nakamura and Madden, 2008). Thermoreceptors also detected in the brain, spinal cord and abdomen, are not as immediately susceptible to changes in environmental temperature as T_{sk} (Morrison, Nakamura and Madden, 2008), and therefore it is the skin that is a major sensory input to cold stress. The initial effect of exposure to cold air is a reduction in T_{sk} that leads to sympathetically-mediated vasoconstriction and subsequently a reduced periphery blood flow (Cheung and Daanen, 2011), displacing blood from the periphery to the core. This vasoconstriction of the peripheral microvasculature facilitates a further reduction in T_{sk} to decrease the skin to environmental temperature gradient and limit further heat loss from the body. The further maintenance of core body temperature is achieved through endogenous heat production, such as shivering and non-shivering thermogenesis. Shivering thermogenesis involves rapid, repeated skeletal muscle contractions (Morrison, Nakamura and 2011) whereas non-shivering thermogenesis comes from the metabolism of brown adipose tissue. Both of these are mechanisms mediated by the sympathetic nervous system and the associated release of noradrenaline via the stimulation of cold stress (Cannon and Nedergaard, 2011).

2.5.3 Cardiovascular responses to cold stress

The responses of the cardiovascular system are under the influence of the two components of the autonomic nervous system (ANS); the sympathetic and parasympathetic nervous system that act antagonistically to control automated body functions such as blood pressure, heart rate, digestion and metabolism (Carnethon and Craft, 2008). The autonomic nervous system's primary role is to maintain blood to the major organs, which is achieved through the control of blood pressure through modulation of cardiac output (CO) and systemic resistance (i.e. blood pressure (BP) = cardiac output (CO) x total peripheral resistance (TPR)). An increase or decrease in body temperature poses a physiological challenge on the autonomic nervous system and how the cardiovascular system responds to this challenge is of great importance. The following section discusses the cardiovascular responses to a cold stress.

As previously mentioned, the lowering of skin temperature and/or a decrease in core body temperature from cold exposure elicits a reflex activation of sympathetically mediated vasoconstriction (Johnson, Minson and Kellogg, 2014). This increase in sympathetic nerve activity (Greaney, Kenney and Alexander, 2016) results in a vasoconstriction of both the peripheral and visceral arteries (Wilson et al., 2007a) and subsequently an increase in vascular resistance. The increase in vascular resistance is observed as a result of skin surface cooling of the whole body (Keatinge et al., 1984; Korhonen, 2006; Wilson et al., 2007a; Kingma et al., 2011), face (Stemper et al., 2002), local skin areas (Korhonen, 2006) and cold air inhalation (Bunker et al., 2016) and is balanced by a typical increase in BP, approximately 5-30 mm Hg in systolic blood pressure (SBP) and 5-15 mm Hg in diastolic blood pressure (DBP) (Ikaheimo, 2018).

Many of the cardiovascular responses to cold stress are altered dependent on the presence of shivering. In the absence of shivering, CO, heart rate (HR) and stroke volume (SV) do not substantially change (Wilson and Metzler-Wilson, 2018), although central venous

pressure increases (Cui et al., 2005). This negligible change in CO is due to the balance of mean arterial pressure and vascular resistance. However, lower air temperatures and cold stress such as skin surface cooling that provokes shivering can induce an increase in CO and SV (Raven et al., 1970; Wilson and Metzler-Wilson, 2018). Once shivering is engaged, there is skeletal muscle vasodilation that can decrease systemic vascular resistance (Wilson et al., 2007b), although systolic blood pressure (SBP) continues to increase (Raven et al., 1975). The way in which HR responds is heavily dependent on the type of cold exposure, but is not generally altered much by whole body cold exposure (Wilson et al., 2010), and whole body skin cooling with and without facial exposure has demonstrated that dependent on SV, HR is either decreased (Keatinge et al., 1984; Kingma et al., 2011; Hintsala et al., 2014) or unaltered (Cui et al., 2005; Wilson et al., 2007b). Intriguingly, during skin surface cooling, there is an increased resistance in the cutaneous and visceral vasculature (Wilson et al., 2007b), while there is a decrease in cerebral vascular resistance (Wilson and Metzler-Wilson, 2018).

The responses to a cold stress are highly dependent on the type, duration, severity and pain involvement of the exposure (Stocks et al., 2004; Alba, Castellani and Charkoudian, 2019). As previously mentioned, research studies investigating the responses to cold stress, different types of cooling methods can be employed including; cold restricted locally to areas of the body such as hands (cold-pressor test), facial region (external application of cold to the forehead) or respiratory tract (inhalation of cold air), and whole-body cooling of the skin in an environmental chamber or via water-perfused suit experiments (Ikaheimo, 2018). The latter provides a well-controlled and reproducible cold stress with the capability to clamp skin temperature, without inducing other issues, such as an increase in hydrostatic pressure associated with water immersion, shivering or decreases in core temperature (Greaney et al., 2014).

2.5.4 Impact of ageing on physiological responses to cold stress

Negative consequences associated with cold exposure such as increases in blood pressure also increase with advancing age (Bots, Grobbee and Hofman, 1991) and so it is important to understand how physiological responses to cold exposure may change throughout ageing. Ageing has been shown to demonstrate a greater pressor response in comparison to their younger counterparts following both skin surface cooling; passing 12-15^{o}C water through a water perfused suit for 20 minutes (Hess et al., 2009) and core temperature reducing cold stress; exposing individuals to 6^{o}C air (Collins et al., 1985; Collins et al., 1995). In support of this, a study by Zhang et al. (2014) identified an increase in biochemical indicators such as noradrenaline and angiotensin II in response to cold air exposure in older individuals suffering with cerebral thrombosis or haemorrhage, coronary heart disease and high blood pressure (age 59 ± 10 years) and older healthy controls (55 ± 9.8 years), identifying the activation of the sympathetic nervous system and renin-antiogensin system, inevitably leading to elevated blood pressure. Human ageing, even in the absence of pathology also displays attenuated blood flow responses to whole body cold and heat stress (Holowatz and Kenney, 2010) and on average older individuals (60-90 years) display a 25-50% attenuation in SkBF compared to 18-30 year olds, rendering them susceptible to heat and cold related illnesses (Hajat, Kovats and Lachowycz, 2007; Holowatz and Kenney, 2010; Shibasaki, Okazaki and Inoue, 2013). The impairment of reflex cutaneous vasoconstriction displayed in older individuals contributes to an inability to maintain core body temperature during even mild (22^{o}C) cold exposure (Degroot and Kenney, 2007). A further potential mechanism to explain the increased pressor response to a cold stress could be related to increased levels of arterial stiffness commonly displayed with ageing (Hess et al., 2009; Sun, 2015). The mechanisms around what cardiovascular responses occur during exposure to cold stress, exercise and in combination with each other are hypothesised in Figure 2.3. However, a meta-analysis of reviews shows that despite the risk of cold exposure for people with underlying cardiovascular disease, there is not enough evidence on the risks to the elderly and the association between cardiovascular morbidity

and cold exposure evident across young, middle aged and elderly populations (Song et al., 2017) and warrants further investigation. Furthermore, epidemiological data also displaying that cerebrovascular diseases also increased with cold exposure (Bunker et al., 2016; Song et al., 2017), and there being evidence of increased stroke incidence associated with decreases in environmental temperature (Hong et al., 2003), little research has been conducted into the cerebrovascular responses to cold exposure.

Figure 2.3 removed due to third party copyright

Figure 2.3. Cardiovascular responses to cold exposure and exercise and their combination in healthy individuals. In addition, the potential mechanisms explaining cardiovascular events in healthy individuals and those with hypertension, coronary artery disease and heart failure. Taken from (Ikaheimo, 2018). Abbreviations: CBF; coronary blood flow, CO; cardiac output, DBP; diastolic blood pressure, HR; heart rate, MI; myocardial infarction, RAAS; renin-angiotensin system, RPP; rate pressure product, SBP; systolic blood pressure, SCD; sudden cardiac death, SV; stroke volume, SVR; systemic vascular resistance.

2.5.5 The effects of cold stress on cerebral blood flow regulation

In contrast with other vascular beds in the body such as the peripheral cutaneous circulation, CBFv increases and/or is maintained during a bout of cold stress (Doering et al., 1996). This has been shown in a study that has applied cold exposure via application of cold packs to the surface of the skin at the forehead (Brown, Sanya and Hilz, 2003) and thigh (Doering et al., 1996), displaying an increase in resting CBF. During skin surface cooling, there is an increased resistance in the cutaneous and visceral vasculature (Wilson et al., 2007a), whilst there is a decrease in cerebral vascular resistance (Wilson and Metzler-Wilson, 2018). Wilson et al. (2002) perfused 15^{o}C water for one minute through a water-perfused suit prior to a 60^{o} head tilt and displayed a maintained CBFv contributing to an improved orthostatic tolerance. This was supported by Durand et al. (2003) who found that perfusing 16^{o}C water through a water-perfused suit for 10 minutes prior to the onset of graded lower body negative pressure initially increased CBFv and subsequently attenuated the fall in CBFv throughout the graded protocol, also contributing to orthostatic tolerance. Rapid skin surface cooling particularly to the face stimulates a “diving reflex” that is characterised by a decrease in limb blood flow and an increase of the muscle nerve fascicles (Fagius and Sundolf, 1986). These cardiovascular changes are believed to divert blood towards the brain (Brown, Sanya and Hilz, 2003) increasing CBF. CBF and cerebrovascular function to an acute cold stress alone, without titling or lower body negative pressure, will be examined in the present thesis to understand the cerebrovascular haemodynamic responses to a cold stress. Moreover, given that ageing is associated with reductions in resting CBFv and impairments in cerebrovascular function, the responses to acute cold will be examined in both young and older individuals. The cerebrovascular responses may provide insight into why cerebrovascular events occur more often in cold temperatures. In this context, some evidence is available from studies that have looked into CBFv as either a primary or secondary measure in response to differing acute cold exposures (Table2.2).

2.5.6 The effects of a combination of a cold stress and exercise on cerebral blood flow regulation

Studies of adults exercising in cold environments are limited and generally use varied methodologies to explore either the control of cutaneous blood flow during exercise (Pergola et al., 1996) or performance related effects of cold acclimation (Galoza et al., 2011; Vieira et al., 2013). The effects of cold and exercise on the cardiovascular system have been extensively reviewed (Manou-Stathopoulou et al., 2015; Ikaheimo, 2018), however little research has been performed on the combination of cold and exercise on the cerebrovascular system.

Cold exposure via application of cold packs to the skin increases resting CBF (Doering et al., 1996; Brown, Sanya and Hilz, 2003) and facial cold-water immersion amplifies the increase in CBFv during moderate intensity exercise compared to no facial immersion (Kjeld, Pott and Secher, 2009; Miyazawa et al., 2012), but this has not been investigated using whole body cooling.

Any method of augmenting the CBF response to an acute exercise bout could optimise any training response. Evidence has suggested that increasing the exercise stimulus (intensity), e.g. with HIIT exercise (Ramos et al., 2015; Chidnok et al., 2020) can magnify the chronic conduit artery responses to exercise training. Therefore, by manipulating environmental conditions using cold stress could increase CBF to a greater extent and thus possibly exacerbate the beneficial chronic vascular exercise training effects.

Table 2.2. Cerebrovascular and blood pressure responses from previously controlled (n=7) studies.

	Subjects	Cold Exposure	Duration of cold exposure	Measured cerebral parameters	Results	Conclusion
Whole Body Cold Exposure						
Wilson et al (2002)	9 healthy individuals (4 males); mean age 32±2 years	Whole body cold exposure, water perfused suit (15°C) prior to 60° NT-tilt or HT-tilt	1 minute	Middle Cerebral Artery velocity using TCD MAP $P_{ET}CO_2$	SSC attenuated decrease in MCAv as displayed with NT-tilt and HT-tilt SSC increased MAP prior to tilt $P_{ET}CO_2$ reduced irrespective of cooling	SSC prior to and during tilting in both normothermic and heat-stressed conditions prevents the fall in cerebral blood flow velocity. The maintenance of cerebral blood flow velocity is likely due to MAP elevation in response to SSC
Durand et al (2004)	8 healthy individuals (4 males); mean age 33±2 years	Whole body cold exposure, water perfused suit (15°C) for 10 minutes prior to and throughout LBNP	10 minutes	Middle Cerebral Artery velocity using TCD MAP $P_{ET}CO_2$	SSC increased MAP prior to LBNP SSC increased MCAv No change in $P_{ET}CO_2$	SSC causes increases in blood pressure, CBFv and perhaps greater sympathetic activation during LBNP, increasing orthostatic tolerance.
Local Cold Exposure						
Brown et al (2003)	17 healthy individuals (3 female); mean age 27±5 years	A thin plastic bag filled with ice and water (0°C) applied bilaterally to the forehead	1 minute	Middle Cerebral Artery velocity using TCD MAP $P_{ET}CO_2$	MAP, MCAv increased during FC No change in $P_{ET}CO_2$ during cold stress	A localised cold stimulus to the face increases cerebral perfusion
Doering et al (1999)	9 healthy individuals; mean age 36±5 years	Cryogel cold packs (8-12°C) applied to both thighs	10 minutes	Middle Cerebral Artery velocity using TCD MAP $P_{ET}CO_2$	MAP, MCAv increased following stimulation period No change in $P_{ET}CO_2$	Cold thermo- applications have an influence on cerebral hemodynamics, displaying an increase in CBFv following cold application possibly due to a change in ABP
Miyazawa et al (2012)	9 healthy males; mean age 20±2 years	4°C water face mist and fanning with a fan placed 50cm from the subject's face	3 minutes	Middle Cerebral Artery velocity using TCD MAP $P_{ET}CO_2$	MAP, MCAv increased during FC No change in $P_{ET}CO_2$	FC induced changes in MAP could have an impact on CBF regulation. FC induces increases in CBFv at rest.

Table 2.2. *Continued*

	Subjects	Cold Exposure	Duration of cold exposure	Measured cerebral parameters	Results	Conclusion
Cold Exposure and Exercise						
Kjeld, Pott and Secher (2009)	9 healthy males; 28 years (21-34 years) (median with range)	180w cycling exercise with facial immersion in a 10°C water bath whilst breathing through a snorkel (without nose clip)	15 minutes	Middle Cerebral Artery velocity using TCD MAP P_aCO_2	No change in MAP MCAv significantly increased No change in P_aCO_2	The increase in CBFv was larger by facial immersion in cold water, independent of P_aCO_2.
Miyazawa et al (2012)	9 healthy males; mean age 20±2 years	During steady state exercise, 4°C water face mist and fanning with a fan placed 50cm from the subject's face	3 minutes	Middle Cerebral Artery velocity using TCD MAP $P_{ET}CO_2$	MAP, MCAv increased during FC No change in $P_{ET}CO_2$	FC induced changes in MAP could have an impact on CBF regulation. FC induces increases in CBFv at both rest and during exercise.
AlSalahi et al (2020)	1. 13 healthy individuals (11 males; 23±4 years) 2. 8 healthy males (23±6 years) 3. 8 healthy individuals (7 males; 24±3 years) 4. 8 healthy males (24±3 years)	1. FC (0°C ice pack application to the face) under poikilocapnic conditions 2. FC (0°C ice pack application to the face) under isocapnic conditions 3. FC (0°C ice pack application to the face) with and without BH 4. BH with or without FC (0°C ice pack application to the face)	1. 3 minutes 2. 3 minutes 3. 1 minute 4. Full length of a BH	Middle Cerebral Artery velocity using TCD MAP $P_{ET}CO_2$	1. MCAv, MAP, $P_{ET}CO_2$ unchanged. Correlation between change in $P_{ET}CO_2$ and MCAv. 2. $P_{ET}CO_2$ held constant, MCAv unchanged, MAP increased. 3. MCAv only increased in presence of a BH where $P_{ET}CO_2$ increased. MAP elevated numerically (P=0.23) during FC-BH, whereas increased during FC+BH (P<0.05 vs FC-BH). 4. BH with and without FC increased MCAv and MAP.	Factors associated with breath holding makes the predominant contribution to the diving-response mediated increase in CBFv.

Abbreviations: TCD; Transcranial Doppler, MAP; Mean arterial pressure, $P_{ET}CO_2$; end tidal carbon dioxide, SSC; Skin surface cooling, MCAv; Middle cerebral artery velocity, NT; Normothermic, HT; Hyperthermic, CBFv; cerebral blood flow velocity, LBNP; Lower body negative pressure, FC; facial cooling, P_aCO_2; arterial carbon dioxide, BH; Breath hold.

2.6 Summary

In summary, understanding the changes in CBF and cerebrovascular function in response to a cold stimulus, that individuals encounter in everyday life, is essential to understand as it may provide insight into prevention of cerebrovascular events that occur more often in the cold (Hong et al., 2003; Lichtman et al., 2013; Lichtman et al., 2016). In addition, better understanding of the responses to cold stress could allow a cold stress to be used in an advantageous way to prevent the age-related decline in CBFv and enhance cerebrovascular function (Chapter 3). Given the studies that have assessed the impact of structured exercise training on CBFv and cerebrovascular function with some evidence of negating the decline and improvement in function. It is possible that adding a cold stimulus to the exercise might enhance the stimulus for greater CBF and cerebrovascular changes (Chapter 4).

Chapter 3

Study 1

The effect of acute cold stress on cerebral blood flow and cerebrovascular function: the impact of ageing.

3.1 Introduction

During the winter months and prolonged periods of low environmental temperatures there is evidence of a higher occurrence of cardiovascular and cerebrovascular morbidity and mortality (Ryti, Guo and Jaakkola, 2016; Song et al., 2017; Ikaheimo, 2018). For example, lower average annual temperatures, such as those experienced during winter months, have been associated with an increased number of hypertensive emergencies (Liu, Yavar and Sun, 2015), cardiac deaths (Ryti et al., 2017) and stroke hospitalizations (Lichtman et al., 2016). Evidence from a case-crossover study suggests that a 17.4^{o}C decrease in environmental temperature increased the risk of stroke onset (Odds Ratio 2.9 [1.5-5.3]) (Hong et al., 2003). A cold temperature related increase in stroke risk is likely more problematic for older individuals as they exhibit an impaired thermoregulatory function, resulting in a lower tolerance to a cold environment compared to their younger counterparts (Shibasaki, Okazaki and Inoue, 2013). Moreover, independent of any changes in environmental temperature, stroke risk increases with age (Kelly-Hayes, 2010), as does blood pressure (Bots, Grobbee and Hofman, 1991), while CBF declines (Stoquart-ElSankari et al., 2007; Ainslie et al., 2008; Chen, Rosas and Salat, 2011; Murrell et al., 2013)

There are a range of physiological adjustments to cold exposure in order to maintain internal temperature within safe limits. Cold stress causes sympathetically mediated vasoconstriction (Cheung, 2015) resulting in a reduction of blood flow to the cutaneous peripheries and a reduction in flow to other vascular beds and organs (Beker et al., 2018). In contrast, CBFv increases and/or is maintained during a bout of cold stress (Doering et al., 1996). Skin surface cooling can prevent the fall in CBFv during upright tilting, lower body negative pressure and improves orthostatic tolerance (Wilson et al., 2002; Durand et al., 2003). The maintenance and/or an increase in CBFv can be viewed as a beneficial response to cold stress, contributed to by an increase in BP during cold exposure, a protective mechanism, to prevent cerebral hypo-perfusion. It is well established that reflex cutaneous vasoconstriction is markedly impaired in aged individuals (Holowatz and

Kenney, 2010) and this contributes to an inability to maintain core body temperature during even mild (22^{o}C) cold exposure (Degroot and Kenney, 2007). There is little research on the effect of age on the CBFv response to cold, however, CBF is controlled via perfusion pressure (e.g., blood pressure) and cerebral autoregulation (the ability of the brain to control its flow locally/independently of changes in blood pressure) and local changes in carbon dioxide concentrations (CO_2 reactivity) (Willie et al., 2014). It is unclear, however, that despite these seemingly beneficial effects of cold stress on CBFv, how the underlying control mechanisms of brain blood flow, e.g., cerebral autoregulation and CO_2 reactivity, are favourably or detrimentally altered by cold stress. Furthermore, given the evidence of reduced CBFv (Stoquart-ElSankari et al., 2007; Ainslie et al., 2008; Chen, Rosas and Salat, 2011; Murrell et al., 2013), elevated BP (Bots et al, 1991) and cerebrovascular dysfunction (Vasilevko et al., 2010; Bailey et al., 2013b; Barnes et al., 2013) with age, it is possible that the cerebrovascular response to cold may be altered in the elderly. Therefore, the aim of this study was to examine the effects of cold stress on cerebral and peripheral vascular function in young and older individuals**.** The secondary aim was to examine cerebral, conduit and skin micro-vascular responses during an acute cold stress in younger and older individuals. It was hypothesised that older individuals would display a blunted CBFv response to cold stress and measurements of cerebrovascular function would be reduced in the older individuals compared to younger individuals.

3.2 Methods

3.2.1 Participants

Twenty-one healthy individuals (12 young [6 female]; 25±5years and 9 older [3 female]; 62±6years, Table 3.1) were recruited. Participants were recreationally active, engaged in low to moderate intensity exercise 2-3 times per week, normotensive, non-smokers, not taking any medication and were free from cerebrovascular or cardiovascular disease. Young females were not on oral contraceptives and were tested in the early follicular phase of their menstrual cycle. All older females were post-menopausal. Participants were

informed of the methods verbally and in writing before providing written informed consent prior to any assessments being performed. The study conformed to the Declaration of Helsinki and was approved by the University Research Ethics Committee (14/SPS/014).

3.2.2 Research Design

Participants visited the laboratory for two visits (separated by a minimum of 2 days). Visit 1 consisted of an incremental treadmill test to assess cardiorespiratory fitness ($\dot{V}O_{2peak}$). Visit 2 consisted of participants being instrumented with a water tube-lined suit and cerebrovascular, conduit and microvascular function tested prior to and following an acute cold stress stimulus. Visit 2 was performed in a temperature-controlled laboratory (19-21°C) at the same time of day to control for diurnal variation in cerebrovascular and cardiovascular function (Ainslie et al., 2007a; Jones et al., 2008; Jones et al., 2010) following an overnight fast (~12 hours), abstaining from exercise for 24 hours and caffeine for 12 hours) (Figure 3.1). Data collection took place during March to August to minimise the possible impact of seasonal variation in the measurements (ref).

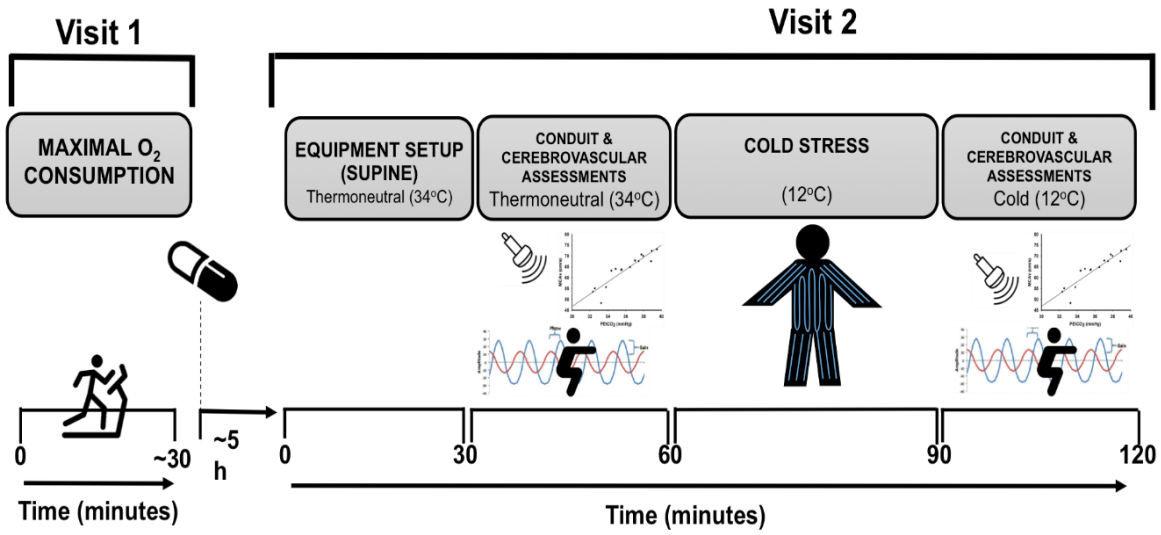

Figure 3.1. Schematic representation of the experimental design.

3.2.3 Measurements

Anthropometrics: Stature and weight were recorded to the nearest 0.1 unit using a stadiometer (SECA) and digital scales (SECA 701, SECA UK), respectively. All

anthropometric measurements were taken three times and an average of the three were recorded. From these variables, body mass index (BMI; mass (kg) / height (m^2)) was calculated. Resting blood pressure was also determined from an average of three measures in the seated position using an automated blood pressure monitor (Dinamap V100, GE Healthcare, Germany).

Cardiorespiratory fitness test (VO_{2peak}): An incremental maximal exercise test was performed on a treadmill (Pulsar 4.0, HP Cosmos, Germany) in a temperature-controlled laboratory. A modified version of the Bruce et al (1973) protocol was adopted as is most suitable for the older participants (Pugh et al., 2013; Sprung et al., 2013; Bailey et al., 2016a). Following a 2-minute warm up at 2.2 $km.h^{-1}$ on a flat gradient, the initial workload was set at 2.7 $km.h^{-1}$ with a 5º gradient. Thereafter, stepwise increments in speed and gradient were made every minute until volitional exhaustion. VO_2 throughout the incremental exercise test was calculated from breath-by-breath analysis of expired gases (Oxycon Pro, Jaeger, Germany) and were expressed as absolute oxygen consumption ($ml.min^{-1}$) and relative to body weight ($ml.kg.min^{-1}$). Heart rate was measured continuously using short-range telemetry (FT1, Polar, Finland) alongside perceived exertion at each exercise stage (RPE; Borg Scale, (Borg, Hassmén and Lagerström, 1987). Maximum oxygen consumption (VO_{2peak}) was calculated as the highest consecutive 15-second period of gas exchange data occurring in the final minute before volitional exhaustion.

3.2.3.1 Impact of acute cold on cerebral and peripheral vascular function

Upon arrival to the laboratory, participants were asked to wear shorts and a vest and were clothed in a water tube-lined jacket and trousers (Med-Eng, Owada, Canada), covering the entire body not including the head, feet and hands (Figure 3.2). The water tube-lined suit allows different temperature of water to be perfused around the suit to manipulate skin and core body temperatures.

Participants rested in a semi-recumbent position whilst 34^{o}C water was perfused through the suit using a temperature-controlled water bath and an electronic water-pump to establish the thermoneutral condition. Following a 15-minute resting period, brachial artery and cerebrovascular function (reactivity to CO_2 and cerebral autoregulation) were assessed. Participants then underwent an acute cold stress where 12^{o}C water was perfused through the suit for 30-minutes with the aim to reduce skin temperature to at least 30^{o}C. Following this cooling period, assessments of brachial artery and cerebrovascular function was repeated whilst 12^{o}C was maintained through the water tube-lined suit. Using the water perfused suit, 12^{o}C was identified during pilot experimentation to be the coldest most tolerable temperature to decrease skin temperature with little effect on core temperature without causing shivering over a period of 30 minutes. During the measurements described above, a water perfused suit was worn throughout. The water perfused suit was not tight fitting to cause compression in limbs in any participant.

3.2.3.2 Vascular and haemodynamic responses during an acute cold stress

MCAv, core temperature (T_c), skin temperature (T_{sk}), and SkBF were monitored continuously during functional assessments and cooling as described below *(see section 3.2.3.3 Cerebral blood flow velocity and Cutaneous Microvascular responses and 3.2.3.4 Temperature Measurements)*. Data were extracted and presented as an average of 1 minute every 5 minutes during the 30-minute acute cold stress.

Figure 3.2. Participant set up in the water perfused tube-lined suit. Instrumentation displayed are (1) Transcranial Doppler Ultrasound, (2) Core Temperature, (3) water perfused tube-lined suit, (4) Finger photoplethysmography and (5) Ultrasound.

3.2.3.3 Cerebral and Vascular Function Measurements

Brachial artery endothelial function: Brachial artery endothelial function was assessed using the flow mediated dilation (FMD) technique strictly following most recent consensus guidelines (Thijssen et al., 2019b). The left arm was extended and positioned ~80° from the

torso. A rapid inflation and deflation pneumatic cuff (D.E. Hokanson, Bellevue, WA) was positioned on the left forearm, immediately distal to the olecranon process to provide a stimulus to forearm ischemia (Thijssen et al., 2019a). A 15-MHz multi-frequency linear array probe, attached to a high-resolution ultrasound machine (T3300; 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. Diameter, flow and shear stress were measured for 1-minute prior to and 3-minutes following 5-minutes of forearm cuff inflation (D.E. Hokanson, Bellevue, WA) (Thijssen et al., 2019b). All images were obtained by the same sonographer with a day-to-day coefficient of variation (CV) in FMD% of 11% and a CV of 3% for baseline artery diameter which is deemed good-excellent based on previous analysis (van Mil et al., 2016).

Analysis of brachial artery diameter was performed using custom-designed edge-detection and wall-tracking software, which is largely independent of investigator bias. From synchronised diameter and velocity data, blood flow (the product of lumen cross- sectional area and Doppler velocity) were calculated at 30 Hz. Shear rate (an estimate of shear stress without viscosity) was calculated as 4 times mean blood velocity/vessel diameter (Parker, Trehearn and Meendering, 2009). Previous articles contain detailed descriptions of this analytical approach (Woodman et al., 2001; Black et al., 2008). Reproducibility of diameter measurements using this semi-automated software is significantly better than manual methods, significantly reduces observer error, and possesses within-day CV of 6.7% (Woodman et al., 2001). FMD analysis was performed (by GM) using a single blinded coding-randomised procedure and allometric scaling to control for baseline diameter was performed (Atkinson and Batterham, 2013).

Cerebral blood flow velocity: Bilateral MCAv was continuously measured using TCD. A 2-MHz pulsed Doppler ultrasound probe (Spencer Technologies, Seattle, WA, USA) was

adjusted through the temporal window until an optimal signal was obtained (Willie et al., 2011) and held in place by a Marc 600 head frame (Spencer Technologies, Seattle, WA, USA). Probe location and parameters (depth, gain and power) were recorded to ensure within-participant consistency of measurement site between visits. The same sonographer obtained the vessel signals throughout the study (GM) and had a between day coefficient of variation of 3.6% for the MCA. The weighted mean MCAv was calculated from the peak envelope of the velocity trace (1/3 systolic + 2/3 diastolic), which accounts for the relative time spent in each phase of the cardiac cycle (Skow et al., 2013). Where two MCA signals were insonated, a mean of the two signals were taken, however if only one was obtainable, the unilateral signal was used. Participants were instrumented with a two-way valve mouthpiece (Hans Rudolph) from which partial pressure of end tidal CO_2 ($P_{ET}CO_2$) was measured using a calibrated gas analyser (ML206 ADinstruments, Colorado Springs, USA). Beat-to-beat arterial blood pressure was measured continuously using finger photoplethysmography (Finapres, Amsterdam, Netherlands) and heart rate was obtained from 3-lead echocardiogram (Powerlab 8.0, AD Instruments, Oxford, UK*).* To validate continuous blood pressure (BP), intermittent arterial BP was measured from brachial auscultation using an automated syphygmanometer (Dinamap V100, Germany). All data were sampled at 50Hz using an analog-to-digital converter (Powerlab, ADInstruments, Oxford, UK) interfaced with a computer and analysed using data acquisition software (Labchart version 8, ADInstruments, Oxford, UK). Resting data were averaged over a 5-minute extract of MCAv, BP and $P_{ET}CO_2$ from LabChart. MCA cerebrovascular conductance (CBVC) was calculated as MCAv/MAP. MCA cerebrovascular resistance (CBVR) was calculated as MAP/MCAv.

Cerebrovascular reactivity to CO_2: Cerebrovascular reactivity to CO_2 was assessed whilst lying in a supine position. Following resting measurements (MCAv, MAP & $P_{ET}CO_2$), participants were coached through a voluntary hyperventilation protocol (approximately 24 breaths per minute for approximately 1 minute) to reach a reduction in $P_{ET}CO_2$ of ~10mmHg

from baseline (<20mmHg). Following this, participants were connected to a prefilled bag (Douglas Bag, Hans Rudolph, Oxford) and were instructed to return to their normal breathing rate whilst inhaling a 5% CO_2 (21%O_2, 5% CO_2, N_2 balance) gas mixture for 3 minutes to reach an equivalent increase in $P_{ET}CO_2$ (Figure 3.3). Resting MCAv, $P_{ET}CO_2$ and MAP were calculated as the mean of the minute before the test commenced, and subsequently MCAv, $P_{ET}CO_2$ and MAP values were collected as 10 second averages throughout the 3-minute rebreathing period. Cerebrovascular CO_2 reactivity was calculated both in absolute and relative (%) terms as the gain of the linear relationship between CBFv and $P_{ET}CO_2$ from baseline to the last 30 seconds of CO_2 inhalation (Battisti-Charbonney, Fisher and Duffin, 2011) using the equations:

$$\text{Absolute } CVR_{CO2} = \Delta MCAv / \Delta PETCO2$$

$$\text{Relative } CVR_{CO2} = \%\ MCAv \text{ change from baseline} / \Delta PETCO2$$

Where Δ is the change from baseline to the 30 seconds of CO_2 inhalation. To correct for any changes in MAP during CO_2 inhalation, cerebrovascular conductance was calculated (CBFv/MAP) and the absolute and relative (%) gains for CVR_{CO2MAP} vs $P_{ET}CO_2$ were also calculated.

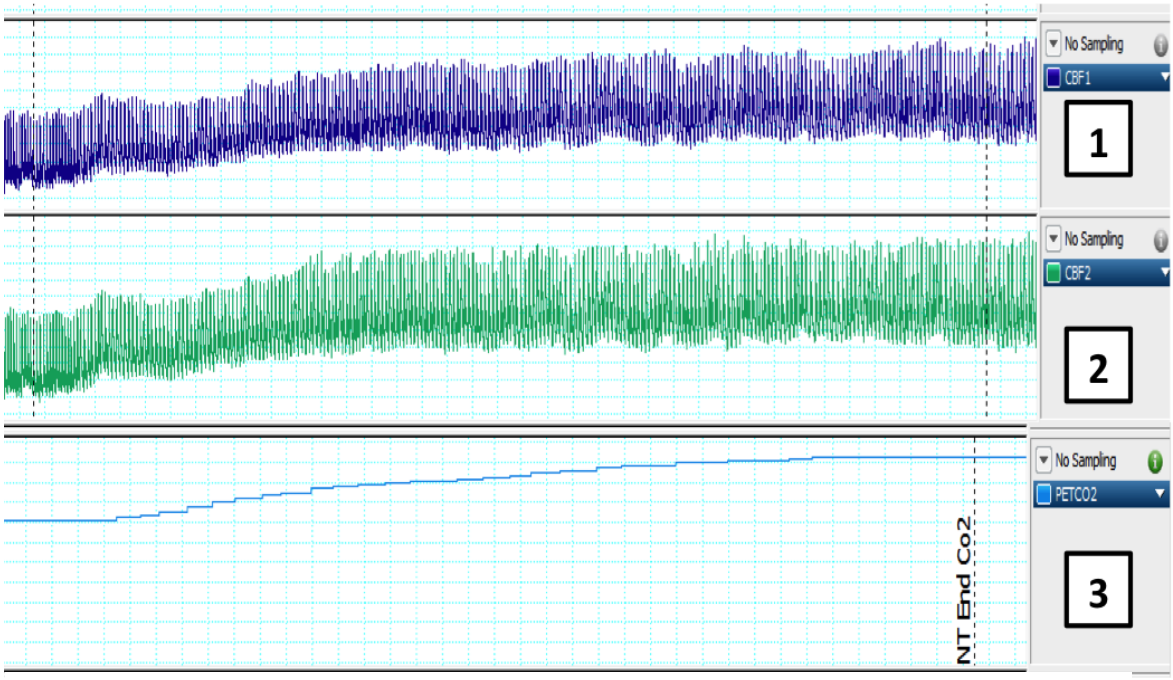

Figure 3.3. Chart file of a real life powerlab tracing during reactivity to 5% CO_2. Variables displayed are (1-2) right and left MCAv, (3) $P_{ET}CO_2$

Concurrently, during resting and CO_2 rebreathing measurements, a 15-MHz multi-frequency linear array probe, attached to a high-resolution ultrasound machine (T3300; Terason, Burlington, MA) was used to image artery diameter and blood flow in the common carotid artery (CCA). The CCA was imaged approximately 2 cm below the point of bifurcation to minimize vascular responsiveness as a result of turbulent flow. Images were acquired in accordance with previous methodological guidelines, including ultrasound measurements being acquired by the same sonographer (Thomas et al., 2015) and were analysed as in the same way as brachial artery diameter. Baseline diameter and blood flow was calculated as the mean of the minute before the test commenced, and subsequently as 10 s averages throughout the 3-minutes of rebreathing.

Cerebral Autoregulation: The dynamic relationship between BP and MCAv, referred to as dynamic cerebral autoregulation (dCA), was assessed using a squat-stand procedure in order to induce transient changes in BP. Participants replicated the experimenter whilst performing these manoeuvres in order to achieve consistent movements. Manoeuvres were performed at 0.10 Hz (5 seconds squat followed by 5 seconds stand) to create physiologically relevant changes in BP that present challenges to the autoregulatory system that are typically experienced in daily life (Simpson and Claassen, 2018). Recent evidence into the day-to-day variation of active vs driven oscillations in blood pressure have identified that that squat stand manoeuvres produce the most reproducible TFA metrics (<20%) and TFA assesses squat stands as a linear system with coherences between 0.99-1.00 (Smirl et al., 2015). The BP-MCAv relationship during these manoeuvres were analysed in accordance with most recent consensus guidelines (Claassen et al., 2016) using Transfer Function Analysis.

Resting measurements of MCAv, BP and $P_{ET}CO_2$ were extracted from LabChart beat-to-beat and averaged over a 5-minute period. Data from 5 min recording of squat to stand

manoeuvres for dCA were extracted from LabChart beat-to-beat using ECG tracing (MAP, MCAv and $P_{ET}CO_2$) before spline interpolation and assessed via TFA. TFA was applied using a provided script (http://www.car-net.org/), via MATLAB (2018a; MathWorks-Inc., Natick, MA) in order to calculate associated power (gain), timing (phase) and linearity (coherence) at the point estimate of the driven frequency (0.10Hz) (Claassen et al., 2016).

Cutaneous microvascular responses: Skin blood flow was assessed using the non-invasive method of Laser Doppler Flowmetry (LDF: Periflux System 5001, Perimed, AB, Sweden), of which absolute flux values provides an index (in arbitrary units, AU) of skin blood flow (Brothers et al., 2010). One 7-laser array probe was attached to the skin on both the non-dominant arm and chest (not covered by the suit) using adhesive stickers and medical tape. Participants were inspected for any abrasions or skin damage that may affect cutaneous blood flow responses and measurement sites were chosen, avoiding visible veins and hair follicles (Cracowski et al., 2006a). The laser Doppler probe signals (flux) were continuously monitored through the data acquisition software. Data were averaged over 60 seconds at baseline and over 60 seconds every 5 minutes during the acute cold stress. Cutaneous vascular conductance (CVC) was also used as an index of skin blood flow that was calculated by the ratio of the LDF data to mean arterial blood pressure (mmHg) during the thermoneutral and cold measurements (CVC=FLUX/MAP).

3.2.3.4 Temperature Measurements

Core body temperature was measured from an ingestible pill telemetry system ingested ~5 h before data collection began (CoreTemp, HQInc; Palmetto, FL, US), as per recommendations for the consistency of temperature measures (Goodman et al., 2009). The ingestible telemetric pill transmits an internal temperature relative to the surrounding gastrointestinal temperature to an external receiver via radio waves for data logging (O'Brien et al., 1998). The telemetry pill has been validated against other measures of core

temperature (O'Brien et al., 1998), including both research setting “gold” standard measures rectal and oesophageal temperature (Gibson, Redman and Belyavin, 1981; Sparling, Snow and Millard-Stafford, 1993; Byrne and Lim, 2007) and is deemed reliable for repeated measurements (Gant, Atkinson and Williams, 2006).

Mean skin temperature was obtained from the weighted average of 4 regional temperatures measured using thermocouples (iButtons data logger, Maxim Integrated; San Jose, CA, US) secured using medical tape (Transpore, 3M, Michigan, USA) to the upper thoracic, anterior forearm, anterior mid-thigh and calf (Ramanathan, 1964b). Wireless iButtons have been identified to provide a valid measurement of skin temperature (Harper-Smith et al., 2010). The calculation of mean skin temperature was calculated using the weighting system for mean surface temperature (Hardy and Dubois, 1938) and the equation (Ramanathan, 1964a):

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

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.

Liu, Yavar and Sun (2015) compared mean skin temperature calculations against a 10-site calculation and deemed the Ramanathan calculation as highly reliable. Core and skin temperature were analysed during baseline measurements and every 5 minutes during the acute cold stress.

3.2.4 Statistical Analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS version 23.0, Chicago, IL). Participant characteristics for each age group was analysed using an independent samples t-test. Differences in age group characteristics were not statistically controlled for due to these naturally occurring as a result of ageing. Linear mixed models were employed to examine the changes in resting values including

CBFv, BP, T_c and T_{sk} and SkBF, CO_2 reactivity and dCA between thermoneutral 34°C and cold 12°C (temperature) and across age groups (age). CBFv, BP, T_c, T_{sk} and SkBF were measured continuously during the acute cold stress and were analysed using linear mixed modelling with age group and time (every 5 minutes throughout cooling). Covariance structure for each model was determined using a chi-square distribution, with the selected structure being the most parsimonious fit. Statistically significant interactions were followed-up with the least significant difference (LSD) approach to multiple comparisons (Perneger, 1998). Statistical significance was defined at $P < 0.05$ and exact P values are cited (P values of '0.000' provided by the statistics package are reported as <0.001). Data are presented as mean and 95% confidence intervals unless otherwise stated.

3.3 Results

3.3.1 Participant Characteristics

By design, there was a significant difference in age between the two groups with a mean difference of 37 years (33, 42; P<0.001); between younger and older individuals. Unequal participant group sizes were established due to resource and practicality constraints. Resting MCAv was higher in the younger individuals compared to the older individuals but this did not reach statistical significance (Table 3.1, P=0.14). MAP was 10 mm Hg lower (-16, -4) and VO_{2peak} was 15 ml.min.kg^{-1} higher (6.9, 24.2) in the younger compared to the older individuals (Table 3.1, P<0.01; P=0.002 respectively). There was no difference in BMI (P=0.18).

Table 3.1. Participant characteristics in the young and older group.

Characteristic	Young	Older	P-value
	n=12 [6 females]	n=9 [3 females]	
Age (years)	25 ± 5	62 ± 6	<0.001*
Body Mass (kg)	72 ± 13	76 ± 15	0.52
BMI (kg/m^2)	24 ± 3	26 ± 3	0.18
SBP (mmHg)	118 ± 6	128 ± 16	0.04*
DBP (mmHg)	64 ± 5	71 ± 5	0.002*
MAP (mmHg)	83 ± 5	93 ± 8	0.002*
MCAv (cm.s^{-1})	65 ± 8	57 ± 15	0.14
VO_{2peak} (ml.min.kg^{-1})	43.1 ± 6.0 [n=10]	27.6 ± 5.9 [n=3]	0.002*

Values are mean ± SD. Abbreviations: BMI; body mass index, SBP; systolic blood pressure, DBP; diastolic blood pressure, MAP; mean arterial pressure. * Denotes significant difference (P<0.05).

3.3.2 Impact of acute cold on cerebral and peripheral vascular function

3.3.2.1 Cerebrovascular Function

Cerebrovascular reactivity to CO_2: There were no main effects of temperature, age or temperature*age interaction on the absolute or relative gain of MCAv to CO_2 inhalation

(P>0.05, Table 3.2). CVR_{CO2} was also studied in absolute and relative terms using CVR_{CO2MAP} to account the effects of MAP changes on CVR_{CO2} estimates. There were no effects of temperature, age or temperature*age interaction on the absolute or relative gain of CVR_{CO2MAP} to CO_2 inhalation (P>0.05, Table 3.2). Resting carotid diameter was 0.07 cm (0.02, 0.11; P=0.006) larger in the older group compared to the younger group and the percentage change were similar in both groups (P>0.05). There were no main effects of temperature or age*temperature interactions on baseline carotid diameter or in response to CO_2 inhalation (P>0.05, Table 3.2).

Cerebral Autoregulation: There were no main effects of temperature, age or temperature*age interaction on $P_{ET}CO_2$, normalised and absolute gain and phase (Table 3.2, P>0.05). Coherence was 0.11 (0.05, 0.18) lower in the cold compared to thermoneutral (Table 3.2, main effect of temperature; P=0.002). There was no main effect of age or temperature*age interaction on coherence (P>0.05).

3.3.2.2 Peripheral vascular function

Brachial Artery Endothelial Function: There were main effects of temperature on FMD%, peak diameter and shear rate area under the curve (SR_{AUC}) (P<0.05; Figure 3.3). FMD reduced by 2.6% (-5.1 -0.1, P=0.05), peak diameter reduced by 0.02 cm (-0.03, 0.00, P=0.05) and SR_{AUC} decreased by 10 (-19 -2 (10^3), P=0.02) in the cold. There were no effects of age or age*temperature interactions and the results did not change following allometric scaling (P>0.05, Figure 3.4). There was also no main effect of age, temperature or age*temperature interactions for artery diameter or time to peak (TTP) (P>0.05; Table 3.3).

Table 3.2. Dynamic cerebral autoregulation using squat-stand manoeuvres (0.10Hz) and cerebrovascular reactivity data in the younger and older age group in a thermoneutral and cold temperature.

Characteristics	Young Group		Older Group		P-value		
	THERMONEUTRAL	COLD	THERMONEUTRAL	COLD	Temp	Age	Temp*Age
CO_2 Reactivity test							
Baseline Carotid Diameter (cm)	0.63 ± 0.03	0.64 ± 0.04	0.71 ± 0.07	0.69 ± 0.05	0.66	0.006*	0.44
Carotid Diameter (cm) (last 30 seconds)	0.62 ± 0.03	0.64 ± 0.04	0.71 ± 0.05	0.69 ± 0.08	0.96	0.001*	0.38
Relative CVR_{CO2} (% cm.s/mmHg^{-1})	3.8 ± 2.1	4.0 ± 2.9	2.9 ± 3.3	2.4 ± 1.3	0.90	0.15	0.68
Absolute CVR_{CO2} (cm.s/mmHg^{-1})	2.5 ± 1.4	2.6 ± 2.2	1.7 ± 2.3	1.5 ± 0.9	0.91	0.13	0.64
Relative CVR_{CO2MAP} (%cm.s/mmHg^{-1}. mmHg^{-1})	2.9 ± 3.8	3.3 ± 2.6 [n=8]	3.9 ± 1.7	3.0 ± 2.1 [n=9]	0.46	0.57	0.61
Absolute CVR_{CO2MAP} (cm.s.mmHg^{-1}. mmHg^{-1})	0.7 ± 0.4	0.8 ± 0.4 [n=8]	0.8 ± 0.4	0.7 ± 0.2 [n=9]	0.98	0.67	0.13
Cerebral Autoregulation test							
$P_{ET}CO_2$ (mm Hg)	37 ± 5	36 ± 5	36 ± 4	35.5 ± 4.5	0.31	0.82	0.76
Gain (cm.s^{-1}/mm Hg^{-1})	0.79 ± 0.16	0.88 ± 0.16	0.71 ± 0.25	0.76 ± 0.38	0.11	0.38	0.60
Normalised Gain	1.20 ± 0.21	1.34 ± 0.28	1.27 ± 0.35	1.32 ± 0.53	0.17	0.88	0.44
Phase (radians)	0.04 ± 0.22	0.03 ± 0.39	0.15 ± 0.22	0.17 ± 0.13	0.98	0.25	0.88
Coherence	0.66 ± 0.13	0.60 ± 0.15	0.73 ± 0.15	0.57 ± 0.19	0.002*	0.81	0.19
CBFv Power (cm.s^2)	458 ± 242	374 ± 173	182± 97	174 ± 106	0.04*	0.01*	0.79
BP Power (mm Hg^2)	657 ± 351	417 ± 132	408 ± 256	359 ± 173	0.01*	0.13	0.20

Values are mean ± SD. Abbreviations: $P_{ET}CO_2$; end tidal volume of CO_2, CVR_{CO2}; cerebrovascular reactivity to CO_2; $CVR_{CO2MAP;}$ cerebrovascular reactivity to CO_2 (MAP accounted) BP, blood pressure. * Denotes significant difference (P<0.05).

Table 3.3. FMD data for participants in the older and younger groups during thermoneutral and cold conditions.

Characteristics	**Young Group** (n=12)		**Older Group** (n=6)		**P-value**		
	THERMONEUTRAL	**COLD**	**THERMONEUTRAL**	**COLD**	**Temp**	**Age**	**Temp*Age**
Artery diameter (cm)	0.34 ± 0.08	0.34 ± 0.08	0.41 ± 0.09	0.42 ± 0.09	0.77	0.12	0.35
Time to Peak (secs)	60.8 ± 28.6	66.2 ± 41.2	61.6 ± 14.1	67.1 ± 29.5	0.64	0.93	0.99

Values are mean ± SD.

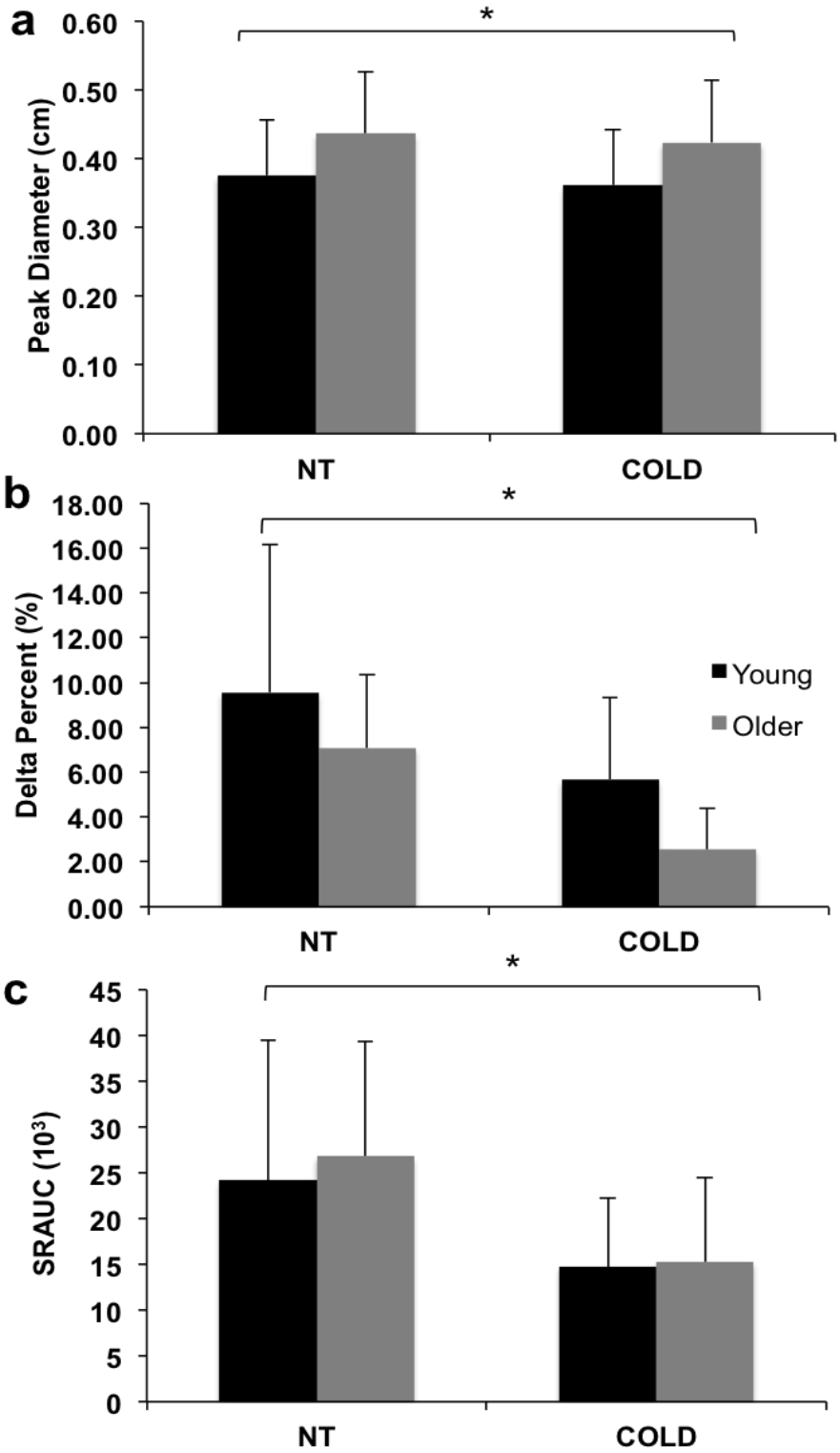

Figure 3.4. Peripheral artery diameter (a), flow mediated dilatation % (b) and shear rate area under the curve (c) for young and older individuals during thermoneutral and following an acute cold stress under cold conditions. * denotes significant main effect of temperature from thermoneutral to cold. Abbreviations: NT; thermoneutral, SRAUC shear rate area under the curve

3.3.3 Vascular and haemodynamic responses during an acute cold stress

3.3.3.1 Temperature Responses

Skin temperature significantly reduced by 6.2°C (-6.9, -5.4) from thermoneutral throughout the acute cold stress (main effect of temperature; P<0.001) with no main effect of age or temperature*age interaction (Table 3.4, P>0.05). Despite this reduction in skin temperature, core temperature increased by 0.04°C (-0.10, 018, main effect of temperature; P<0.001). There was a significant group*temperature interaction effect for core temperature with older individuals displaying significantly lower core temperatures at 10, 15, 20, 25, 30 minutes of the acute cold stress compared to the younger individuals (Figure 3.4, P<0.001). Combining these temperatures, mean body temperature significantly reduced by 5.5°C (-6.2, -4.8) from thermoneutral throughout acute cold stress (main effect of temperature; P<0.001) with no main effect of age or temperature*age interaction (Table 3.4, P>0.05).

3.3.3.2 Cerebral blood flow velocity and haemodynamics

There was a main effect of age with the older individuals displaying 8.4cm.s^{-1} (-16.9, -0.1) reduction in MCAv compared to younger individuals (Figure 3.5, P=0.05). There was a main effect of temperature as MCAv decreased from thermoneutral temperature throughout the acute cold stress (P<0.001). There were no main effect of age or temperature*age interaction effects (Figure 3.5, P>0.05).

Mean arterial pressure increased from the thermoneutral condition throughout the acute cold stress (main effect of temperature; P<0.001) and was significantly higher in the older compared to younger individuals (13 mmHg [7, 18], Figure 3.5; P<0.001). There was no temperature*age interaction effect (Table 3.5, P>0.05).

MCA cerebrovascular conductance (CBVC) significantly decreased during the acute cold stress (main effect of temperature; P<0.001) and was 0.16 cm.s^{-1}/mmHg^{-1} lower in the older individuals compared to younger individuals (-0.26, -0.01; Figure 3.5, main effect of age; P=0.005). There were no temperature*age interaction (P>0.05, Figure 3.5). MCA

cerebrovascular resistance (CBVR) significantly increased during the acute cold stress (main effect of temperature; P<0.001) and was 0.54 $mmHg^{-1}/cm.s^{-1}$ (0.20, 0.90) higher in older individuals compared to younger individuals (main effect of age; P=0.004, Figure 3.5,). There were no temperature*age interactions (P>0.05, Figure 3.5).

3.3.3.3 Skin Blood Flow

There was no main effect of temperature, age or temperature*age interaction on CVC at the arm and chest (Table 3.5, P>0.05).

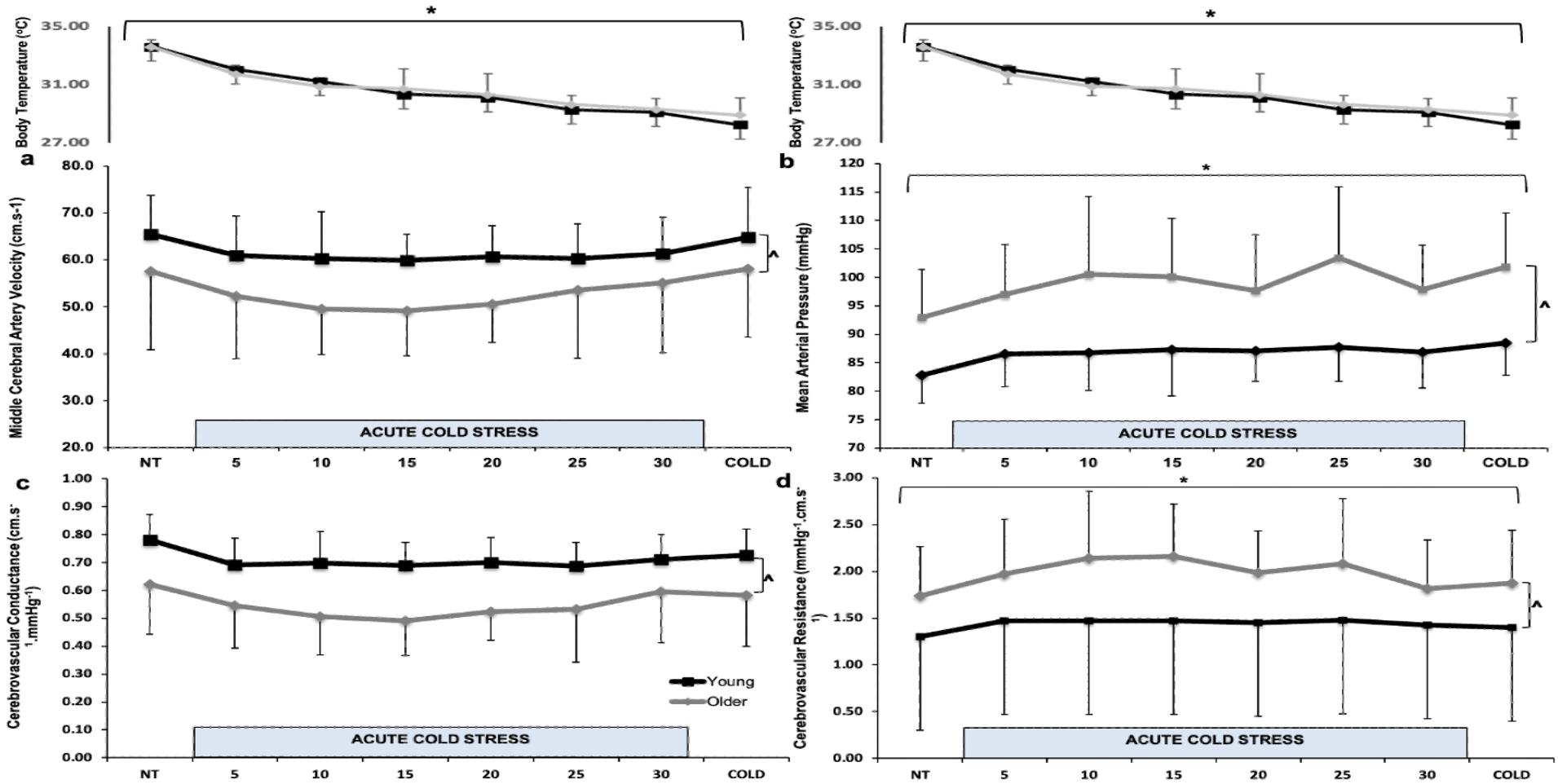

Figure 3.5. The responses of body temperature, resting middle cerebral artery velocity (a), mean arterial pressure (b), cerebrovascular conductance (c) and cerebrovascular resistance (d), in a thermoneutral condition, during 30 minutes of acute cold stress and in a cold condition. ^ denotes significant main effect of age (P<0.05). * denotes significant effect of temperature (P<0.05).

Table 3.4. Skin blood flow and temperature responses before and after 30 minutes of cooling.

Characteristics	Group	NT	5	10	15	20	25	30	COLD	P-value Temp	P-value Age	P-value Temp*Age
CVC Arm (PU/mmHg)	Young	0.27±0.11	0.32±0.09	0.39±0.41	0.40±0.39	0.32±0.34	0.34±0.27	0.29±0.27	0.22±0.13	0.18	0.71	0.51
	Older	0.34±0.22	0.27±0.16	0.33±0.31	0.30±0.22	0.27±0.20	0.28±0.21	0.21±0.11	0.23±0.14			
CVC Chest (PU/mmHg)	Young	0.56 ± 0.50	0.86±0.86	0.73±0.81	0.87±0.68	0.63±0.61	0.60±0.47	0.55±0.46	0.46±0.40	0.68	0.79	0.17
	Older	0.86±0.29	0.68±0.20	0.64±0.18	0.65±0.19	0.69±0.26	0.59±0.17	0.61±0.34	0.69±0.34			
Core Temperature (°C)	Young	37.0±0.2	37.3±0.3	37.4±0.3	37.4±0.3	37.4±0.3	37.4±0.3	37.4±0.3	37.1±0.3	<0.001*	0.008*	<0.001*
	Older	36.8±0.2	36.9±0.1	37.0±0.1	37.0±0.1	37.1±0.1	37.1±0.2	37.1±0.1	36.9±0.2			
Skin Temperature (°C)	Young	32.9±0.5	31.3±1.5	30.3±1.8	29.5±1.6	28.8±1.5	28.4±1.4	27.9±1.4	26.9±1.4	<0.001*	0.35	0.92
	Older	32.8±0.4	30.8±1.0	29.6±0.5	28.9±0.4	28.3±0.3	27.9±0.3	27.5±0.4	26.5±0.5			
Mean Body Temperature (°C)	Young	33.3±0.5	31.8±1.5	31.0±1.6	30.1±1.5	29.7±1.3	29.1±1.2	28.9±1.2	28.0±0.9	<0.001*	0.34	0.96
	Older	33.2±0.4	31.4±0.9	30.3±0.5	29.7±0.4	29.2±0.3	28.8±0.3	28.4±0.4	27.6±0.4			

Values are mean ± SD. Abbreviations: NT; thermoneutral; Temp: temperature; MCAv; middle cerebral artery velocity, MAP; mean arterial pressure; LDF; laser Doppler flux. CVC; cutaneous vascular conductance.* Denotes significant difference (P<0.05).

3.4 Discussion

The aim of this study was to examine the effects of cold stress on cerebral and peripheral vascular function and to examine cerebral, conduit and skin micro-vascular responses during an acute cold stress in young and older individuals. The results of the current study suggest an acute bout of cold stress did not alter measurements of cerebrovascular function but reduced brachial artery endothelial function in both age groups. The lower FMD was characterised by a lower shear rate and reduced peak diameter, in response to flow mediated dilation assessment. Moreover, in contrast to the hypothesis, MCAv did not increase during cold stress but displayed a similar MCAv response during cold stress in both age groups. Taken together, the findings indicate that cold stress did not mediate changes in cerebrovascular function and acute cold stress is unlikely to be a risk to cerebrovascular haemodynamics with healthy aging.

The main finding from this study was that following an acute cold stress, MCAv, nor cerebrovascular function were altered. The lack of increase in MCAv was somewhat surprising given that previous research studies suggested CBFv increases during a bout of cold stress (Doering et al., 1996; Brown, Sanya and Hilz, 2003) and improves orthostatic tolerance (Wilson et al., 2002; Durand et al., 2003). In the current study MCAv demonstrated a trend for an initial decrease, with a gradual increase towards pre-cooling levels towards the end of the 30 min cooling period. Differences in experimental design between the current and previous studies may explain the lack of observed increase in MCAv. Firstly, the duration of cold stress in the current study was 30 mins, which was much longer than previous studies examining an acute cold stress (Wilson et al., 2002; Durand et al., 2003) or in other studies cold stress was used in conjunction with passive heating (Wilson et al., 2002). Whilst a short and brief increase to MCAv in response to cold is plausible, examination of the data during the first minute of cooling in the current study did not show evidence of an increase in MCAv ($P<0.05$) (Appendix 7.2). Secondly, a water-perfused suit

was also employed to mediate whole body skin surface cooling rather than facial cooling or local cooling such as the cold pressor test (Brown, Sanya and Hilz, 2003; Fluck et al., 2017). It has been suggested that MCAv increases are a consequence of direct cooling of the head/face skin surface, which is extremely thermally sensitive, containing the trigeminal nerve (Brown, Sanya and Hilz, 2003). However, a recent study displayed no differences in MCAv during facial cooling or a cold pressor test under poikilocapnic conditions, whereas an increase in CBFv was displayed during a cold pressor test under isocapnic conditions (AlSalahi et al., 2020). This suggests a likely hyperventilation-induced fall in $P_{ET}CO_2$ blunts an increase in CBF during the cold pressor test. In the current study, despite $P_{ET}CO_2$ being unchanged following the acute cold stress, this was not measured throughout the cooling period. Initiation of whole body cooling may have caused hyperventilatory induced hypocapnia, supported by the initial reduction in cerebral perfusion and paralleled increase in CBVR, gradually returning to pre-cooling levels. Finally, the comparison in the current study was to thermoneutral body temperatures rather than hyperthermic temperatures (Doering et al., 1996). Passive heating has been shown to increase cerebrovascular resistance causing sympathetically mediated cerebral vasoconstriction (Wilson et al., 2006) and reducing MCAv (Wilson et al., 2006; Fan et al., 2008), therefore, it is difficult to make comparisons regarding the differences in MCAv responses (Doering et al., 1996). Taken together, the current data suggests MCAv does not increase in response to prolonged whole body skin cooling thus cold stress applied to the body in this manner is not a mechanism to increase cerebral blood flow.

Given the lack of change in MCAv in response to acute cold, the lack of change in cerebrovascular function might seem unsurprising. There are few previous studies examining the impact of cold on cerebrovascular function. In a study by Doering et al. (1999) dCA was assessed by autoregulatory index in response to cooling vs. hyperthermia using water immersion. Whilst in that study they observed that reducing core temperature by 0.3^{o}C using cold water immersion decreased dCA, a recent study examining the impact of

the cold pressor test, brief aggressive cooling of one limb (that reduced just skin but not core temperature), found no change in dCA in healthy young men (Washio, Watanabe and Ogoh, 2020a). Despite this, the differences in measurement techniques of autoregulatory index and rate of regulation make it difficult to make direct comparisons with these previous study findings. The current data herein suggests that the cold has negligible impact on the underlying control mechanisms of CBFv in the young or old and is unlikely to contribute to increased risk of cerebrovascular function and/or stroke onset observed with reductions in environmental temperature (Hong et al., 2003; Lichtman et al., 2016). It is important to note that the data providing evidence of increased cardiovascular and cerebrovascular morbidity and mortality during periods of low environmental temperatures have come from case-crossover studies that have not directly measured the responses of cerebral flood flow or cerebrovascular function to an acute cold stress but how incidence is elevated during winter seasons (Brennan et al., 1982; Hong et al., 2003; Gasparrini et al., 2015; Manou-Stathopoulou et al., 2015; Bunker et al., 2016). The data in the current study was collected throughout the spring and summer months in the United Kingdom (March – August), to limited the potential impact of seasonal variation (Widlansky et al., 2007), however the underlying mechanisms of CBFv and cerebrovascular function in response to cold during changing seasons warrants further investigation.

The cold stress did have an impact on peripheral vascular function. Brachial artery flow mediated dilatation was lower in the cold when compared to thermoneutral and this was explained by a reduction in peak diameter and shear rate area under the curve in response to the 5 min limb occlusion. The lower shear rate suggests there is less stimulus for vasodilation during cold stress and together with the higher MAP and decreasing skin temperature more central drive for vasoconstriction and reduced blood flow to the peripheral arteries during the cold stress. Another suggestion could be the provoked pattern of shear stress elicited by the cold stress. Short-term exposure to stressors such as an increase in sympathetic nervous activity can increase the pattern of retrograde shear, (ref Casey et al, 2012) that has been shown to result in a reduction in conduit artery endothelial function

(Thijssen et al., 2009; Tinken et al., 2009; Schreuder et al., 2015a). Nevertheless, the data within the current study focused on mean shear rate and therefore, the impact of skin surface cooling on the pattern of shear rate warrants further investigation. In support of the current FMD data, this could also support data that displays an association of endothelial function with season, FMD was observed to be lowest during the coldest outdoor temperatures (Widlansky et al., 2007). Mechanistically, there is some evidence that prolonged skin surface cooling induces increases in Rho Kinase, down regulating endothelial nitric oxide synthase and resulting in a decrease in nitric oxide production (Thompson-Torgerson, Holowatz and Kenney, 2008). Regardless of the mechanism, the data suggests prolonged skin surface cooling impacts on peripheral conduit arteries. The cold stress did not, however, have the same effect on the central conduit arteries. During the CVR_{CO2} assessment, carotid diameter at rest and diameter in response to inhalation of CO_2 was measured continuously and were unaffected by temperature. Thus, cold stress likely affects the peripheral and central conduit arteries differently. This has been observed in previous studies where carotid and coronary arteries respond differently in response to sympathetic activation induced by the cold pressor test and lower body negative pressure (van Mil et al., 2018) compared to the peripheral brachial artery (Dyson, Shoemaker and Hughson, 2006). Carotid and coronary artery responses to the cold pressor test display a moderate-to-strong correlation, dilation in healthy individuals or constriction in those with coronary artery disease (van Mil et al., 2017; van Mil et al., 2018), whereas peripheral conduit arteries have displayed negligible changes in diameter (Lind, Johansson and Hall, 2002; Dyson, Shoemaker and Hughson, 2006). Furthermore, recent work has also identified that there is no significant relationship between the endothelium-dependent diameter responses of the internal carotid arteries and brachial arteries (Carr et al., 2020), highlighting that physiological challenges imposed on different vascular beds may not elicit comparable adaptation.

The experimental design of the current study included younger and older individuals with distinct differences in their cerebrovascular and cardiovascular haemodynamics including lower resting MCAv (Yazici, Erdogmus and Tugay, 2005; Ainslie et al., 2008; Murrell et al., 2013), higher resting blood pressure (Bots, Grobbee and Hofman, 1991) and larger resting carotid diameters (Yazici, Erdogmus and Tugay, 2005; van Mil et al., 2017) compared to younger individuals. Furthermore, although not significant, the current data supports some previous data that CVR_{CO2} is lower in older individuals (Bailey et al., 2013b; Barnes et al., 2013), whereas when this was controlled for blood pressure (CVR_{CO2MAP}) these potential age differences were no longer evident. This could support the notion that blood pressure can have an impact on CVR_{CO2} and when comparing individuals with differing resting blood pressure, this should be accounted for. Despite this, the older individuals displayed similar dynamic CA compared with younger individuals (van Beek et al., 2008) and neither measures of cerebrovascular function were affected by temperature, highlighting that despite key differences in resting cerebrovascular and cardiovascular haemodynamics with aging, cold stress has no detrimental effect on cerebrovascular function, irrespective of age. Ageing is associated with decreasing thermoregulatory efficiency, as vasoconstriction and shivering, two imperative responses that protect individuals during cold stress are less effective in older individuals (Frank et al., 2000). When exposed to mild cold, older individuals fail to maintain core temperature compared to younger individuals with similar anthropometric characteristics (Degroot and Kenney, 2007). Although, in the current study the older group did not display an inability to maintain core temperature, despite displaying lower core temperatures overall and cutaneous vascular conductance was similar with ageing. It is important to outline that the method of whole-body skin surface cooling that was employed in the current study was sufficient in reducing skin temperature, as mean skin temperature decreased by 6.2^{o}C from thermoneutral to cold, similarly in both young and older individuals. The technique of a water-perfused suit cools the torso and limbs but does not directly cool the head and face. But despite the observed decrease in skin temperature, an increase in core temperature during the acute cold stress was evident. A

transient rise in core temperature at the start of a cold stress with reductions in core temperature occurring after 60 minutes has been reported previously, speculatively due to warm blood at the periphery being redistributed back to the core (Tikuisis, 2003). Moreover, detriments in core temperature were observed at more extreme cold stress temperatures than used in the current study, such as cold air ranging between 5-10^{o}C (Collins et al., 1985; Collins et al., 1995) and often caused shivering. The method used in the current study aimed to not severely challenge the thermoregulatory system but to reduce skin temperature without initiating the shivering response. Therefore the methodology of eliciting a cold stress could provide a rationale for the differences between the current data and other studies using cooling to the face (Brown, Sanya and Hilz, 2003), and immersion of a limb into cold water (Washio, Watanabe and Ogoh, 2020b).

It is important to acknowledge a number of limitations associated with the present study. Whilst the data takes into consideration both younger and older individuals it was limited to individuals who were healthy and free from any cardio and cerebrovascular diseases. Acute and chronic cold exposure can affect cardiovascular responses and can be modified by cardiovascular disease, potentially increasing the risk of a cardiovascular event (Figure 2.3). Therefore, it is important to investigate this in individuals with CVD risk factors for and with cardiovascular disease. Secondly, TCD was used to assess blood velocity rather than blood flow as TCD does not allow visualisation of arterial diameter. Research evidence has suggested that MCAv is a reliable index of cerebral blood flow if the insonated vessel maintains a constant diameter across experimental conditions and it is assumed that the dilation of extra-cranial arteries matches the dilatation in the cerebrovasculature (Smith et al., 2019b). Due to their being no effect of temperature on carotid artery diameter in the current study it was assumed that the MCA diameter remained constant. Another consideration to the rise in core temperature observed in the current study is the order at which measurements were taken. Thermoneutral core temperature was measured prior to the participants performing squat to stands for a dCA assessment. Therefore, we anticipate

that the core temperature rise could be at least partly explained by an acute bout of exercise performed prior to cooling.

In summary, the results of this study suggest that an acute cold stress, that reduced skin temperature but not core temperature has little impact on MCAv and measurements of cerebrovascular function in the young or older individuals. Whilst cold stress did cause a reduction in peripheral artery endothelial function, this response was similar in both age groups. Therefore, cold stress is unlikely to be a risk to cerebrovascular haemodynamics with healthy ageing, although the impact of more severe acute cold stress warrants further investigation.

Chapter 4

Study 2

Exercise training in the cold improves cerebral autoregulation more than exercise training in a thermoneutral environment in young healthy individuals

4.1 Introduction

Cerebral blood flow declines with age (Stoquart-ElSankari et al., 2007) and reductions in CBF are associated with clinical conditions including stroke (Markus et al., 2004), cognitive impairment (Benedictus et al., 2017) and Alzheimer's disease (Kisler et al., 2017). Higher cardiorespiratory fitness is associated with an increased CBFv across a broad age range (Ainslie et al., 2008; Brown et al., 2010; Bailey et al., 2013a), thus exercise may be a useful non-pharmacological intervention for offsetting age-related CBFv reductions and ultimately improving cerebrovascular health. Research studies examining the impact of exercise interventions on CBFv have however, yielded contrasting results. For example, 8-weeks of moderate intensity exercise increased CBFv in post-menopausal women (Akazawa et al., 2012), whilst 8-12 weeks of exercise did not alter CBFv in healthy young or older individuals (Murrell et al., 2013; Lewis et al., 2019c). Further, 6 weeks high intensity interval training mediated a small attenuation in dynamic cerebral autoregulation (dCA) (Drapeau et al., 2019a) whereas moderate intensity endurance exercise training improved cerebrovascular reactivity (CVR_{CO2}) in both healthy older and younger individuals (Murrell et al., 2013) and also in stroke survivors (Ivey et al., 2011), but not in those with chronic obstructive pulmonary disease (Lewis et al., 2019c).

During an acute bout of sub-maximal continuous exercise, CBFv increases linearly with exercise intensity from rest up to ~60-70% of maximal oxygen uptake (Moraine et al., 1993; Smith et al., 2014), although the magnitude of effect appears greater in younger individuals (Klein et al., 2019b). Increases in CBFv mediate elevations in shear stress to the cerebral vessel walls, likely resulting in shear mediated intracellular signalling cascades similar to that observed in the peripheral vasculature (Carter et al., 2016; Hoiland et al., 2017; Barnes and Corkery, 2018). In conduit arteries and micro vessels of the skin, episodic increases in shear stress provides the mechanical stimulus for enhanced vascular function and adaptation (Green et al., 2017). Increasing the exercise stimulus during an acute bout of

exercise (e.g., exercise intensity) or manipulating the environmental conditions may mediate larger blood flow increases, and thus shear stress responses, in specific vascular beds. This may enhance vascular adaptations to chronic exercise training. Acute cold exposure mediates several integrated cardiovascular responses, including, sympathetically mediated cutaneous vasoconstriction (Alba, Castellani and Charkoudian, 2019) and blood pressure increases (Modesti, 2013). The cutaneous vasoconstrictor response represents a primary mechanism to limit heat loss and maintain core temperature at a cost of reduced peripheral tissue temperature (Castellani and Young, 2016). In the cerebrovasculature, acute exposure to a cold stimulus or environment has elicited an increase in CBFv (Doering et al., 1996; Brown, Sanya and Hilz, 2003), although this was not evident from the findings in Chapter 3 that displayed no change in CBFv following 30 minutes of skin surface cooling. A contributing factor to this difference could be due to the cold stimulus used. The studies mentioned previously used direct cooling of the face (Brown, Sanya and Hilz, 2003), whereas the head and face was excluded from cooling in chapter 3. Furthermore, facial cooling during exercise has displayed an augmented MCAv response to a breath hold, independent of P_aCO_2 (Kjeld, Pott and Secher, 2009). Therefore, performing a bout of exercise in a cold environment, where the head and face will also be exposed, may cause a greater increase of CBFv relative to a normothermic environment and may translate to enhanced shear-stress mediated functional improvements in the cerebrovasculature.

The primary aim of this study was to assess the impact of 8 weeks of exercise training in a cold environment on CBFv and cerebrovascular function compared to exercise training in a thermoneutral environment. We also aimed to examine the acute effects of a single exercise bout in the different environmental conditions on CBFv.

4.2 Methods

4.2.1 Participants

Twenty-one (16 males & 5 females) physically active individuals who engaged in low to moderate intensity exercise 2-3 times per week were recruited. Participants were normotensive (BP <140/90 mm Hg), non-smokers with no history of cardiovascular disease. Female participants were initially assessed during the early follicular phase of their menstrual cycle. Two female participants recruited were currently taking hormonal contraception; these participants were also tested during the 7-day gap from hormonal contraception (Shenouda et al., 2018). All participants were informed of the methods verbally and in writing before providing written informed consent prior to any assessments being performed. The study conformed to the Declaration of Helsinki and was approved by the local university ethics committee (15/SPS/033).

4.2.2 Research Design

Participants attended a temperature-controlled laboratory (19-21^{o}C) for two experimental visits no more than 5 days apart having fasted overnight (12 hours), abstained from exercise for 24 hours and caffeine for 12 hours. Experimental visit 1 consisted of an assessment of CBF, measures of cerebrovascular function, brachial artery endothelial function and cutaneous microvascular function. Experimental visit 2 consisted of an incremental treadmill test to assess maximal cardiorespiratory fitness ($\dot{V}O_{2peak}$). Participants were then randomly assigned (computer generated sequence) to either 8-weeks of exercise training in a cold (5^{o}C, n=10) or a thermoneutral (15^{o}C, n=11) environment (Figure 4.1). Experimenters were blinded from the group allocations throughout recruitment, consent taking and initial visits until requesting the participants allocation from a member of the departmental technical team. Visits 1 and 2 were then repeated in the same order and at the same time of day following the intervention (Ainslie et al., 2007a; Jones et al., 2010) (Figure 4.2a; ***chronic experiment***). In addition, as part of their normal training intervention, participants attended

at the midpoint of the intervention for the examination of cerebral, conduit and cutaneous responses during an acute bout of exercise in their randomised environment (***acute experiment)*** (Figure 4.2b). Data collection took place during October to December to minimise the possible impact of seasonal variation in the measurements.

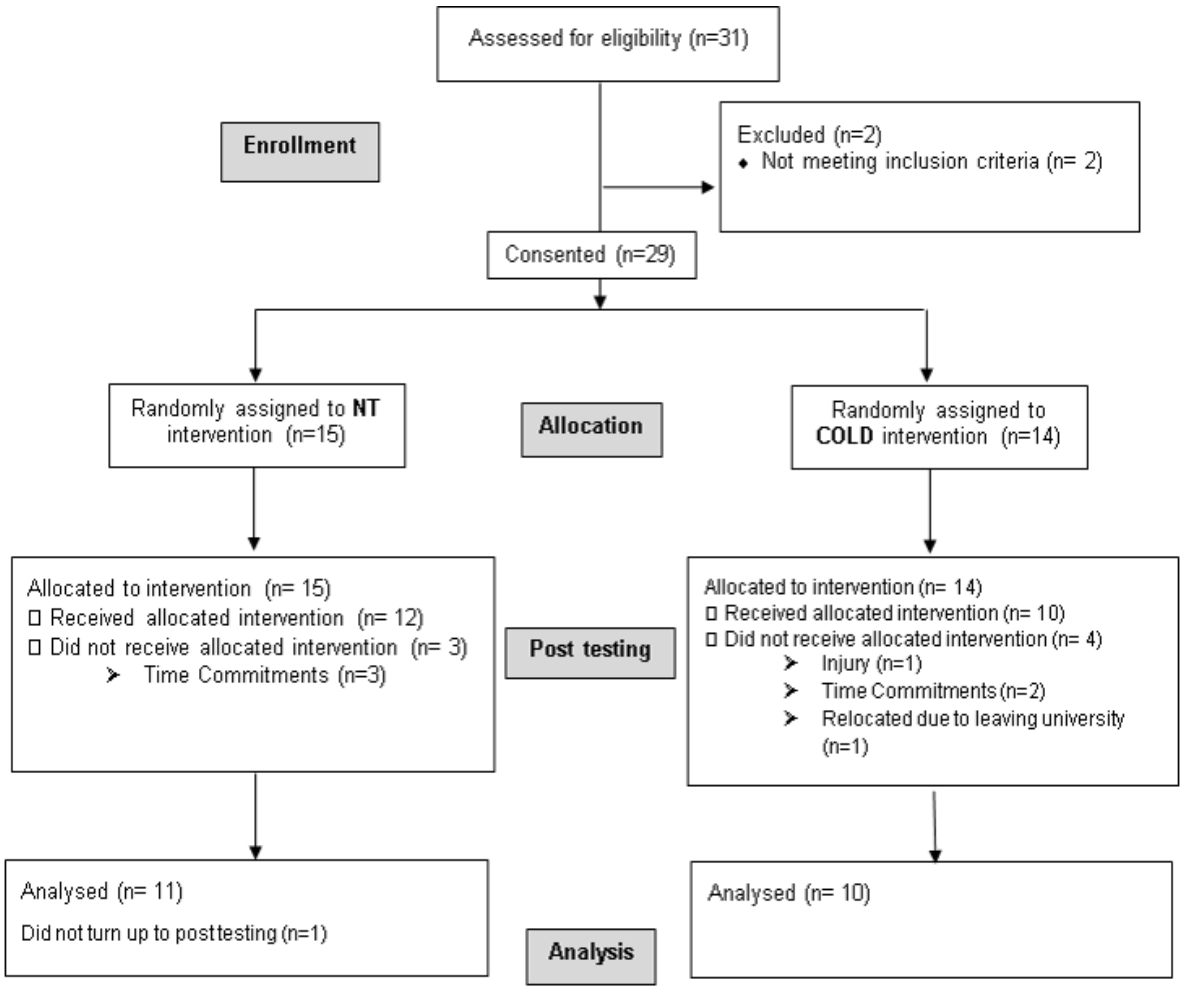

Figure 4.1. Flowchart of the study design. Abbreviations; NT; thermoneutral

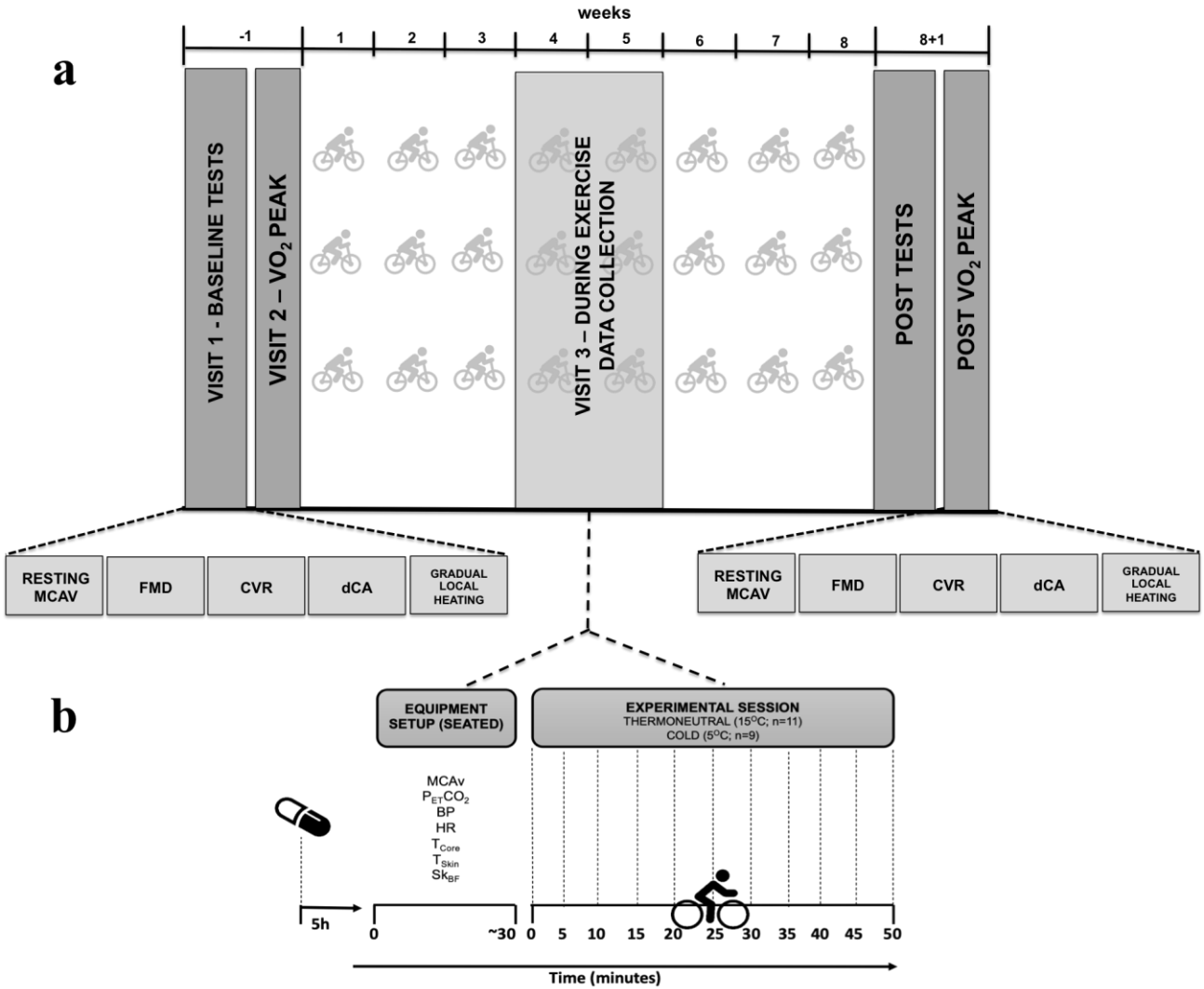

Figure 4.2. Schematic of experimental design (**a**) and experimental procedures of during exercise data collection (**b**). Abbreviations; MCAv; Middle cerebral artery velocity, FMD; flow mediated dilatation, CVR_{CO2}; cerebrovascular CO_2 reactivity, dCA; dynamic cerebral autoregulation, $P_{ET}CO_2$; Partial pressure end tidal carbon dioxide, BP; Blood Pressure, HR; Heart rate, T_{core}; Core temperature, T_{skin}; skin temperature & SkBF; skin blood flow.

4.2.3 Measurements

4.2.3.1 Chronic experiment (n=21): the effects of moderate intensity cycling exercise on cerebral, conduit and micro-vascular function in a cold vs neutral environment.

Following completion of experimental visits 1 and 2, participants cycled (Wattbike Pro/ Trainer, Wattbike, UK) at ~70% of maximum heart rate (HR_{max}), which was calculated from the cardiorespiratory fitness test, for 50 minutes, 3 times per week for 8 weeks. All exercise sessions were supervised by a member of the research team and took place within an environmental chamber (Sporting Edge & TISS, UK) that controlled for the temperature of the exercise sessions (thermoneutral; 15°C and cold; 5°C). Humidity was standardised across interventions at 20% relative humidity. Heart rate (FT1, Polar, Finland), rate of perceived exertion (Borg Scale, UK) and a measure of thermal comfort were recorded every 5 minutes throughout each exercise session.

Anthropometrics: Stature and weight were recorded to the nearest 0.1 unit using a stadiometer (SECA) and digital scales (SECA 701, SECA UK), respectively. All anthropometric measurements were taken three times and an average of the three were recorded.

Cardiorespiratory fitness: An incremental maximal exercise test was performed on a treadmill (Pulsar 4.0, HP Cosmos, Germany). After a 5-minute warm-up (8 $km.h^{-1}$), treadmill speed and incline gradient increased until volitional exhaustion. Breath by breath expired gases were measured (Oxycon Pro, Jaeger, Germany) for oxygen consumption ($ml.kg.min^{-1}$) and data were averaged over 15 second blocks. Maximum oxygen consumption was calculated as the highest consecutive 15-second period of gas exchange data occurring in the final minute before volitional exhaustion. HR was measured continuously using short range telemetry (FT1, Polar, Finland) alongside perceived exertion at each exercise stage (RPE; Borg Scale, (Borg, Hassmén and Lagerström, 1987)).

Brachial artery endothelial function: Following a minimum of 15 minutes supine rest, FMD was performed on the left arm of each participant as per the information in Chapter 3, Section 3.2.3, Measurements, *Brachial artery endothelial function.*

Cerebral blood flow velocity and cerebrovascular function: Measurements of CBFv, CVR_{CO2} *and dCA:* were completed as per the information in Chapter 3, Section 3.2.3, Measurements, *Cerebral blood flow velocity, Cerebrovascular reactivity to* CO_2 and *Cerebral autoregulation.*

Cutaneous microvascular function: Cutaneous flux was assessed using laser Doppler flowmetry (LDF) on the non-dominant arm and thigh; one 7-laser array probe was secured into a heating disc and attached to the skin. Skin sites were inspected for any abrasions or skin damage that may affect cutaneous blood flow responses and measurement sites were chosen, avoiding visible veins and hair follicles (Cracowski et al., 2006b). The placement of these probes was recorded to ensure accuracy of placement for the repeat measurement. Following placement and resting measurements (15-minutes at 33^{o}C), the heating discs were gradually heated using an incremental protocol up to 44^{o}C (33^{o}C to 42^{o}C; 1^{o}C every 5-minutes, then 30-minutes at 42^{o}C and 20-minutes at 44^{o}C) (Black, Green and Cable, 2008a; Roberts et al., 2017). Mean arterial pressure (MAP) and heart rate (HR) data was measured from brachial auscultation using an automated syphygmanometer (Dinamap V100, Germany) on the non-dominant arm every 5-minutes throughout. Cutaneous vascular conductance (CVC) was assessed by the ratio of LDF FLUX/ MAP. CVC was also calculated as a percentage of max ($CVC\%_{max}$ = (CVC/CVC_{max}) x100).

4.2.3.2 Acute experiment (n=21): the effects of acute exercise on cerebral, conduit and cutaneous micro-vascular responses.

Participants underwent measurements of MCAv, $P_{ET}CO_2$, BP, HR, T_{sk}, T_c, SkBF and radial artery diameter and velocity during a single exercise session at the midpoint of the training programme. The assessments were performed in the environmental condition to which the individual had been allocated (n=10 cold and n=11 thermoneutral). Measurements were performed pre exercise and every 5-minutes during the 50-minute exercise bout, excluding $P_{ET}CO_2$ and radial artery diameter and velocity that were assessed prior to exercise and every 10-minutes throughout the 50-minute exercise (Figure 2b).

Measurements: MCAv, $P_{ET}CO_2$ were measured as per the information in Chapter 3, Section 3.2.3, BP was monitored from the brachial auscultation using an automated syphygmanometer (Tango, SunTech, England) and HR was measured continuously using short range telemetry (FT1, Polar, Finland).

Core body temperature was measured from an ingestible pill telemetry system and mean skin temperature was obtained from the weighted average of 4 regional thermocouples as per information in Chapter 3, Section 3.2.4, *Temperature Measurements*. Core and skin temperature were analysed during baseline measurements and every 5 minutes during the acute bout of exercise.

Skin blood flow was assessed using Laser Doppler Flowmetry at both the right forearm and thigh of each participant as per the information in Chapter 4, Section 3.2.3, CVC was calculated every 5-minutes during the 50-minute exercise and values were also expressed as percentage increases from the baseline [Percentage change (%) = (time point value – baseline value)/(baseline value)*100].

Radial artery diameter and velocity was recorded from the middle third of the forearm using a 15-MHz multi-frequency linear array probe, attached to a high-resolution ultrasound machine (T3300; Terason, Burlington, MA). Images were acquired in accordance with previous methodological guidelines, including ultrasound measurements being acquired by the same sonographer (Thomas et al., 2015) and were analysed as in the same way as brachial artery diameter as per information in Chapter 3, Section 3.2.3, *Brachial artery endothelial function*. Images of the radial artery were obtained every 10 minutes during the acute bout of exercise.

4.2.4 Statistical Analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS version 23.0, Chicago, IL). Between-group participant characteristics were explored using independent samples t-tests. Fishers exact tests were used to compare the between group proportions of males and females between groups. To examine the changes with exercise training (i.e. not stratified by training environment) two-factor linear mixed models (intervention*time) were employed to analyse resting haemodynamic variables, fitness, and brachial artery and cerebrovascular function. Three-way linear mixed models (intervention*time*temperature) were employed to analyse cutaneous microvascular function. Changes following exercise (pre- vs. postexercise) were examined using student's paired t tests. Covariance structure for each model was determined using a chi-square distribution, with the selection determined by the most parsimonious structure that optimised model fit. To examine the changes during an acute exercise session two-way linear mixed models were employed with group (cold vs thermoneutral) and time (rest, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 minutes) as fixed effects. Statistically significant interactions between were followed-up with the least significant difference (LSD) approach to multiple comparisons (Perneger, 1998). Distribution data are presented as mean±SD and

outcomes of linear mixed models as mean (95% CI). Statistical significance was assumed at P<0.05.

4.3 Results

4.3.1 Participant Characteristics

At baseline, there were 21 participants with a mean age of 22 ± 5 years, BMI of 23.0 ± 2.5 kg/m^2 and VO_{2peak} of 50.7 ± 7.6 $ml^{-1}.min.kg^{1}$. There were no significant differences between the groups at baseline in age, body mass, BMI, MAP or VO_{2peak} (P>0.05, Table 4.1).

4.3.2 Chronic experiment (n=21): the effects of moderate intensity cycling exercise on cerebral, conduit and cutaneous micro-vascular function in a cold vs neutral environment.

Adherence to the 24-session exercise intervention was 98 ± 3% in thermoneutral group and 99 ± 2% in the cold group (P=0.51). The efficacy of the training intervention is evident by a decreased resting HR by 4 $beats.min^{-1}$ (-7, -1; P=0.03) and improved VO_{2peak} by 2.91 $ml.min.kg^{-1}$ (0.49, 5.3; P=0.02) after 8 weeks of exercise training, changes that were similar in both groups (P>0.05, Table 4.1). The exercise interventions did not alter body mass, BMI, SBP, DBP or MAP (P>0.05, Table 4.1).

Table 4.1. Descriptive characteristics of participants in the thermoneutral exercise and cold exercise-training group pre and post exercise intervention.

Characteristic	Thermoneutral Group (n=11)		Cold Group (n=10)		P-value			
	Pre	Post	Pre	Post	T-test (on entry)	Time	Group	Time*Group
Females	3		2		0.64	-	-	-
Age (years)	22±5		22±5		0.84	-	-	-
Body mass (kg)	66.4±5.1	66.2±5.1	72.8±12.5	72.2±12.1	0.18	0.26	0.16	0.43
BMI (kg/m^2)	22.3±1.5	22.2±1.6	23.6±3.2	23.4±3.0	0.23	0.44	0.20	0.43
SBP (mmHg)	118±11	114±8	122±10	120±12	0.44	0.17	0.28	0.66
DBP (mmHg)	63±5	64±6	63±5	62±6	0.82	0.96	0.57	0.49
MAP (mmHg)	86±7	83±5	84±6	83±7	0.68	0.13	0.93	0.47
Resting HR ($beats.min^{-1}$)	62±8	57±6	57±9	56±6	0.24	0.03*	0.19	0.20
Absolute VO_2 ($l.min^{-1}$)	3.4±0.5	3.6±0.7	3.6±0.6	3.8±0.7	0.54	0.03*	0.36	0.96
Relative VO_2 ($ml^{-1}.min.kg^{1}$)	50.9±6.9	53.8±9.3	50.4±8.7	53.3±9.2	0.57	0.02*	0.90	0.99

Abbreviations: BMI; body mass index, SBP; systolic blood pressure, DBP; diastolic blood pressure, MAP; mean arterial pressure. *P<0.05.

4.3.2.1 Cerebrovascular Function

Cerebral blood flow and cerebrovascular reactivity: Resting MCAv, $P_{ET}CO_2$, MAP and absolute or relative gain of MCAv to CO2 inhalation were not altered by exercise training in either environment with no main effects or group*time interactions (P>0.05, Table 4.2). CVR_{CO2} was also studied in absolute and relative terms using CVR_{CO2MAP} to account the effects of MAP changes on CVR_{CO2} estimates. There were no effects of group, time or group*time interaction for absolute or relative gain of CVR_{CO2MAP} to CO_2 inhalation or MCA CBVC and MCA CBVR (P>0.05, Table 4.2).

Cerebral autoregulation: Normalised gain decreased by 0.192 %cm.s^{-1}.%mmHg^{-1} (95% CI =-0.318, -0.065; P=0.01) following exercise training in the cold and increased following exercise training in the thermoneutral environment [0.129 %cm.s^{-1}.%mmHg^{-1} (0.011, 0.248; P=0.04; group*time interaction; P=0.001], which were greater than the level of the MCID of 0.07 and 0.26. Absolute gain decreased by 0.124 cm.s^{-1}.mmHg^{-1} [-0.217, -0.031; P=0.01] following exercise training in the cold and was unaffected (0.016 cm.s^{-1}.mmHg^{-1}; 95% CI = -0.072, 0.103; P=0.71; intervention*time interaction; P=0.03) following exercise training in the thermoneutral environment. Phase increased by 0.072 radians [-0.007, 0.152; P=0.07)] following exercise training in the cold and decreased by 0.065 radians following exercise training in the thermoneutral environment (-0.144, 0.014; P=0.10; intervention*time interaction; P=0.02, Table 4.2; Figure 4.3). There were no main effects or intervention*time interactions for coherence (P>0.05, Table 4.2).

4.3.2.2 Peripheral vascular function

Brachial Artery Endothelial Function: Brachial FMD, peak response, time to peak (TTP) or shear rate area under the curve (SR_{AUC}) were not influenced by exercise itself nor when participants were stratified by environment, before or after allometric scaling (P>0.05; Table 4.3).

Cutaneous microvascular function: MAP was not different between groups (P=0.66) and did not significantly change following the exercise training in either environment (P=0.47). A significant main effect of time was evident in the forearm $\%CVC_{max}$ decreasing by 5% following exercise training (-9, -1; P=0.03) (Figure 4.4). Although not significant, a paired t-test displayed skin blood flow max (44^{o}C) at the forearm was 60 PU higher (-26, 147; P=0.16) and forearm CVC max was 0.49 PU/mmHg higher (-0.61, 1.59) following exercise training (P=0.36). There were no main effects of intervention*time or intervention*time*temperature interactions for $\%CVC_{max}$ at the arm (P>0.05). No significant main effects or interactions were evident for $\%CVC_{max}$ at the thigh (P>0.05).

Table 4.2. Cerebral blood flow, cerebrovascular reactivity and transfer function analysis outputs of dynamic cerebral autoregulation for participants in the thermoneutral exercise and cold exercise-training group pre and post exercise intervention.

Characteristic	Thermoneutral Group (n=11)		Cold Group (n=10)		P-value		
	Pre	Post	Pre	Post	Time	Group	Time*Group
Resting							
MCA CBVC ($cm.s^{-1}/mmHg^{-1}$)	0.74±0.11	0.76±0.10	0.80±0.13	0.79±0.10	0.95	0.22	0.68
MCA CBVR ($mmHg^{-1}/cm.s^{-1}$)	1.38±0.21	1.34±0.19	1.28±0.19	1.29±017	0.85	0.23	0.72
CO_2 Reactivity test							
Relative CVR_{CO2} (% $cm.s/mmHg^{-1}$)	4.4±2.1	4.1±2.1	4.0±2.4	3.5±3.0	0.55	0.56	0.82
Absolute CVR_{CO2} ($cm.s/mmHg^{-1}$)	2.8±1.2	2.6±1.6	2.6±1.8	2.5±2.1	0.79	0.82	0.94
Relative CVR_{CO2MAP} (%$cm.s/mmHg^{-1}$. $mmHg^{-1}$)	2.7±4.8	2.4±3.8 [n=10]	3.3±1.9	2.1±3.5 [n=8]	0.18	0.78	0.39
Absolute CVR_{CO2MAP} ($cm.s.mmHg^{-1}$. $mmHg^{-1}$)	1.0±0.6	0.8±0.3 [n=10]	0.9±0.2	0.9±0.2 [n=8]	0.41	0.79	0.50
Cerebral Autoregulation test							
Absolute Gain ($cm.s^{-1}/mmHg^{-1}$)	0.81±0.18	0.80±0.18	0.87±0.16	0.76±0.12	0.09	0.95	0.03*
Coherence	0.7±0.1	0.8±0.1	0.7±0.2	0.7±0.2	0.76	0.09	0.61
CBFv Power	214±104	182±95	201±132	176±131	0.08	0.61	0.28
ABP Power	322±193	250±88	346±149	280±128	0.03*	0.95	0.32

Abbreviations: MCA; Middle Cerebral Artery, CBVC; Cerebrovascular Conductance, CBVR; Cerebrovascular Resistance, CVR_{CO2MAP}; Cerebrovascular reactivity to CO_2, CVR_{CO2MAP}; Cerebrovascular reactivity to CO_2 (MAP accounted for).*$p<0.05$.

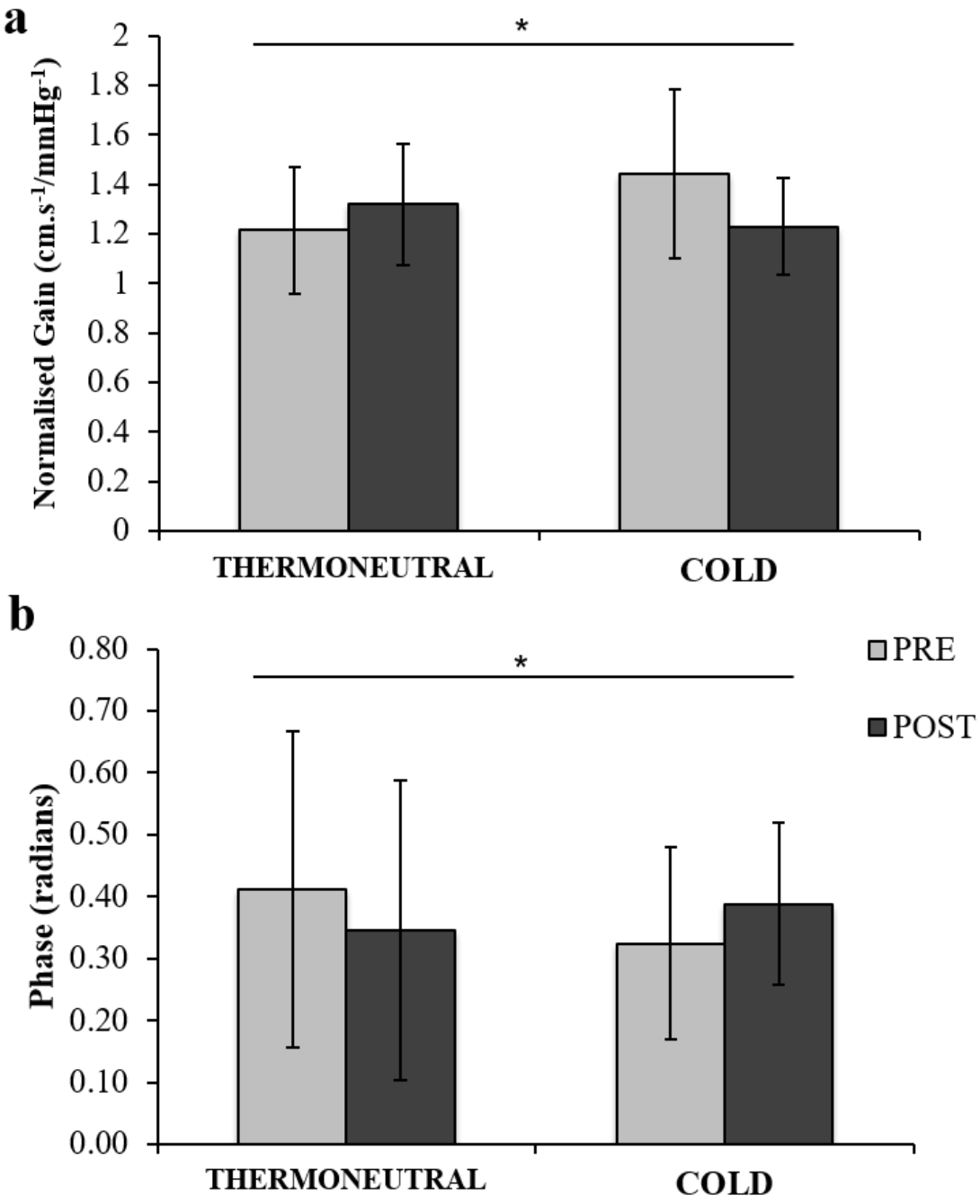

Figure 4.3. Normalised gain (a) and phase (b) for participants in the thermoneutral exercise and cold exercise-training group pre and post exercise intervention. * denotes significant interaction condition*time ($P<0.05$). Error bars represent SD.

Table 4.3. Vascular function for participants in the thermoneutral exercise and cold exercise-training group pre and post exercise intervention.

Characteristic	Thermoneutral Group (n=10)		Cold Group (n=7)		P-value		
	Pre	Post	Pre	Post	Time	Group	Time*Group
Artery diameter (mm)	3.6±0.7	3.6±0.6	3.8±0.7	3.8±0.7	0.91	0.50	0.92
Peak artery diameter (mm)	3.8±0.7	3.9±0.5	4.1±0.8	4.2±0.8	0.63	0.33	0.72
Time to Peak (secs)	47.6±16.8	46.2±13.5	50.0±14.4	43.9±15.1	0.50	0.98	0.66
SR_{AUC} ($x10^3$)	20.7±11.5	15.7±8.2	21.1±14.9	20.7±7.1	0.33	0.56	0.61
FMD (%)	6.7±2.8	6.8±4.4	6.6±2.6	8.0 ± 2.1	0.13	0.64	0.44

Abbreviations: SR_{AUC}; Shear rate under the curve, FMD; Flow mediated dilation.

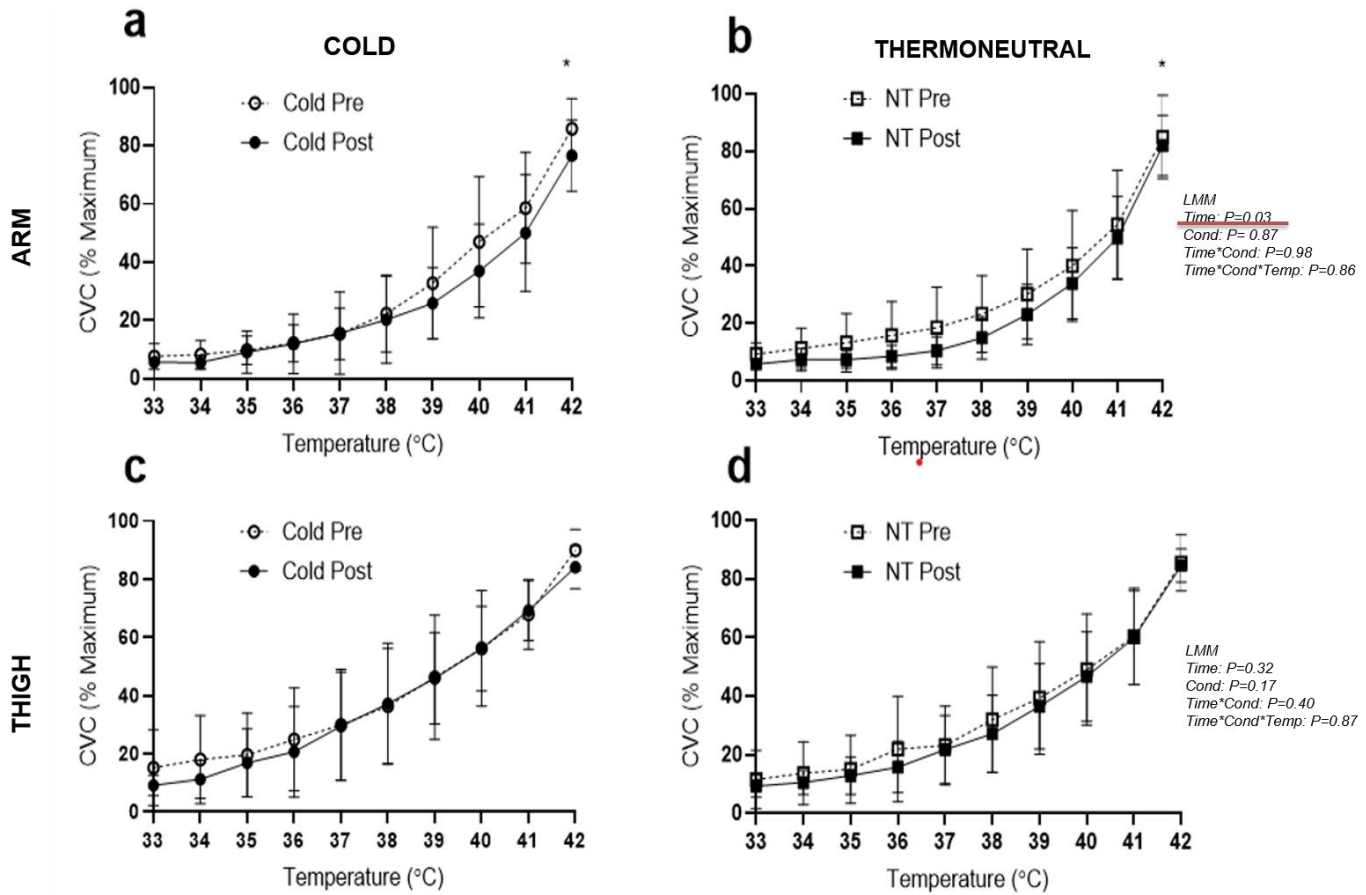

Figure 4.4. Cutaneous vascular conductance normalised to maximum CVC (%CVCmax) across time points (from baseline 33ºC to maximal plateau at 44ºC) following gradual local heating in the arm (a) and thigh (c) in the cold training group and arm (b) and thigh (d) in the thermoneutral training group. * denotes significant difference (P<0.05) from pre to post exercise intervention at time point. Error bars represent SD.

4.3.3 Acute experiment (n=21): the effects of acute exercise on cerebral, conduit and cutaneous micro-vascular responses.

Participants maintained their heart rate at approximately 70% HR_{max} (Thermoneutral; 138±6 beats.minute^{-1}, cold; 137±5 beats.minute^{-1}) throughout the exercise. There was no group*time interaction for adherence to target HR (P>0.05, Table 4.4). There was a trend for a lower power output in the NT group throughout the exercise (-27 watts [-55, 1], P=0.06) but there was no group*time interaction for power output (P>0.05, Table 4.4). Rating of perceived exertion for the exercise increased throughout the exercise session from 10±2 to 11±2 (P<0.001), but there was no between group differences (P>0.05, Table 4.4).

4.3.3.1 Cerebral blood flow velocity and haemodynamics

MCAv and $P_{ET}CO_2$ increased by 8.4 cm.s^{-1} and 9.1 mmHg during the exercise (P<0.001) but was there was no between group differences (Figure 4.5; P>0.05). MCA CBVC increased by 0.12 cm.s^{-1}/mmHg^{-1} (0.03, 0.21, P<0.01) and MCA CBVR decreased by 0.24 mmHg^{-1}/cm.s^{-1} (-0.39, -0.86; P<0.01) but there was no between group differences (Table 4.4).

4.3.3.2 Blood Pressure

SBP increased and DBP increased significantly (P<0.001) during exercise with no effect of group or group*time interaction (P>0.05, Table 4.4). However, MAP was not altered by exercise (P=0.73) or group allocation (P=0.56).

4.3.3.3 Temperature Responses

T_c increased by 0.68^{o}C (0.55, 0.80; P<0.001) during exercise, which was comparable across environmental settings (P>0.05, Figure 4.5). T_{sk} was lower at resting baseline and during all exercise time points in the cold compared to the thermoneutral temperature (-

4.76°C [-6.89, -2.63], P<0.001) (Figure 4.5). T_{sk} significantly decreased by 2.82°C (-4.03, -1.60) during exercise in the cold group but was unchanged during exercise in the thermoneutral group (0.69°C [-0.34, 1.73]) (group*time; P<0.001, Figure 4.5). Thermal comfort increased during the exercise (P<0.001) but was significantly lower in the cold group at every time point during exercise up until the 40-minute time point (group*time; P<0.001, Figure 4.5).

4.3.3.4 Skin Blood Flow

Forearm and thigh SkBF significantly increased during the exercise in both environmental groups (main effect of time; P<0.001), but was significantly lower in the cold group (main effect of group; P<0.05). There was a significant group*time interaction for forearm and thigh SkBF as the rate of increase in flux was higher in the thermoneutral group compared to the cold group (group*time; P<0.001, P=0.01 respectively). Congruent findings were observed when SkBF was expressed as CVC.

4.3.3.5 Radial artery responses

Radial artery diameter increased by 0.54 mm (0.28, 0.80; P=0.001), blood flow velocity increased by 15.3 $cm.s^{-1}$ {8.4, 22.1; P<0.001) and SR_{AUC} increased by 7.6 (4.1, 11.1; P=0.001) during exercise, comparable across environmental settings with no effect of group or group*time interaction (P>0.05). Radial artery blood flow was not altered by exercise (P=0.26) or group allocation (P=0.13) (Table 4.5).

Table 4.4. Vascular and haemodynamic responses during exercise in a cold or thermoneutral environment.

Characteristic													P-value		
	Group	REST	5	10	15	20	25	30	35	40	45	50	Time	Group	Time* Group
SBP (mmHg)	NT	125±9	159±15	143±44	150±24	158±13	159±16	158±14	151±15	151±14	161±25	148±27	<0.001*	0.94	0.90
	COLD	133±14	157±20	152±22	148±23	148±18	163±33	154±28	144±29	151±36	149±31	141±21			
DBP (mmHg)	NT	74±8	61±13	67±12	63±15	68±13	60±13	66±9	67±10	74±18	62±15	65±12	<0.001*	0.32	0.08
	COLD	75±9	61±13	58±15	57±6	63±14	63±19	61±11	65±9	57±14	60±11	63±7			
HR (beat.min^{-1})	NT	-	141±6	144±3	144±6	142±5	141±5	144±5	142±9	142±8	142±6	142±7	0.02*	0.004*	0.15
	COLD	-	129±9	137±8	136±6	134±7	138±8	138±7	139±6	139±7	139±7	139±7			
Watts	NT	-	118±23	116±25	112±21	111±21	109±21	110±20	103±22	105±20	100±22	97±26	0.04*	0.06	0.47
	COLD	-	140±37	138±37	137±39	135±48	135±40	135±41	135±41	133±43	131±37	130±40			
RPE	NT	-	10±2	10±2	10±2	11±2	11±2	11±2	11±2	11±2	11±2	11±2	<0.001*	0.79	0.90
	COLD	-	10±2	10±2	10±2	10±2	11±2	11±2	11±2	11±2	11±2	11±2			
CBVC (cm. s^{-1}.mmHg^{-1})	NT	0.67±0.11	-	0.79±0.21	-	0.72±0.13	-	0.72±0.14	-	0.72±0.09	-	0.76±0.12	0.003*	0.84	0.77
	COLD	0.65±0.10	-	0.77±0.18	-	0.79±0.15	-	0.81±0.12	-	0.79±0.13	-	0.77±0.12			
CBVR (mmHg^{-1}.cm.s^{-1})	NT	1.53±0.22	-	1.33±0.30	-	1.42±0.22	-	1.44±0.27	-	1.40±0.17	-	1.33±0.19	0.002*	0.85	0.74
	COLD	1.58±0.22	-	1.36±0.31	-	1.31±0.27	-	1.27±0.20	-	1.29±0.22	-	1.33±0.20			

Abbreviations: COND, Condition; NT, Thermoneutral; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; HR, Heart Rate; CBVC, cerebrovascular conductance.

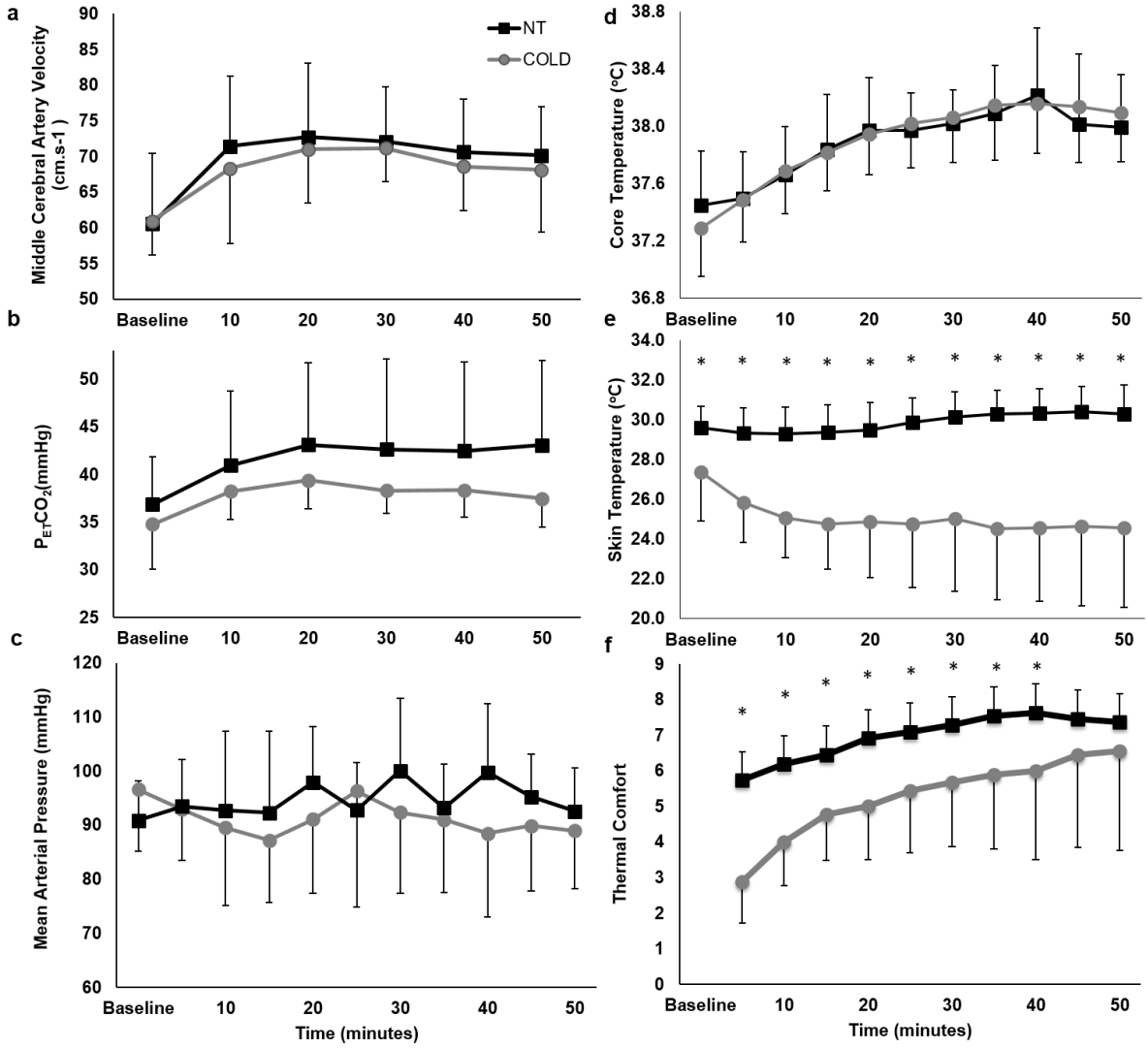

Figure 4.5. Cerebral artery velocity in the middle cerebral artery (MCA) (a), end tidal volume of CO_2 (b), mean arterial pressure (c), core temperature (d) skin temperature (e) and thermal comfort (f) in response to cycling in a thernoneutral environment vs cold environment (10-minute intervals). * denotes significant difference between groups at time point ($P<0.05$). Error bars represent SD.

Table 4.5. Radial artery responses during exercise in a cold (n=6) or thermoneutral (n=8) environment.

Characteristic								P-value		
		Baseline	10	20	30	40	50	Time	Group	Time*Group
Artery Diameter (mm)	**NT**	4.6±0.4	4.5±0.5	5.0±0.8	5.0±0.7	4.7±0.1	5.1±0.8	0.001*	0.27	0.23
	COLD	4.0±0.1	4.2±0.9	4.2±0.8	4.3±1.3	4.6±1.0	4.4±0.9			
Velocity ($cm.s^{-1}$)	**NT**	4.9±5.3	14.0±10.0	15.1±10.3	23.8±7.8	18.2±9.8	20.9±11.0	<0.001*	0.22	0.71
	COLD	2.1±1.9	6.7±7.5	13.1±7.2	21.7±11.9	17.1±8.6	13.9±9.3			
Flow ($ml.min^{-1}$)	**NT**	2.9±5.4	2.7±2.4	3.8±4.1	5.1±3.4	3.8±3.9	4.6±3.3	0.26	0.13	0.86
	COLD	0.3±0.3	1.1±1.2	2.0±1.4	4.0±2.5	2.9±1.7	2.2±1.5			
SR_{AUC} ($x10^3$)	**NT**	2.7±2.8	6.9±4.1	6.2±4.0	11.4±2.4	9.0±3.5	9.8±4.8	<0.001*	0.46	0.29
	COLD	1.6±1.3	2.2±1.1	6.3±3.9	12.1±5.0	9.6±6.3	9.6±6.0			

Abbreviations: SRAUC; shear rate area under the curve, NT; thermoneutral. *P<0.05.

4.4 Discussion

The aim of this study was to investigate whether the combination of exercise training in a cold environment could maximise the positive effects of exercise training on CBFv and cerebrovascular function compared to exercising in a thermoneutral environment. The novel finding from the study is that exercise training in the cold elicits an improvement in cerebral autoregulation compared to exercise training in a thermoneutral environment. It was also evident that changes in autoregulation with exercise training in the cold were not directly explained by greater CBFv or haemodynamics during acute exercise. Taken together, this data suggests that adding cold exposure during exercise could be a potential strategy to enhance or maintain cerebrovascular function that can occur with ageing and/or cerebrovascular disease and warrants investigation in these populations.

The impact of exercise training on cerebral autoregulation is limited with evidence of a small detriment in endurance trained males following high intensity interval training (Drapeau et al 2019) and no change following 8-weeks of aerobic training in chronic obstructive pulmonary disorder patients and in healthy aged-matched controls (Lewis et al., 2019c). The data from the current study suggests a small but significant improvement in dCA induced by exercising in a cold environment compared to exercising in a thermoneutral environment in young healthy individuals. Minimum clinical important difference for LF gain LF gain was between 0.07 and 0.26%cm s^{-1}%.$mmHg^{-1}$mm Hg/%. This was based on studies showing differences between healthy and diseased populations (van Beek et al., 2012; Lewis et al., 2019c) due to the limited intervention studies to date. Interestingly, the improvements in dCA were accompanied by enhancements in both normalised gain and phase indicating that cold exercise training improved both the magnitude of the CBFv response and temporal alignment to forced BP oscillations (van Beek et al., 2008; Claassen et al., 2016). The mechanism by which exercise training in the cold induced dCA improvements is difficult to establish and remains unclear, with research evidence indicating contributions from neurogenic, metabolic, myogenic and endothelial adaptation (Tzeng and

Ainslie, 2014). Whilst these findings are encouraging, the extent of adaptation in cerebral autoregulation across this 8-week exercise intervention is small and may have a limited physiological or clinical relevance in healthy individuals. Furthermore, more participants would have been required for adequate statistical power; therefore, the data should be interpreted with caution.

Interestingly, the function of peripheral conduit and skin vessels did not improve nor did CVR_{CO2}, all assessed by measurements that provoke endothelial mediated responses (Ainslie et al., 2007b; Pugh et al., 2013; Sprung et al., 2013; Carter et al., 2016; Hoiland et al., 2017; Thijssen et al., 2019b). The exact neural contribution to dCA is still debated (Ainslie and Brassard, 2014), and intriguingly the cold pressor test which is used to induce a large sympathetic response, did not alter dCA in a previous study (Washio, Watanabe and Ogoh, 2020a). From one previous exercise intervention study, Drapeau et al. (2019a) speculated that the observed change in dCA may have been as a result of transient changes in BP during the exercise, however BP was not monitored. Our acute exercise data suggests that this is unlikely to explain changes in dCA, given that the acute BP response, along with MCAv and MCA conductance and resistance responses, during exercise were not different between environmental temperatures. This highlights the brains' ability to effectively control brain blood flow under various demanding situations (exercise and environmental challenges). However, as continuous beat-to-beat BP monitoring was not used we cannot quantify the dynamics of this pressure-flow relationship of dCA. Indeed the acute dCA response to exercise is complex and poorly understood (Ogoh and Ainslie, 2009a) and to what extent the acute dCA response influences chronic adaptations warrants further investigation.

There was no observed change in CVR_{CO2} following either intervention. CVR_{CO2} reflects the capacity of blood vessels to dilate and represents a marker for brain vascular reserve (Yezhuvath et al., 2009). Additional mechanistic studies have identified the role of shear-

stress mediated vasodilation as a result of increased CO_2 during CVR_{CO2} assessment, suggesting CVR_{CO2} can be used as an indirect measurement of cerebrovascular endothelial function (Carter et al., 2016; Hoiland et al., 2017). Indeed, this observation of no change in CVR_{CO2}, which provides an index of cerebrovascular endothelial function, coincides with the data on endothelial function in conduit arteries and microvessels with no change apparent following either intervention in the young healthy study population. Thermoneutral exercise interventions have enhanced CVR_{CO2} in sedentary young and old individuals (Murrell et al., 2013) and stroke survivors (Ivey et al., 2011), but it is not a consistent finding (Tanne et al., 2005; Miller et al., 2018; Lewis et al., 2019c). A noteworthy observation from the current during exercise data was that the MCAv response in the cold environment did not differ from thermoneutral exercise, thus suggesting that the shear stress stimulus, which plays a major role in vascular adaptation (Green et al., 2017) and regulation of the CVR_{CO2} response (Carter et al., 2016; Hoiland et al., 2017), was not different between groups, potentially explaining this lack of change in CVR_{CO2}. Similarly, neither intervention mediated a change in resting CBFv. In thermoneutral environments, exercise training has been shown to increase resting CBFv in some (Akazawa et al., 2012; Alfini et al., 2019b) but not all studies (Murrell et al., 2013; Drapeau et al., 2019a; Lewis et al., 2019c). The characteristics of the recruited cohort (i.e. young, healthy and low-moderately active) may be a factor influencing our results, given that the aforementioned studies that demonstrated improvements in CVR_{CO2} (Murrell et al., 2013) and CBFv (Akazawa et al., 2012; Alfini et al., 2019a) were in older or individuals with overt disease. Additionally, exercise modality may also have impacted on CBFv outcomes, especially given recent evidence showing that interval exercise training elicits greater increases in CBFv compared to moderate continuous training (Klein et al., 2019b). The CBF response to exercise is bi-phasic, with CBFv increasing linearly with exercise intensity from rest up to ~60-70% of maximal oxygen uptake (Moraine et al., 1993; Smith et al., 2014), thereafter plateauing or even displaying a progressive decline, dependent on the level of hyperventilatiory-induced hypocapnia {Smith, 2017 #911}. Within the currrent study, participants were required to exercise at 70%

HR_{max} and therefore may have blunted the MCAv response as it could have been above the plateau threshold. Nevertheless, there was no effect of the cold environmental stimulus on the acute responses of CBFv to the exercise intensity when compared to thermoneutral. On balance, our data indicate the addition of a cold environmental stimulus did not yield a greater exercise training mediated response in CVR_{CO2} and CBFv in young healthy individuals. Nevertheless, further research is warranted to examine increasing the exercise dose further with differing intensities/interval exercise and also investigating the responses in elderly and/or populations with cardiovascular risk factors or cerebrovascular disease.

There were negligible differences in the change in conduit endothelial function between the two interventions as well as in cutaneous vascular function. Exercise interventions are strongly associated with increases in vascular endothelial function and enhanced NO bioavailability (Green et al., 2004), yet our study shows no increase in FMD from either intervention. The young healthy participants recruited are likely to be a factor in this lack of response, although we acknowledge studies have shown that short-term exercise training increases FMD in young healthy individuals after 2 weeks of endurance exercise followed by a return to pre-exercise training baseline alongside increases in structural adaptations (Green et al., 2004; Tinken et al., 2010; Birk et al., 2012; Schreuder et al., 2015c). However, these assessment time points and structural markers were not employed in the current study. The acute responses of the radial artery to exercise in the current study highlight that despite an increase in diameter, velocity and shear rate in response to acute exercise, there was no differences between environmental groups. Skin microvessel endothelial function was assessed by the responsiveness to prolonged local heating, a process largely mediated by NO. Exercise training has been shown to enhance NO mediated skin microvascular function in older individuals and those with cardiovascular risk factors (Black, Green and Cable, 2008b; Pugh et al., 2013; Sprung et al., 2013). In the current study, in young healthy individuals the exercise training mediated response was not enhanced, with some evidence of a reduction in forearm responsiveness which could be influenced by the expression of

the skin blood flow data. The CVC and skin blood flow data indicted that flow at 42-44 deg C (e.g., maximal values) was higher post-training. This is consistent with previous studies suggesting improved NO function (Black, Green and Cable, 2008b; Pugh et al., 2013; Sprung et al., 2013) due to regular elevations in skin temperature/or shear stress. In the current study regular elevations in skin blood flow (shear) were likely the key mechanism for the above findings due to the lower skin temperatures in the cold. Elevations in max flow but similar absolute responses to local temperature will result in lower %max values as evident in the current study. These findings are in agreement with one recent previous study in young men (Atkinson et al., 2018) that suggested structural adaptations such as increased capillarity could occur in the cutaneous circulation secondary to exercise training (Atkinson et al., 2018). Such adaptations to the skin microvasculature could prolong transit time of red blood cell flux and thus result in lower skin blood flow responses post-exercise training as assessed via laser Doppler flowmetry (Argarini et al., 2020).

Interestingly, the reduction in forearm responsiveness following exercise was not apparent at the thigh, displaying no difference following exercise training. This could be due to the different hemodynamic and vascular adaptations in the thigh during the regular exercise bouts. For example, relative to the forearm, the thigh was active during cycling exercise, conduit blood flow was much higher and skin temperature was also lower. Such between limb differences in flow and temperature responses to exercise, could modify the cutaneous vascular adaptations to exercise training (Carter et al., 2014; Atkinson et al., 2018). Although skin temperature responses were clearly different between neutral and cold environments, moderate to large elevations in skin blood flow still occurred in the cold which are key drivers of microvascular adaptations.

It is important to acknowledge several limitations associated with this present study. Firstly, TCD assesses blood velocity rather than blood flow as arterial diameter is not measured. Research evidence suggests that MCAv is a reliable index of cerebral blood flow if the

insonated vessel maintains a constant diameter across experimental groups. MCA diameter has been shown to be consistent during modest changes in CO_2 (±5 mmHg) (Ainslie and Hoiland, 2014). Secondly, it is limited to individuals who are young, healthy and free from any cardio and cerebrovascular diseases. The study also did not include a non-exercising control group that would have eliminated the bias of performing the exercise intervention on the measurements. Finally, implementing a repeated measures design for the acute experiment would have enhanced our statistical power and reduced individual participant variability.

It is also important to note the environmental temperatures in which the two interventions were implemented represented temperatures reflecting 2 distinct seasons in the UK whereby many individuals are likely to exercise, highlighting some strong external validity for our environmental temperature choice. Although, the lack of difference identified between two groups could be due to the temperatures not differing enough. Moreover, examining the responses during an acute bout of exercise at the midpoint of the exercise training program ensured that the participants were somewhat accustomed to the exercise and the temperature, potentially resulting in cold habituation or acclimation (ref; young) and therefore blunting the responses to the exercise performed in the cold (ref; makinen). This may have influenced the similarity in the rate of increase in CBFv in response to exercise in the two environmental conditions. The exercise elicited the same responses of blood pressure and $P_{ET}CO_2$, primary factors that influence the CBF response to exercise as well as similar responses of core temperature. However, it is possible that haemodynamic responses to acute exercise in the cold might have been exaggerated during the early exercise sessions and in fact the improvement in cerebral autoregulation may have occurred early in the exercise training intervention (i.e. between weeks 1-4). This is supported in other vascular beds that display that functional and structural adaptations follow different time-courses, with functional adaptations identifiable in as little as two weeks before structural adaptations ensue (Tinken et al, 2008).

Exercise training in a cold environment increased dCA compared to a small drop with training in a thermoneutral environment in a cohort of young healthy individuals. Our data suggests exercise training in cold temperatures improved the efficacy of the pressure-flow relationship aspect of CBF regulation and therefore exercise in a cold environment may be a novel strategy to implement in aged individuals or individuals with impaired dCA and certainly warrants further investigation.

Chapter 5

General Discussion

Previous literature established that acute exposure to a cold stimulus or environment was found to elicit an increase in CBFv (Doering et al., 1996; Brown, Sanya and Hilz, 2003) or attenuate the decline in CBFv usually seen during heat stress and orthostatic intolerance (Wilson et al., 2002; Durand et al., 2003). Therefore, it was hypothesised within this thesis that cold exposure would increase CBFv in both young and older healthy individuals and that by increasing the "dose" during exercise with a cold stimulus could enhance functional improvements in the cerebrovasculature. The main findings from the thesis are summarised below.

5.1 Summary of main findings

A single bout of acute cold stress did not enhance CBFv or cerebrovascular function in young and older healthy individuals (chapter 3).

In contrast to the hypothesis, an acute cold stress using a water-perfused suit did not increase CBFv nor did it increase either measurement of cerebrovascular function. Importantly, there was no effect of ageing on the responses to cold stress, highlighting that whilst there was no increase in CBFv and cerebrovascular function, there was no detrimental effect of the cold stress on CBFv and measures of cerebrovascular function in older individuals.

Peripheral vascular function was reduced during cold in young and older healthy individuals (chapter 3).

During cold stress, brachial artery peripheral vascular function was reduced and was explained by a reduction in peak diameter and shear rate area under the curve in response to the 5 min limb occlusion. This finding was evident across both younger and older individuals, highlighting that cold stress could have a detrimental effect on the peripheral vasculature. Interestingly, this finding was evident in younger and older individuals.

Performing an 8-week exercise intervention in a cold environment did not enhance CBFv but could provide beneficial improvements in dynamic cerebral autoregulation compared to a thermoneutral environment in young healthy individuals (chapter 4).

Given the lack of consistent changes that exercise and lifestyle interventions in healthy individuals have had on CBFv (Murrell et al., 2013; Drapeau et al., 2019b; Lewis et al., 2019a), it is not surprising that the exercise intervention used in chapter 4 did not alter CBFv. The novel finding from the study is that exercise training in the cold elicits an improvement in dCA. Interestingly, the improvements in dCA were represented by both normalised gain and phase indicating that cold exercise training improved both the magnitude of the CBFv response and temporal alignment to forced BP oscillations (van Beek et al., 2008; Claassen et al., 2016). This finding also indicated that performing moderate intensity exercise training for 8 weeks in a thermoneutral environment was insufficient to produce an improvement in dCA, similar to that seen in the healthy aged-matched controls in a previous study (Lewis et al., 2019a) but the added stimulus of a cold environment displayed beneficial improvements on dCA.

Performing an 8-week exercise intervention in a cold or thermoneutral environment did not enhance skin microvessel endothelial function in young healthy individuals (chapter 4).

The skin microvascular response was not enhanced with exercise training, with some evidence of a reduction in forearm responsiveness, which could be influenced by the expression of the skin blood flow data. The data displayed elevations in max flow but similar absolute responses to local temperature will result in lower %max values as evident in the current study. The CVC data showed that flow at 42-44 deg C (e.g., maximal values) was higher post-training consistent with previous studies and improved NO function (Black, Green and Cable, 2008b; Pugh et al., 2013; Sprung et al., 2013). The findings could also be in agreement with one previous study in young men (Atkinson et al., 2018) that

suggested the decreased SkBF response to local heating observed following exercise training could be due increased cutaneous capilliarisation, meaning a reduction in SkBF and decreased red blood cell transit time. This has been supported by the suggestion that capillary beds adapt to stimuli such as shear by increasing density (Brown, 2003). Nevertheless, the skin microvascular responses to exercise training were similar following training in both environmental conditions despite the differences in skin temperature between environmental conditions.

Performing exercise in a cold environment did not enhance CBFv response compared to performing exercise in a thermoneutral environment in young healthy individuals (chapter 4).

During exercise, P_aCO_2 increases linearly until the exercise intensity increases above this threshold (60-70%VO_2max), followed by a plateau/decrease with higher exercise intensities (Smith et al., 2014). In contrast to the hypothesis, performing exercise in a cold environment did not elicit an increased CBFv response to exercise. This finding suggests that whilst there was no effect of environmental temperature on the CBFv response to exercise, there was no detrimental effect of the cold environment on CBFv and highlights the ability of the brain to effectively control blood flow under various demanding conditions (environmental and exercise).

5.2 General discussion of major findings

Overall the thesis findings suggest that cold stress alone poses no benefit to CBFv and measures of cerebrovascular function. Notably though, combining exercise training in a cold environment elicits small but significant improvements in dynamic cerebral autoregulation in young healthy individuals. This suggests that in contrast to previous research suggesting that older individuals or those at risk of cerebrovascular disease need to avoid exposing themselves to lowering temperatures in winter months, exercising in these temperatures

may pose no cerebrovascular risk and could be beneficial to their cerebrovascular function and needs further investigation.

5.2.1 Cold

The body of literature surrounding environmental cold stress comes primarily from epidemiological data and systematic reviews that highlight possible complications associated with seasonal variation on cardiovascular risk factors and; the reduction in environmental temperature that is experienced in winter months for older individuals and people with existing cardiovascular and cerebrovascular diseases. The increase in cardiovascular disease risk is presumed to be in part due to vascular dysfunction (Widlansky et al., 2007), sympathetic overactivity (Liu, Yavar and Sun, 2015; Grassi and Ram, 2016) and increased blood pressure (Goodiwn et al., 2001; Hayashi et al., 2008). Despite evidence for the association between low ambient temperature and adverse cardiovascular and cerebrovascular effects, there has been few controlled laboratory studies to try to identify the potential pathophysiolocal mechanisms responsible for the association of higher wintertime cardiovascular morbidity and mortality. Of the controlled laboratory studies, many have utilised different types of cold exposure such as whole body cooling (Greaney, Matthews and Wenner, 2015; Greaney, Kenney and Alexander, 2017), local cooling (Smith, Santhanam and Alexander, 2013; Prodel et al., 2017) and the cold pressor test (Nabel et al., 1988) to examine thermoregulatory responses to cold stress. Of these studies, they have primarily focused on individuals with cardiovascular risk factors such as hypertension (Smith, Santhanam and Alexander, 2013; Greaney, Matthews and Wenner, 2015; Greaney, Kenney and Alexander, 2017; Prodel et al., 2017) or existing cardiovascular diseases such as coronary artery disease (Nabel et al., 1988) and compared the responses to cold exposure to healthy controls.

Epidemiological and clinical studies have also observed temporal patterns of stroke occurrence, with peaks often displayed in wintertime (Kelly-Hayes, 2010). Evidence from a case-crossover study identified that a 17.4^{o}C decrease in environmental temperature increased the risk of stroke onset (Hong et al., 2003). Clinical conditions including stroke are strongly associated with reductions in cerebral blood flow velocity and impaired cerebrovascular function (Markus et al., 2004). However, despite this, little research has been conducted into the cerebrovascular responses to cold exposure and the possible implications that could arise with ageing or disease. Research into cerebral haemodynamics has primarily used cold stress as a mechanism to reduce the attenuation in CBFv observed during heat stress causing orthostatic intolerance (Doering et al., 1996; Wilson et al., 2002) or as a way of studying the diving reflex (Brown, Sanya and Hilz, 2003; Kjeld, Pott and Secher, 2009) but little research has focused on the responses of CBFv and cerebrovascular function to cold stress as a primary aim.

The responses to a cold stress are highly dependent on the intensity, duration and type of cold stress and on the methodology employed. The data from this thesis utilised both skin surface cooling using a water-perfused suit to identify whether an acute cold stress (Chapter 3) or performing an exercise training intervention during whole body exposure in cold using an environmental chamber (Chapter 4) to examine if cold could enhance cerebral blood flow velocity and subsequently cerebrovascular function. The data from this thesis suggests that utilising these methods of cooling found no improvement in MCAv following acute (chapter 3) and chronic (chapter 4) exposure to cold, nor did it increase the MCAv response to acute exercise (chapter 4). However, combining the cold environment and exercise elicited an increase in dynamic CA, a mechanism of cerebrovascular function that was not observed following exercise training in a thermoneutral environment (chapter 4). These findings may contrast previous research that identified an increase in MCAv during cold stress (Brown, Sanya and Hilz, 2003). However, these differences may be due to a difference in the type of cold stimulus used. It was thought that MCAv increases could be a

consequence of direct cooling to the head/face skin surface with previous research using cold face stimulation (Brown, Sanya and Hilz, 2003; Kjeld, Pott and Secher, 2009). Recent research has proposed that increases in CBFv and internal carotid artery blood flow, with and without facial cooling, are due to physiological factors associated with breath holding, and not facial cooling per se. Apnoea causes an increased perfusion pressure and accumulation of arterial CO_2 (AlSalahi et al., 2020), which could contribute to the increases displayed during facial cooling. Within this thesis, externally valid cold stressors were utilised, with an acute cold stress that reduced skin temperature by approximately 6.2^{o}C to stimulate the sympathetic nervous system and provoke vasconstriction but without compromising core temperature. This methodology did not include cooling of the face and did not impact resting $P_{ET}CO_2$, although this was not measured throughout the acute cooling period and may have impacted on the MCAv response to cooling. Also in chapter 4 it was aimed to compare externally valid environmental temperatures that correponded to the median environmental temperatures observed in Liverpool, UK during the winter months for cold (5^{o}C) and the median environmental temperatures seen through spring, summer and autumn for thermoneutral (15^{o}C)(*Time and Date*, 2015). Furthermore, during pilot testing in these two temperature settings, participants were asked to described their feeling of thermal comfort and deemed on average throughout the session to be “neutral” during 15^{o}C and “cold” 5^{o}C as assessed on a 1-9 point thermal comfort scale (Appendix 7.1). Therefore, it was suggested that performing a bout of exercise in a cold environment, where the head and face was also exposed, may have caused a greater increase of CBFv relative to a normothermic environment. Despite this, MCAv was not enhanced following an exercise intervention in the cold environmental conditions nor was the acute MCAv response increased. There was no measurement of skin temperature of the face during exercise and so it cannot be determined whether the cold environment provoked an increase in facial cooling compared to the thermoneutral environment. Furthermore, during exercise, $P_{ET}CO_2$ was not different between the two environmental conditions although as continuous $P_{ET}CO_2$

monitoring was not used we cannot infer that exercise in the cold environment provoked the same ventilatory responses as the thermoneutral environment throughout exercise.

5.2.2 Systemic Vascular Function

It is important to collectively assess the cerebral, peripheral and micro vascular function to provide a representation for overall systemic vascular function. Minimal research has examined the effects of cold stress in a range of vascular beds simultaneously. Throughout this thesis each of the studies were designed to assess whether the possible changes in one vascular bed interchanged with changes in another. The ability of the endothelium to produce and respond to nitric oxide is imperative for optimal systemic vascular function. Evidence from previous physiological studies have identified techniques that are valid measures of endothelium dependent and NO-specific indexes of endothelial function, such as FMD in the peripheral vasculature (Thijssen et al., 2011; Thijssen et al., 2019a), hypercapnic cerebrovascular reactivity in the cerebral vasculature (Carter et al., 2016; Hoiland et al., 2017) and the dilation response to local heating in the microvasculature (Black, Green and Cable, 2008b). Chapter 3 identified that despite cold stress having displayed a decrease in peripheral vascular function, through a reduction in shear stress and decreased NO bioavailability, the study reported that this effect of cold does not extend to the cerebral circulation, as no change was observed in cerebral perfusion during cold, nor was there a difference of temperature in absolute or relative CVR_{CO2}.

Due to the natural decline in CBF observed in the aged population and the increased disease risk this poses, interventions that target attenuating this reduction in CBF and cerebrovascular function are of upmost importance (van der Kleij et al., 2018). Life-long exercise has been proven to mitigate the effects of age on CBF (Ainslie et al., 2008) and exercise interventions have been implemented to target the improvement of CBF and cerebrovascular function, but findings have been inconsistent (Akazawa et al., 2012; Murrell

et al., 2013; Drapeau et al., 2019a; Lewis et al., 2019c). In chapter 4 the aim was to assess the impact of 8 weeks of exercise training in a cold environment (5^{o}C) on CBFv and cerebrovascular function compared to exercise training in a thermoneutral environment (15^{o}C). The cold exercise intervention mediated an improvement in dCA compared to a slight attenuation in dCA following the exercise intervention in the thermoneutral environment. The attenuation in dCA displayed following exercise training in the thermoneutral condition supports the findings of previous research that also identified a decrease in dCA displayed by a reduced TFA gain during 0.10 Hz squat to stands following exercise training (Drapeau et al., 2019b) and in individuals with increased cardiorespiratory fitness (Lind-Holst et al., 2011; Labrecque et al., 2017). However, the influence of a higher cardiorespiratory fitness has displayed contradicting results on TFA gain, with lower (Ichikawa et al., 2013) and comparable (Aengevaeren et al., 2013) TFA gain also being reported compared to untrained controls. Drapeau et al. (2019a) speculated that the observed change in dCA may have been as a result of transient changes in BP during the exercise, however BP was not monitored. Our acute exercise data suggests that this is unlikely to explain changes in dCA, given that the acute BP response, along with MCAv and MCA conductance and resistance responses, during exercise were not different between environmental temperatures.

Interestingly, despite the observation of a reduced peripheral vascular function following an acute cold stress in chapter 3 there was no observed change in function in peripheral conduit vessels following 8 weeks of exercise training in a cold or thermoneutral environment (chapter 4). This suggests that the possible combination of exercise and cold could eliminate compromised endothelial function induced by cold stress alone. This is supported by recent data that established that FMD was not impaired following an acute bout of exercise performed in a cold environment (-15^{o}C) in patients with coronary artery disease (Valtonen et al., 2020). Exercise induced changes in shear have been shown to provide the principal physiological stimulus to adaptation in flow mediated endothelial

function (Tinken et al., 2010). The acute exercise data (chapter 4) indicates that despite differing environmental conditions, the shear rate in the radial artery did not differ. Therefore, these findings could indicate that despite lower skin temperatures and skin blood flow exhibited during exercise in the cold, exercise may allow the maintenance of adequate shear rate and blood flow to ensure that endothelial function is not compromised. Although, this also highlights a potential risk of passive cold exposure to peripheral vascular function and requires further investigation.

5.3 Methodological Considerations and Limitations

There are a number of strengths in the methodology of this thesis. The participants recruited for the experimental studies in this thesis give a good representation of younger and older healthy individuals. Evidence has shown that exercise is beneficial for CBFv and measures of cerebrovascular function (Ainslie & Bailey, 2013) and that exercise training can improve CBFv in healthy and diseased populations (Murrell et al, 2011). Although, sedentary individuals or individuals that had risk factors or were diagnosed for CVD were not included, the findings are limited to a healthy population and those with overt disease require further investigation.

The studies performed within the current thesis ensured strict inclusion and exclusion criteria in addition to the control of diet and exercise prior to and during laboratory visits. Repeated measurements within studies were performed during the same time of day to control for participant circadian rhythms. Each measurement applied within this thesis was performed adhering to the most recent published guidelines for that specific measurement. For example, FMD assessments were undertaken according to the latest peer-reviewed consensus guidelines (Thijssen et al., 2019a), together with the use of custom-designed edge-detection and wall-tracking analysis software, the accuracy, validity and prognostic value of FMD outcomes were maximised. Dynamic CA measurements and TFA analysis were all performed following cerebral autoregulation network recommendations (Claassen

et al., 2016) and micro vascular function was performed following guidance for gradual local heating (Black, Green and Cable, 2008b).

Despite these methodological strengths, there are a number of considerations and limitations. Both male and female participants were included throughout, this could be observed as both a strength and a weakness. Evidence has shown that females can display differences in vascular function due to differences in sex hormones (Green et al., 2016) and for this reason it is advised that females should be assessed alongside males during the early follicular phase of the menstrual cycle when oestrogen and progesterone are at their lowest or during the placebo phase of the menstrual cycle (Stanhewicz and Wong, 2020). In chapters 3 and 4, measurements were performed on the younger females during the early follicular phase of their menstrual cycle to try to minimise any sex differences due to hormonal variation. Recent research has also found that dynamic CA was not different between biological sexes when measured using squat stand manoeuvres if females are tested within the early follicular phase of the menstrual cycle (Burma et al., 2020). More recently this has been questioned as to whether it decreases external validity, especially as whether the menstrual cycle affects measurements of vascular function is unclear (Stanhewicz and Wong, 2020). Statistical analysis was not employed to compare sex differences as this was not the primary focus in the chapters. Another limitation in the current thesis is the methodology used to assess the menstrual phase of the female participants. A calendar approach was adopted, with self-report.

Another potential limitation to consider within the present thesis is the order at which the studies were conducted and presented. The originally proposed chapters within the thesis were to include three chapters; 1) First, to investigate the responses of cerebral blood flow when performing an acute bout of exercise in a cold environment versus performing this in a thermoneutral environment and then 2) to assess the impact of 8 weeks of exercise training in a cold environment on CBFv and cerebrovascular function compared to exercise training in a thermoneutral environment. However, the acute measurements were assessed

at the midpoint of the exercise training intervention. These measurements could have been implemented during week 1 and 8 of the exercise training intervention as a repeated measures design to enhance our statistical power and reduce individual participant variability. This would also allow identification of any possible training or acclimation effects that may have occurred. Due to these measurements being performed during the exercise training intervention, the two chapters were merged within the thesis to try to help the reader understand the research design.

It is possible that the data within the thesis lacks statistical power due to the small sample sizes and that the variability within the data due to “noisy” measurement techniques may have induced a type II error and therefore more subjects are required in order to guard against this risk (ref; Atkinson & Batterham, 2005). Furthermore, unequal group sizes can also require larger sample sizes and must be taken into consideration for the data from Chapter 3 and 4.

A constant limitation within this thesis is the use of TCD for the measurement of CBF and cerebrovascular function. As previously discussed in a number of reviews (Willie et al., 2011; Ainslie and Hoiland, 2014), TCD provides a measurement of CBFv as an index of CBF, as TCD does not provide feedback regarding vessel diameter. Changes in vessel diameter can affect measurement accuracy of CBF with TCD (Heus et al., 2018). Research evidence suggests that MCAv is a reliable index of cerebral blood flow if the insonated vessel maintains a constant diameter across time and experimental conditions (Ainslie and Hoiland, 2014). Research studies have shown that the dilation of extra-cranial arteries matches the dilatation in the cerebrovasculature (Smith et al., 2019b). Assessment of carotid artery diameter as an extra cranial vessel was employed in the thesis during the CO_2 reactivity test. The use of additional imaging techniques such as functional magnetic resonance imaging and arterial spin labelling have been used to detect changes in global and regional perfusion, however their temporal resolution is currently too low to assess the

dynamics of dCA (Heus et al., 2018), such as in exercise and are either expensive or invasive techniques. The MCA diameter is unlikely to change under resting conditions or during moderate changes in BP (Serrador et al., 2000b), similar to that induced during repeated squat stand manoeuvres. Furthermore, when utilising TCD for repeated measurements it is not possible to ensure complete accuracy in insonating the same part of the MCA. However, every effort was made to document Doppler probe position, record depth and velocity for repeated measurements.

5.4 Summary

The incidences of cardiovascular events, such as stroke, appear to have seasonal variation, increasing in the cold winter months, particularly in older adults (Gao et al., 2012). Clinical conditions such as stroke are strongly associated with a reduction in cerebral blood flow velocity and impaired cerebrovascular function (Markus et al., 2004), something that is also evident with ageing (Bejot et al., 2019). It has also been suggested that older individuals and those with underlying disease may be most at risk during cold weather (Bunker et al., 2016). Regular exercise is important for health and has been suggested to be an efficient form of secondary prevention in delaying disease (Piepoli et al., 2010; Schneider et al., 2017). Additionally, regular life-long exercise is shown to attenuate the decline in CBF (Ainslie et al., 2008) and can improve endothelial function (Green et al., 2008). There is little research that focuses on the direct effects of cold exposure on CBFv with studies identifying that cold exposure could display increases in CBFv (Brown, Sanya and Hilz, 2003). This thesis suggests that whole body cold stress does not have a negative impact on CBFv or cerebrovascular function measured using reactivity to CO_2 and dynamic cerebral autoregulation. There is evidence of an improvement in dynamic cerebral autoregulation following 8 weeks of exercise training in a cold environment. Therefore, it could be advised that individuals should maintain exercise all year round, including outdoors during winter months. However, it is necessary to further understand the impact of cold on cerebral blood

flow and cerebrovascular function in older individuals and those with pre-existing risk factors.

5.5 Recommendations and future direction

Several potential areas of future research have emerged following the studies detailed within this thesis.

1. Establish whether the increase in CBFv observed in response to a cold stimulus, in contrast to what was displayed in this thesis, is primarily dependent on face cooling stimulation and assess whether this technique can alter cerebrovascular function in younger and older individuals.
2. Establish whether cold stress exerts its effects differently depending on health status. Expanding on the work contained within this thesis, future research should explore the effects of cold stress on a broad range of clinical groups (e.g. patients with hypertension, type 2 diabetes, cognitive impairment or early onset dementia) and elderly individuals with cardiovascular risk factors to gain an understanding of how different individuals respond to changes in temperature.
3. Based on the work in chapter 4 in this thesis, identify whether an exercise intervention in a cold environment could elicit different responses in CBFv and cerebrovascular function in older individuals, similar to that evident in younger individuals.
4. It has been suggested that identifying the type and format of exercise that could provide the greatest acute increase in MCAv could optimise exercise programs for the enhancement of cerebrovascular function (Lucas et al., 2015). By designing large-scale trials with a range of different intervention protocols, this should in turn identify the optimal dose of exercise in terms of duration (e.g. days, weeks or months) and frequency (e.g. daily or weekly) and intensity (e.g. temperature and exercise mode).

Chapter 6

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Chapter 7

Appendices

Appendix 7.1 Thermal Comfort Scale

THERMAL COMFORT SCALE

1	**Very Cold**
2	**Cold**
3	**Cool**
4	**Slightly Cool**
5	**Neutral**
6	**Slightly Warm**
7	**Warm**
8	**Hot**
9	**Very Hot**

Appendix 7.2 Supplemental data

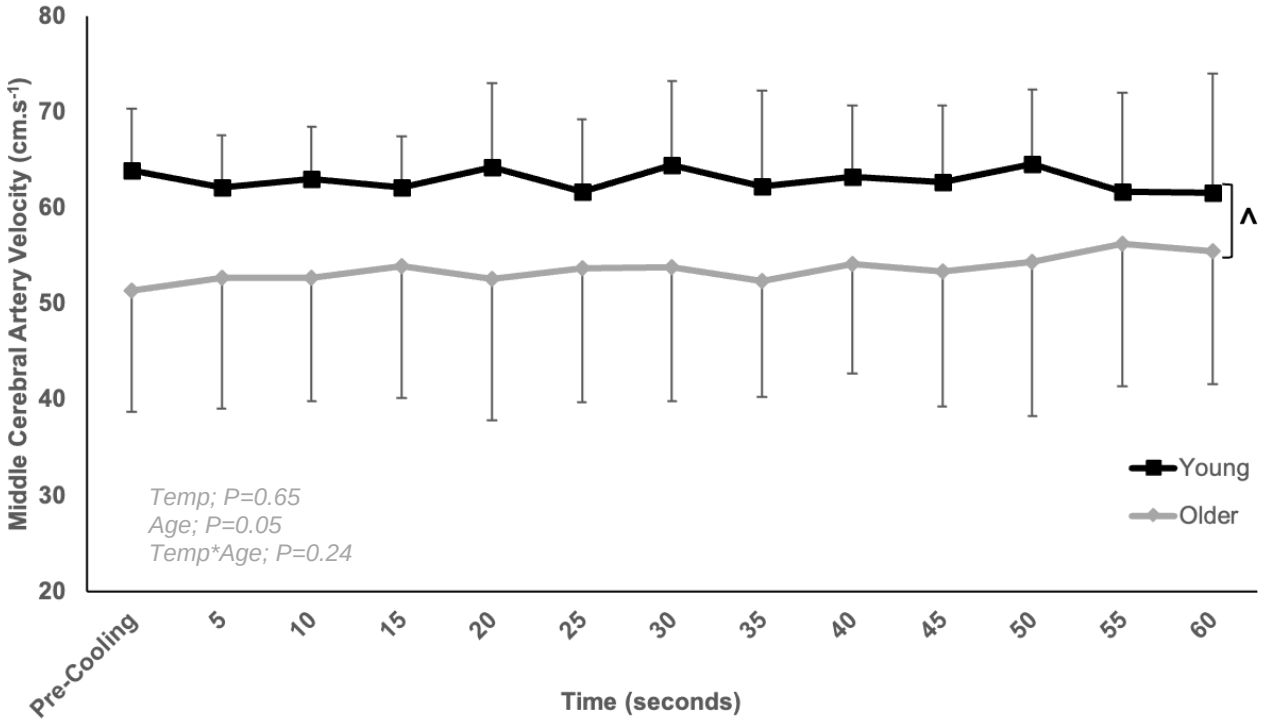

Figure 3.6. The responses of middle cerebral artery velocity during the first minute of a 30 minute acute cold stress in young and older individuals. ^ denotes significant main effect of age (P<0.05).