The impact of ischaemic preconditioning on cerebrovascular function in healthy individuals

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Abstract

Introduction: Remote ischaemic preconditioning (RIPC) refers to non-lethal repeated bouts of ischaemia followed by reperfusion applied to a vascular bed, tissue or organ rendering protection to future ischaemic events. RIPC has been reported to enhance functional recovery from heart attacks and strokes, as well as improve conduit artery and microvessel function. Remarkably, very few human studies have examined the impact of RIPC on cerebral blood flow (CBF) or cerebrovascular function. The overall aim of this thesis was to assess, in healthy individuals, the impact of bilateral arm RIPC or SHAM on resting CBF, cerebrovascular reactivity, cerebral autoregulation, and autoregulation during hypercapnia.

Methods: Nine healthy participants (age 32.6±11.1 yrs; BMI 25.9±4.4 kg/m^2) underwent two laboratory visits; RIPC and SHAM. RIPC consisted of 4 x 5 min periods of cuff inflation (to 200 mmHg) around the upper arm, with cuff inflation alternated between arms every 5 mins. SHAM consisted of the same protocol, but with cuff inflation to a pressure of 10 mmHg. CBF was measured using transcranial Doppler ultrasonography. Cerebral autoregulation was assessed using the squat to stand technique (5 second squat-5 second stand) while cerebral CO_2 reactivity was assessed via a period of hypercapnia involving inhalation of 5% CO_2. Finally, cerebral autoregulation was again assessed during hypercapnia.

Results: CBF was not affected during either RIPC or SHAM ($P>0.05$). RIPC did not significantly affect cerebral autoregulation or CO_2 reactivity, compared to SHAM ($P>0.05$). Hypercapnia reduced transfer function phase (39 to 16) radians during autoregulation, with no difference between the IPC and Sham conditions.

Conclusion: These data indicate that an acute bout of bilateral RIPC does not affect CBF, cerebral autoregulation or cerebral reactivity. Additionally, an acute bout of RIPC does not attenuate the impairment to cerebrovascular function caused by hypercapnia.

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Declaration

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

Publications completed by the candidate during MPhil tenure

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Oral communications

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Table of Contents

List of Abbreviations

ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BP	Blood pressure
CABG	Coronary artery bypass surgery
CBF	Cerebral blood flow
CBVC	Cerebrovascular conductance
CO_2	Carbon dioxide
FMD	Flow mediated dilation
HR	Heart rate
IAS	Intra-arterial stenosis
ICA	Internal carotid artery
IPC	Ischaemic preconditioning
IRI	Ischaemia reperfusion injury
MAP	Mean arterial pressure
MCA	Middle cerebral artery
MCAv	Middle cerebral artery velocity
NO	Nitric oxide
O_2	Oxygen
$PaCO_2$	Partial pressure carbon dioxide
PCI	Percutaneous coronary intervention
$PETCO_2$	Partial pressure of end-tidal carbon dioxide
RIPC	Remote ischaemic preconditioning
TCD	Transcranial Doppler
VA	Vertebral artery

List of Figures

List of Tables

1. CHAPTER 1

Literature Review

1.1. Introduction

Ischaemic preconditioning (IPC) refers to the phenomenon whereby 3-4 brief periods of ischaemia, followed by tissue reperfusion, confers subsequent protection against the magnitude of tissue injury following ischaemia. This concept was introduced 30 years ago in a study which demonstrated that cycles of ischaemia and reperfusion of coronary arteries are able to protect the myocardium from subsequent prolonged ischaemia and reperfusion, leading to a reduction in infarct size (Murry et al., 1986). A follow-up study, by Przyklenk and co-workers (Przyklenk et al., 1993), demonstrated that cycles of coronary ischaemia and reperfusion also protect remote cardiac tissue not directly exposed to the ischaemia-reperfusion cycles. This study stimulated substantial research that resulted in the clinical application of IPC of a limb to protect *remote* tissue and/or organs, such as the heart, against the magnitude of tissue loss following an ischaemic event (Pickard et al., 2015). A reduction in myocardial damage by remote IPC (RIPC), including improvement in clinical outcomes, has been shown when applied in patients prior to cardiac surgery (Thielmann et al., 2013) and in patients with suspected myocardial infarction treated with RIPC during ambulance transition (Botker et al., 2010).

A second potential benefit of RIPC has emerged from studies that have explored the effects of repeated RIPC (i.e. daily episodes of the 4 bouts of ischemia and reperfusion) on systemic vascular function. Improvement in vascular function as a consequence of repeated IPC may contribute to a reduction in the risk of future ischaemic events. Hence, RIPC may be of direct benefit in terms of reducing the impact of infarction on affected cardiac muscle, but also reducing the likelihood of atherothrombotic events occurring in the first instance by virtue of this impact on endothelial and vascular function. Importantly, very few studies have evaluated the effects of RIPC on the cerebrovascular system in terms of function, protection against cerebral ischaemia and/or prevention against cerebrovascular dysfunction. This

literature review will provide a brief overview of the research studies to date investigating the impact of RIPC on the cardio and cerebro- vascular systems.

1.2. Ischaemic Preconditioning

Murry *et al.* introduced the potential cardioprotective benefits of an episode of IPC (Murry et al., 1986). In this study, the IPC protocol involved occlusion of the left anterior descending artery of dogs 4 times (for 5-minutes per occlusion), alternated with 5-minutes of reperfusion. This was followed by 40-minute ischaemia of the same artery. A 75% smaller infarction size was evident after IPC, compared to control animals that underwent a sham-intervention. This finding provided experimental support for clinical observations in the mid-1980s, which suggested that post-myocardial infarction patients with a prior history of angina (i.e. myocardial ischaemia) demonstrated better ejection fraction (Matsuda et al., 1984). These data contributed to the concept that exposure to (non-lethal) cardiac ischaemia in the period preceding coronary ischaemia may protect against the impact of reperfusion of the occluded artery on the magnitude of myocardial damage. Subsequent studies provided further clinical evidence that "pre-conditioning" of the myocardium before an acute myocardial infarction, for example through prodromal angina, leads to a smaller infarct size (Ottani et al., 1995) and improves (in-hospital) outcome (Kloner et al., 1995, Nakagawa et al., 1995).

Despite these intriguing observations, direct clinical application of IPC is challenging and associated with some limitations. Compared to control groups undergoing traditional coronary artery bypass surgery (CABG), smaller post-surgery release of cardiac troponins was observed after IPC applied to human coronary arteries preceding CABG (Jenkins et al., 1997), along with attenuated impairment in cardiac function (Wu et al., 2000). Nevertheless, the direct application of IPC to coronary vessels can only be applied to planned ischaemic injury or surgery and is obviously impractical in humans.

1.2.1. Remote IPC and the Heart

Przyklenk and colleagues performed a landmark study in 1993, in which they demonstrated that cyclical ischaemia and reperfusion of the circumflex coronary artery was associated with protection of cardiac territory supplied by the left anterior descending artery (i.e. an area remote from the distribution of the circumflex coronary artery) (Przyklenk et al., 1993). They provided support for the notion that IPC can afford infarct-sparing protection for distinct areas within the heart; and initiated several investigations to explore the potential effects of *remote* IPC (RIPC).

Preclinical studies exploring the effects of RIPC have typically collected perfusate from ischaemic preconditioned tissue/animals and subsequently perfused naïve hearts using a Langendorff preparation. Interestingly, several studies have demonstrated that infarct sizes were significantly smaller in both donor hearts subjected to RIPC and the naïve recipient hearts that received the perfusate from a pre-conditioned donor (Huffman et al., 2008, Dickson et al., 1999). This demonstrates the ability of RIPC to reduce damage upon ischaemic injury in remote areas, possibly through a blood-borne pathway. Although the underlying protective mechanisms may differ, the efficacy of RIPC and IPC seem comparable. Furthermore, evidence is present for between-species protection of RIPC, since rabbit hearts demonstrated protection against prolonged ischaemia when perfused with human preconditioned serum (Michelsen et al., 2012, Shimizu et al., 2009). This suggests a similarity in the factor/s conferring protection across species, and that such agent/s remain conserved during such procedures, allowing binding to the recipient receptors.

Experimental observations on the impact of RIPC have been supported by clinical studies demonstrating the potential of RIPC to prevent or attenuate (ischaemia-induced) tissue

damage in the heart and various other organs (e.g. liver, brain, vascular endothelium and skeletal muscle) (Yoshizumi et al., 1998, Stenzel-Poore et al., 2003, Pang et al., 1995, Jabs et al., 2010). Kharbanda *et al.* used a swine model of RIPC of the limb (using a blood pressure cuff) and evoked reduction in the magnitude of myocardial damage against prolonged ischaemia (Kharbanda et al., 2002).

Studies have typically explored clinical effects by applying an episode of cyclical RIPC on a limb using a blood pressure cuff, before (planned) prolonged myocardial ischaemia. Several studies have examined the impact of RIPC in patients undergoing impending CABG or percutaneous coronary interventions (PCI), as these strategies induce global myocardial ischaemia (and subsequently cardiac damage) as reflected by a post-surgery elevation in cardiac troponins. Accordingly, strategies that can attenuate the global myocardial ischaemia have clinical relevance. Most of these studies reported lower peri-/post-operative levels of troponins in patients undergoing CABG and elective PCI (Heusch, 2013), findings reflected by meta-analyses (D'Ascenzo et al., 2012, Brevoord et al., 2012). More importantly, RIPC may reduce peri-operative myocardial infarction (Brevoord et al., 2012, Thielmann et al., 2013) as well as post-operative atrial fibrillation (Candilio et al., 2015). For example, Thielmann and co-workers demonstrated in 329 patients undergoing CABG that preceding RIPC reduced post-CABG troponin levels, and also lowered all-cause mortality following 1.5-years follow-up (Thielmann et al., 2013). RIPC may therefore possess potential long-term clinical benefit in humans.

Instituting RIPC *before* prolonged ischaemia does not seem to be a pre-requisite for cardioprotection, since RIPC, applied to the lower limbs of pigs *during* cardiac ischaemia, was also associated with reduced severity of myocardial infarction and improved indices of cardiac function (Schmidt et al., 2007). In 2010, Bøtker and colleagues explored this concept

in humans by randomising patients with suspected acute myocardial infarction to RIPC or a control intervention during transport to the hospital for primary PCI (Botker et al., 2010). They found that RIPC before hospital admission was associated with better myocardial salvage, measured by myocardial perfusion imaging. A follow-up study of this patient population indicated that, after a median follow-up of 3.8 years, the RIPC-treated patients experienced fewer major adverse cardiac and cerebrovascular events (Sloth et al., 2014). The potential of RIPC to improve outcomes in patients with acute myocardial infarction undergoing primary PCI was supported by subsequent studies (Munk et al., 2010, Rentoukas et al., 2010).

1.2.2. Remote IPC and the Vasculature

There is also evidence to suggest that (R)IPC can have a direct impact on the vasculature. Studies have been performed on large conduit and small microvessels including the cutaneous vasculature. One study, using RIPC applied to the upper arm, measured tissue oxygen saturation and capillary blood flow (by means of laser Doppler) in the thigh and observed an increase in both while the RIPC procedure was being performed (Kraemer et al., 2011). More recently it has been shown that RIPC applied to the upper arm of patients who have recently undergone a tissue transfer operation improved blood flow (laser Doppler) and tissue oxygen saturation, aiding the recovery of the transferred tissue (Kolbenschlag et al., 2016). Taken together, research is now strongly suggesting that an acute bout of RIPC can improve blood flow in peripheral and cutaneous vessels as well as the myocardium.

The effects of RIPC within the vasculature have not been limited to changes in blood flow. Changes in endothelial function have been observed, with significantly improved brachial artery flow mediated dilation (FMD) following RIPC (Moro et al., 2011). Remarkably, this

improvement in FMD was detected both when the IPC was applied to the same arm that was used for FMD assessment, but also when the stimulus was applied to the upper thigh (RIPC) (Moro et al., 2011). In contrast, Loukogeorgakis et al. (2005) reported no effect of RIPC on brachial artery FMD when RIPC was applied to the contralateral arm, along with no change in resting blood flow. This finding was further supported by both van den Munckhof et al. (2013) and Seeger et al. (2016) when it was reported there was no difference in FMD % change, nor brachial artery diameter when FMD was performed post IPC compared to SHAM. Similarly, response to increased acetylcholine was found to be unchanged following a bout of RIPC (Kharbanda et al., 2002).

Whilst it is not possible to induce an ischaemia-reperfusion injury (IRI) within a human myocardium, it is possible to induce a non-lethal IRI to human conduit peripheral arteries by means of causing reversible endothelial dysfunction using a blood pressure cuff inflated to a supra-systolic pressure for 20 minutes. Previous research studies have shown that RIPC can attenuate the dysfunction caused by IRI's with Kharbanda *et al* identifying that IPC applied before a 20 minute forearm occlusion significantly reduced the endothelial dysfunction measured via FMD compared to controls (Kharbanda et al., 2001). This protection against endothelial dysfunction was further supported in a study by Loukogeorgakis et al. (2005). Interestingly, the RIPC effect on IRI may be age dependent. Attenuation of endothelial dysfunction measured by FMD, induced by a 15 minute brachial artery occlusion (IRI) was reported in younger individuals (20-25 yr), but this protection was not detected in older individuals (68-77 yr) (van den Munckhof et al., 2013). More recently it was found that patients with heart failure showed an exaggerated decline in endothelial function during an IRI compared to healthy controls and that RIPC does not reduce the endothelial dysfunction caused by an IRI within heart failure patients but does in healthy individuals despite no differences in baseline FMD between RIPC and SHAM conditions (Seeger et al., 2016). The

lack of response to the RIPC stimulus within the heart failure patients was believed to be largely as a result of this exaggerated decrease in endothelial function compared to healthy controls, which RIPC was simply not able to attenuate.

1.2.3. Remote IPC and the Brain

Whilst the beneficial effects of (R)IPC on the cardiovascular system has been extensively researched, its effect on the cerebrovascular system has received less attention. Nevertheless, there is a growing body of animal research that supports the notion that RIPC provides protection against ischaemic injuries in the cerebrovascular system and provides neuroprotection. This is of enormous clinical relevance as brain ischaemia is one of the leading causes of morbidity and mortality in the world (Liu et al., 2009).

Hoyte *et al*., (2006) conducted a study in mice and employed 15 minutes of middle cerebral artery (MCA) occlusion 72 hours prior to inducing a 45-minute injurious cerebral ischemia. They reported an increase in regional brain blood flow during the injurious occlusion, measured via magnetic resonance perfusion and laser Doppler flowmetry, in the mice that received the IPC as well as a reduction in infarct size (Hoyte *et al.*, 2006). Ren and colleagues extended this work by applying RIPC to the left femoral artery of rodents, 2 days prior to inducing cerebral ischaemia. They found that RIPC was associated with a reduction in infarction size compared to the animals with no preconditioning (Ren et al., 2008). Interestingly, Ren *et al* identified that an IPC protocol of 2 x 15 minutes of occlusion-reperfusion generated strong protection, whilst 2 x 5 minutes of occlusion-reperfusion did not provide protect against ischaemic injury. Taken together this data provides evidence that RIPC can offer cerebrovascular protection against cerebral ischaemia, with some suggestion that longer periods of ischaemia confer a stronger protective effect. Jensen and colleagues

also observed that pigs who received RIPC to the limb, displayed an accelerated recovery in neurological function and ECG pattern and demonstrated a lower brain lactate concentration compared to controls following ischaemic injury as a result of hypothermic circulatory arrest (Jensen et al., 2011). Taken together, these data provide evidence that (R)IPC can offer cerebrovascular and neural protection against cerebral ischaemia. Nevertheless, research work in humans is warranted to be able to translate this into clinical practice.

Research examining the impact of repeated RIPC on cerebrovascular function and clinical outcomes in humans is limited. Two large scale clinical trials have been conducted examining the potential benefits of repeated RIPC on human cerebrovascular function in patients with intracranial arterial stenosis (IAS). The studies employed bilateral limb RIPC twice daily for 300 days and found a significant reduction in stroke recurrence along with a significant increase in cerebral perfusion (Meng et al., 2012). Remarkably, the same group more recently observed a significant reduction in ischaemic events following a course of 180 days of bilateral limb IPC, twice daily, applied within an older population of IAS patients, with no adverse changes to heart rate or arterial blood pressure (Meng et al., 2015). Whilst these studies provide indirect evidence that RIPC may have an impact on cerebrovascular function in humans, no study to date has directly assessed this concept.

1.3. The Cerebrovasculature

The next section of this literature review will outline how to assess cerebral blood flow non-invasively using transcranial Doppler in humans, and how to examine cerebrovascular function.

1.3.1. Anatomy of the Cerebral Vasculature

Blood flow to the brain is supplied via two large extra-cranial arteries bilaterally; the internal carotid (ICA) and vertebral arteries (VA). The ICA bifurcates from the common carotid artery whilst the vertebral arteries originate from the subclavian arteries and join to form the basilar artery. These arteries rise to form the anterior and posterior components of the circle of Willis, an anastomotic ring at the base of the brain. The ICA continues upwards to the base of the brain to form the anterior and middle (MCA) cerebral arteries bilaterally (Cipolla 2009), whilst the basilar artery bifurcates to eventually form the posterior cerebral arteries. These three primary intracranial arteries are all connected via communicating arteries (Boyajian et al., 1995). The anterior cerebral artery supplies the frontal lobe of the brain whilst the MCA, the largest branch of the internal carotid artery, supplies blood to a small portion of the frontal lobe as well the lateral face of the temporal and parietal lobes. Finally, the posterior cerebral artery extends laterally before turning in a posterior direction to deliver blood to the hindbrain (occipital lobe, cerebellum ,medulla oblongata) (Willie et al., 2011). These three main arteries that branch from the circle of Willis continue to progressively split into smaller arteries and arterioles (e.g. pial vessels) that run along the surface of the brain until they ultimately penetrate the tissue in order to supply blood to the brain (Cipolla 2009).

1.3.2. Measurement of Cerebral Blood Flow

The ability to measure CBF can provide valuable information on the functional status of the blood vessels that supply the brain, which is of obvious importance to clinicians and researchers. Nevertheless, assessing CBF is difficult due to the brain being enclosed by the cranium, which limits the effectiveness of non-invasive imaging techniques. Transcranial Doppler (TCD) ultrasound has become the most commonly utilised method to examine CBF

in humans (Purkayastha and Sorond, 2012). The following section provides an overview of the use of TCD for the measurement of CBF.

1.3.2.1. Transcranial Doppler (TCD)

Transcranial Doppler was first introduced in 1982 by Aaslid and colleagues and offers a safe, non-invasive, non-ionising, portable and inexpensive method for the assessment of CBF using a pulsed Doppler transducer (Aaslid et al., 1982) (Sarkar et al., 2007). TCD offers three windows for the assessment of intra cranial blood flow; (i) orbital (ii) temporal (iii) foramen magnum. Unlike Duplex ultrasound that offers a B mode image, TCD can be described as a 'blind' technique as the vessels cannot be visualised, and identification of vessels is established via; the window used, position of the probe, depth of the ultrasonic beam, and the values and shape of the velocity waveforms (Sarkar et al., 2007).

TCD ultrasonography uses probes that emit ultrasonic beams that can be focused onto vessels of interest. The assessment of cerebral blood velocity is derived from the Doppler shift, created by the reflection of ultrasound waves from moving erythrocytes (red blood cells) within the blood vessel that are returned to the receiver unit in the Doppler probe. Simply, the Doppler shift refers to the difference between the transmitted and received ultrasound signals (Aaslid, 1986), with faster erythrocyte movement associated with higher velocities, and by extension, blood flow.

The relative success and accuracy of a TCD sonography scan depends heavily on two main factors; (i) the sonographer's technical ability and (ii) the sonographer's anatomical and spatial knowledge of the cerebral vasculature. Knowledge of typical vessel depths, correct

directions and mean blood flow velocities all affect the chances of collecting valid cerebral measurements (Arnolds and von Reutern, 1986) (Table 1.1).

Table 1-1: Typical patterns for identification of cerebral arteries

Artery	Window	Depth (mm)	Direction	Mean Flow Velocity
MCA	Temporal	30 to 60	Toward probe	55 ± 12 cm/s
ACA	Temporal	60 to 85	Away	50 ± 11 cm/s
PCA	Temporal	60 to 70	Bidirectional	40 ± 10 cm/s

Abbreviations: **MCA**, middle cerebral artery; **ACA**, anterior cerebral artery, **PCA**, posterior cerebral artery.

1.3.3. Cerebrovascular Control and Regulation

In order to maintain normal functioning, the brain requires a constant adequate nutritional flow due to its high metabolic demand. In resting conditions the brain is accountable for approximately 25% of total oxygen (O_2) consumption and receives 15-20% of total cardiac output (Q) (Franco Folino, 2007). Despite the high metabolic demand, the brain has a very limited ability to store energy, therefore CBF needs to be highly regulated in order to maintain a constant supply of nutrients and O_2 (Peters et al., 2004). The main regulatory features of the cerebral circulation applicable to this thesis are detailed below.

1.3.3.1. Cerebral autoregulation

Autoregulation of CBF refers to the brain's ability to maintain a relatively constant blood flow despite changes in arterial blood pressure (BP). A rapid and unopposed drop in BP, and subsequently CBF, can lead to dizziness and fainting. Alternatively, a similarly rapid rise in BP and CBF can lead to increased mechanical stress on the smaller fragile cerebral blood

vessels, which can be fatal. Indeed, evidence shows that brain injury occurs when autoregulatory mechanisms are lost (Euser and Cipolla, 2007, Novak et al., 1998).

It was traditionally believed that cerebral autoregulation allowed for CBF to remain entirely constant within certain high-low limits of blood pressure (Bayliss et al., 1895, Franco Folino, 2007, van Beek et al., 2008). Nonetheless, recent studies have shown that such a tight regulation, and across such a large range, is not strictly accurate.

The mechanisms involved in the intrinsic proficiency of cerebral vessels to maintain a constant CBF over a range of systemic BP levels is yet to be fully elucidated, yet most likely involves myogenic and/or autonomic mechanisms (Paulson et al., 1990, Aries et al., 2010, Strandgaard and Paulson, 1984). The myogenic reflex is the intrinsic ability of the vascular smooth muscle to respond to changes in pressure or mechanical load, with a constriction in response to increased pressure and dilation in response to decreases in pressure, referred to as the 'Bayliss effect' (Mellander, 1989). The cerebral vessels are also heavily innervated by neural sympathetic fibres that has also been shown to be involved in the autoregulatory responses. Whilst transient changes in arterial BP occur throughout the cerebrovasculature as well as within extra-cranial vessels, the pial arterioles appear to be the most responsive to these changes (Willie et al., 2014).

1.3.3.2. Measurement of Cerebral Autoregulation

The assessment of cerebral autoregulation requires changes in arterial BP that induces transient alterations in CBF. Whilst there is no 'gold standard' method available for the assessment of cerebral autoregulation (Panerai, 1998), a number of techniques have been previously used to achieve these rapid fluctuations in BP. Traditionally, sudden deflation of bilateral thigh cuffs after a period of inflation to supra-systolic levels was used to induce

transient decreases in BP (Sorond et al., 2009), however this technique is associated with high levels of discomfort as well as a variability in the levels of BP change with each cuff deflation. More recently, a simple squat to stand procedure has been found to produce transient changes in BP, yet with less discomfort than the traditional bilateral thigh cuffs (Lipsitz et al., 2000). Work by Serrador et al. (2005) identified that the sit to stand procedure could be successfully performed by elderly patients and the results were similar to that produced by the thigh cuff deflation technique. Additionally, Sorond and colleagues established that the sit to stand protocol was better tolerated and the CBF response was larger compared to the thigh-cuff technique (Sorond et al., 2009).

1.3.3.3. Arterial blood gases

The cerebrovasculature is also highly sensitive to changes in blood gas content, particularly the partial pressure of carbon dioxide (Pa_{CO2}) (Brian et al., 1996). Kety and Schmidt (1948) were the first to document the effect of CO_2 on CBF, outlining that inhalation of 5% CO_2 mix increased CBF by 50% in humans and that a 7% CO_2 mix increased CBF by 100%. This response in CBF to changes in Pa_{CO2} is referred to as cerebrovascular CO_2 reactivity, which acts as a crucial homeostatic function aiding the maintenance and regulation of central pH, affecting the respiratory central chemoreceptor stimulus (Ainslie and Duffin, 2009). The most common method of tracking arterial CO_2 levels is via the measurement of the partial pressure of end-tidal CO_2 in exhaled air ($PETCO_2$). Typical resting end tidal CO_2 values in humans are between 35-40 mmHg. The sensitivity of the cerebrovasculature to CO_2 is highlighted by recent studies showing a 1 mmHg change in $PETCO_2$ can alter CBF by up to 4-6% (Regan et al., 2013). It is now known that increases in arterial CO_2 cause a relaxation of the vascular smooth muscles in cerebral vessels, with the smaller cerebral vessels being the most sensitive to these changes resulting in a greater response. A number of potential mechanisms involved

in the vasodilation response to hypercapnia (a state of increased levels of CO_2 in the blood) have been suggested, however are yet to be fully elucidated. One of these mechanisms involves changes in pH as a result of increased CO_2, which may activate K^+ channels in the vascular smooth muscle and stimulate relaxation of cerebral vessels (Kitazono et al., 1995, Jackson, 2005). Additionally, evidence is suggesting a role of nitric oxide (NO) in the vasodilation response to hypercapnia (Toda et al., 2009) with previous work showing that the dilatory response of cerebral vessels during hypercapnia is attenuated when a NO synthase inhibitor was used (Smith et al., 1997, Schmetterer et al., 1997)

Whilst cerebral blood vessels are also sensitive to changes in Oxygen (O_2), the range in which this sensitivity is different to CO_2. Whilst small changes in CO_2 cause significant changes in CBF, significant changes as a result of O_2 occur when stretched further than normal values. The increase in CBF as a result of hypoxia occurs via direct effects on vascular cells of cerebral arteries and arterioles. When in a hypoxic state, this induces reductions in ATP levels and opens K_{ATP} within the vascular smooth muscle, resulting in both a hyperpolarization and vasodilation on cerebral vessels (Tomiyama et al., 1999).

1.3.3.4. Measurement of Carbon Dioxide Reactivity

Measuring the cerebrovascular response to changes in CO_2 concentration reflects an index of the ability of the cerebrovascular beds to dilate or constrict (Ainslie and Duffin, 2009). With the use of TCD, a significant amount of previous research has evaluated cerebrovascular reactivity in healthy individuals but also in clinical settings, such as patients with heart failure, head trauma, hypertension and following strokes (Puppo et al., 2008, Maeda et al., 1994, Wijnhoud et al., 2006). Relationships between cerebrovascular CO_2 reactivity and systemic endothelial function have been discovered, suggesting an increased CO_2 reactivity response is

positively associated with all-cause mortality and decreased cerebrovascular risk (Lavi et al., 2006, Ainslie et al., 2007b).

CO_2 reactivity can be assessed in three potential ways. The earliest technique used was breath-holding, whereby participants would hold their breath in order to progressively increase CO_2. This technique has many limitations. Individual variability between individuals in many factors such as; metabolic, size of lungs, age all affected the CBF response to breath holding (Fierstra et al., 2013). Following this, the rebreathing of exhaled gas became, and remains, a common technique to achieve increases in $PETCO_2$. This method requires minimal equipment with just an exhaled gas reservoir and gas sensors necessary and allows assessment of ventilatory response. Nevertheless, this method does have limitations, rebreathing of exhaled gas causes a ramp-like response with each breath causing an increase in $PETCO_2$, therefore not allowing a stable measurement of increased CBF. Thirdly, researchers have adopted the use of external CO_2 supplied to a participant by means of a non-rebreathing face mask, with inspired CO_2 concentrations ranging from 2-7% (Fierstra et al., 2013). This method is favourable as it can induce a standardised hypercapnic stimulus (Vernieri et al., 2004).

1.4. Summary

This literature review has sought to provide a historical perspective around the concept of ischaemic preconditioning from animal to human models, and aimed to highlight the evidence that shows RIPC has beneficial effects on both the heart and peripheral blood vessels in humans. The (R)IPC induced mechanisms responsible for these beneficial adaptations are poorly understood and beyond the scope of this review (Heusch et al., 2015). Importantly, whilst animal studies have shown that RIPC can improve aspects of

cerebrovascular function and improve neural recovery from ischaemia, very little is known about its effect on the human cerebrovascular system. Two clinical trials have revealed that RIPC reduces stroke recurrence, suggesting that it may affect cerebral blood flow and/or function, yet no study has examined this in healthy humans to date.

Therefore, the aims of this thesis were to assess the impact of 40 minutes of bilateral arm IPC, or SHAM, on both CO_2 reactivity, cerebral autoregulation and its effect on a hypercapnia-induced dysfunction of cerebral autoregulation. It was hypothesised that an acute bout of RIPC would enhance CO_2 reactivity and cerebral autoregulation, and that RIPC would also attenuate a hypercapnia-induced dysfunction in cerebral autoregulation.

2. CHAPTER 2

The Impact of Ischaemic Preconditioning on Cerebrovascular Function

2.1. Introduction

Ischaemic Preconditioning (IPC) is characterised by brief cycles of ischaemia followed by reperfusion, and has been shown to have cardioprotective effects beyond the vascular bed directly exposed to the IPC stimulus, known as remote (R)IPC (Jones et al., 2014, Przyklenk et al., 1993, Kharbanda et al., 2002). These protective effects attained from RIPC have been demonstrated in both animal and human models, and have typically focused on the heart. Nevertheless, there is accumulating evidence showing that RIPC offers protection to other vascular beds and organs (Koch and Gonzalez, 2013, Jensen et al., 2011).

One of these areas that may also benefit from RIPC relates to the brain. Research investigating the acute impact of RIPC on the brain is limited to animal models, which have indicated that IPC has neuroprotective effects (Jensen et al., 2011). Hoyte *et al* reinforced these findings, by conducting a study in mice and employing 15 minutes of MCA occlusion 72 hours prior to inducing cerebral ischaemia. They reported an increase in regional brain blood flow following the ischaemic injury, measured via magnetic resonance perfusion and laser Doppler flowmetry, in the mice that received the RIPC as well as a reduction in infarct size (Hoyte *et al.*, 2006). Ren and colleagues extended these findings to a RIPC model by applying RIPC to the left femoral artery of rodents, 2 days prior to inducing cerebral ischaemia. It was observed that RIPC caused a reduction in infarction size compared to the animals with no preconditioning (Ren et al., 2008). Interestingly, Ren *et al* identified that the dosage of RIPC has an effect on the protection attained with an RIPC protocol of 2 x 15 minutes of occlusion-reperfusion generated strong protection, whilst 2 x 5 minutes of did not provide protection against ischaemic injury. Taken together this data provides evidence that RIPC can offer cerebrovascular protection against cerebral ischemia.

The only study to date in humans, involving stroke survivors, supports the general notion that RIPC has neuroprotective effects. Meng *et al.,* (2012) employed daily RIPC for 300 days and found improved cerebral perfusion, faster recovery and a 70% lower stroke recurrence compared to standard care (Meng et al., 2012). Despite these observations, no previous study has directly explored the impact of RIPC on cerebrovascular function, and the ability to reduce or prevent the impact of potentially harmful stimuli on the cerebrovascular circulation in humans *in vivo*.

The typical approach to test if RIPC can protect against ischaemia *in vivo* in humans is to induce vascular endothelial dysfunction via ischaemia reperfusion injury. When applied to the forearm, this involves using a blood pressure cuff inflated to a supra-systolic pressure on the upper arm for 20 mins, followed by 20 mins of reperfusion (van den Munckhof et al., 2013). Such procedures are impractical to induce cerebrovascular ischaemia-reperfusion injury. Previous studies have suggested that a sustained period of hypercapnia (inhalation of higher levels of carbon dioxide) transiently impairs cerebral autoregulation (Birch et al., 1995, Zhang et al., 1998), and thus cerebrovascular function. The aim of the current study, therefore was to independently assess the impact of 40 mins of bilateral arm IPC, or SHAM, on CO_2 reactivity, cerebral autoregulation and finally on the effect of hypercapnia-induced dysfunction of cerebral autoregulation. It was hypothesised that RIPC would i) enhance CO_2 reactivity and cerebral autoregulation and ii) attenuate the hypercapnia-induced dysfunction in cerebral autoregulation, compared to SHAM. This study would i) demonstrate the potential impact of RIPC on the cerebrovasculature, and ii) introduce hypercapnia as a potential stimulus to induce cerebrovascular dysfunction that can be prevented by RIPC.

2.2. Methods

2.2.1. Participants

Ten participants **(**8 males and 2 females, Table 2.1) were recruited for the study. Participants were recreationally active, engaging in low to moderate intensity exercise 2-3 days per week. Smokers and individuals with a history of cardiovascular diseases, including diabetes, hypertension or hypercholesterolemia were excluded. Participants were informed of the study protocol verbally and in writing before providing written informed consent. The study was approved by the Liverpool John Moores University ethics committee and adhered to the standards set out in the Declaration of Helsinki (2000).

Table 2-1: Descriptive characteristics of participants.

Characteristics	Group
Age (years)	32.6 ± 11.1
Height (cm)	176.5 ± 6.6
Weight (kg)	81.0 ± 17.3
BMI (kg/m^2)	25.9 ± 4.4
Systolic Blood Pressure (mmHg)	125 ± 10
Diastolic Blood Pressure (mmHg)	61 ± 5
Mean Arterial Pressure (mmHg)	85 ± 7

2.2.2. Research Design

Participants were required to attend two laboratory visits (separated by a minimum of 3 days), that were performed at the same time of day in order to control for diurnal variation in cerebrovascular function (Ainslie et al., 2007a). All participants arrived for testing following an overnight fast and were asked to refrain from alcohol and exercise for 24h and caffeine for 12h before each visit. Visits were randomised and counterbalanced to receive either the

bilateral upper arm RIPC or SHAM condition. Each visit consisted of the bilateral assessment of middle cerebral artery blood flow velocity (MCAv) during RIPC or SHAM. Following a 5 min seated rest period, cerebral autoregulation was assessed using a 5 min squat-stand protocol and a 5 min seated rest period followed by 9 mins of hypercapnia, 4 mins whilst seated, followed by 5 mins of cerebral autoregulation, as above (see Figure 2.1).

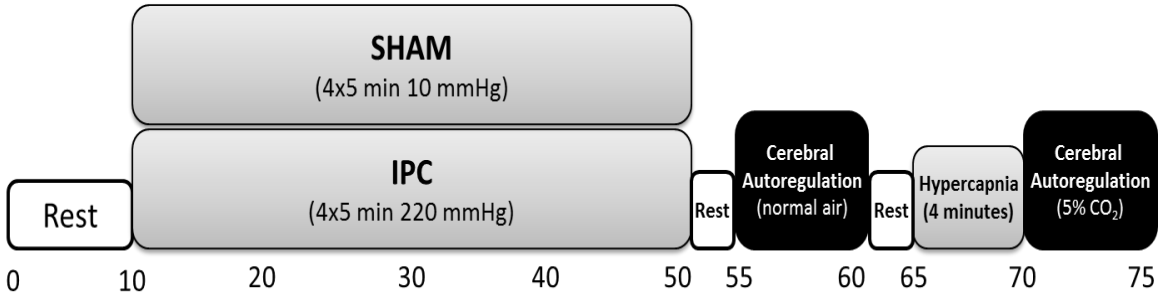

Figure 2.1: Schematic of research design

2.2.3. Measurements

2.2.3.1. Remote Ischaemic Preconditioning and Sham

The RIPC condition consisted of 8 bouts in total involving the inflation of a blood pressure cuff on the upper arm using a rapid inflator (EC-20; D.E Hokanson) to 220 mmHg for 5 minutes. Cuffs were placed bilaterally however were independently inflated, after which the cuff on the contralateral arm was inflated. This allowed for a 5 minute period of reperfusion for each arm. The SHAM condition consisted of an identical protocol, however with the difference that the cuff was inflated to a pressure of 10 mmHg.

2.2.3.2. Middle Cerebral Artery Velocity

Following 10 minutes rest in the supine position, MCAv was continuously measured through the temporal window using transcranial Doppler ultrasonography (TCD). A 2-MHz Doppler probe (Spencer Technologies, Seattle WA, USA) was adjusted until an optimal signal was

identified, as described in detail previously (Willie et al., 2011), and held in place using a Marc 600 head frame (Spencer Technologies, Seattle, USA) to prevent subtle movement of the Doppler probe and maintain insonation angle accuracy. Once the optimal MCA signal was attained in the temporal window, the probe location and machine settings (depth, gain and power) were recorded to identify the same imaging site for both assessments. MCAv was measured bilaterally and the signal that was free of noise or artefact was selected for analysis. Participants were instrumented with a two-way valve-breathing (MLA1028, ADInstruments, Colorado Springs, Colorado, USA) mouthpiece (MLA1026, ADInstruments) from which end tidal CO_2 ($PETCO_2$) was measured using a calibrated gas analyser (ML206, ADInstruments). Continuous beat-by-beat blood pressure was obtained from a digit (Finapres, Amsterdam, Netherlands) and heart rate acquired from a 3 lead electrocardiogram (Powerlab, AD Instruments, Oxford, UK). An index of cerebrovascular resistance (CBVC) was calculated using the ratio of MCAv to mean arterial pressure (MAP). All data was sampled at 50 Hz with a data acquisition system (PowerLab, ADInstruments, Oxford UK) and displayed on LabChart (ADInstruments, Colorado Springs, Colorado, USA).

2.2.3.3. Cerebral Autoregulation

Changes in blood pressure and MCAv was assessed using a squat to stand procedure in order to induce transient changes in arterial blood pressure. Participants replicated the experimenter whilst performing these manoeuvres. Participants performed two sets of squat-stand manoeuvres at 0.10 Hz (5 second squat- 5 second stand) whilst breathing normal atmospheric air, and again whilst breathing a gas concentration of 5% CO_2. The first set of squat stands was preceded by a 5-min of seated rest period. The second set of squat stands followed 4 minutes of hypercapnia, and the order of squats (normal air – 5% CO_2) was counterbalanced.

When the squat-stand with 5% CO_2 mix was performed first, it was ensured that $PETCO_2$ had returned to baseline values before commencing the second set of squats.

2.2.3.4. Hypercapnia

A period of 2 minutes of baseline breathing of normal room air was followed by 4 minutes inhalation of a 5% CO_2, 21% oxygen and nitrogen balance mix from a Douglas bag in order to induce temporary cerebral dysfunction whilst sat in a rested seated position. Cerebral CO_2 reactivity was taken from the final 30 seconds of the 4 minutes of hypercapnia.

2.2.4. Data Reduction

Data was extracted from LabChart averaged over 5 minutes periods prior to and during RIPC or SHAM. Averages of variables were also extracted during each phase of the protocol (e.g. average during 4 mins hypercapnia and 5 min squat-stands). For CO_2 reactivity an average of 3 mins resting baseline and the last 30 secs of the 4 min period of hypercapnia were used. The reactivity was estimated by calculating the slope of the line between increases in MCAv and $PETCO_2$ using linear regression analysis. Data collected from the squat- stands was extracted from LabChart every 0.1 seconds across the 5 minute period. Specifically, MCAv, $PETCO_2$, MAP were the variables used for transfer function analysis.

The relationship between changes in MCAv and arterial BP was assessed via the transfer function analysis in accordance with standardised guidelines (Claassen et al., 2016). Transfer function analysis was performed using MATLAB (2010b; MathWorks-Inc., Natick, MA) in order to calculate associated power (gain) and timing (phases) over three different frequencies; very low (0.02 – 0.07 Hz), low (0.07 – 0.20 Hz) and high (0.20 – 0.50 Hz) (Claassen et al., 2016). Transfer function also produces an estimated reliability of the relationship between the two signals (coherence) (Triedman and Saul, 1994).

2.2.5. Statistical Analysis

A two factor (intervention*time) general linear model was employed to analyse resting MCAv and MAP during the RIPC and SHAM protocol. Similarly, a two factor (intervention*time) general linear model was employed to analyse variables in response to hypercapnia and squat-stands. Statistically significant interactions were followed up with the least significant difference (LSD) approach to multiple comparisons. A paired sample t-test was used to analyse averaged variables during hypercapnia and squat-stands, the relative change in MCAv, $PETCO_2$, MAP during hypercapnia and the reactivity slopes between MCAv and $PETCO_2$. Analysis was conducted using Statistical Package for Social Sciences (Version 17; SPSS Inc., Chicago, IL). Statistical significance was delimited 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 in the text as mean (95% confidence interval) unless otherwise stated.

2.3. Results

2.3.1. Exposure to RIPC or SHAM

MCAv did not change throughout either the 40 min RIPC or SHAM, with no significant main effect for time (*P*=0.728), condition (*P*=0.958) or interaction between condition*time (*P*=0.463, Figure 2.2). A significant main effect for time (*P*=0.016) was evident for MAP across the RIPC and SHAM conditions with MAP increasing 5 mmHg (95% CI: 84, 92) over the 40 min period. There was no main effect for condition (*P*=0.825), or condition*time interaction (*P*=0.762, Figure 2.2).

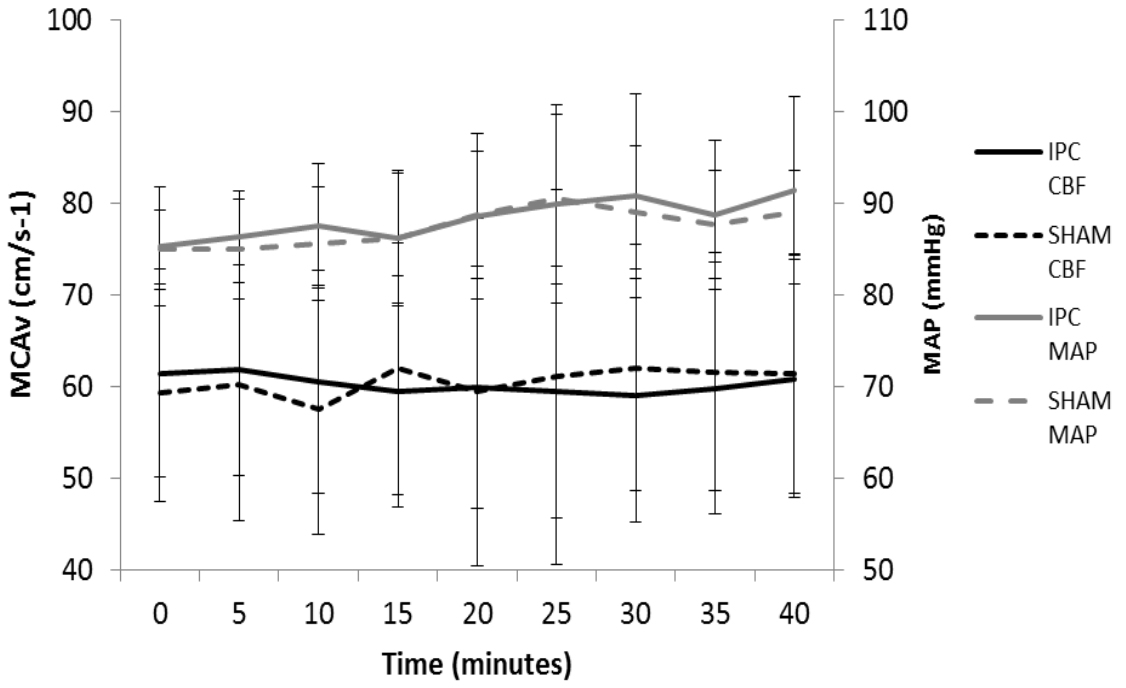

Figure 2.2: Middle cerebral artery velocity (MCAv) and mean arterial blood pressure (MAP) at rest and every 5 minutes during RIPC or SHAM (mean ± SD).

2.3.2. Cardiorespiratory variables measured throughout each cerebrovascular assessment

MAP was significantly higher in the SHAM condition (106 mmHg) during hypercapnia compared RIPC (101 mmHg) (P=0.027, 95% CI: 9.58, 0.76 mmHg). There was no significant difference between conditions for any other cardiorespiratory measurements taken throughout the protocol (Table 2.2).

Table 2-2: Cardiorespiratory variables measured throughout each cerebrovascular assessment in RIPC and SHAM.

	REST			***Cerebral Autoregulation***			***Hypercapnia***			***Hypercapnic Cerebral Autoregulation***		
	RIPC	SHAM	P Value	RIPC	SHAM	P Value	RIPC	SHAM	P Value	RIPC	SHAM	P Value
MAP (mmHg)	85 ± 7	85 ± 4	0.925	99 ± 5	98 ± 8	0.847	101 ± 5	106 ± 6	**0.027**	109 ± 6	110 ± 7	0.720
HR (bpm)	63 ± 7	65 ± 6	0.214	76 ± 9	76 ± 9	0.942	79 ± 11	70 ± 17	0.743	81 ± 7	83 ± 9	0.301
MCAv (cm/s^{-1})	61 ± 11	59 ± 12	0.586	61 ± 11	58 ± 8	0.251	77 ± 11	79 ± 15	0.763	83 ± 13	81 ± 13	0.530
CBVC	0.72 ± 0.10	0.69 ± 0.11	0.419	0.61 ± 0.13	0.59 ± 0.12	0.401	0.76 ± 0.15	0.75 ± 0.11	0.419	0.76 ± 0.15	0.74 ± 0.14	0.536
F_b (breathes /min)	16 ± 3	16 ± 3	0.370	19 ± 3	19 ± 3	0.401	20 ± 4	19± 3	0.771	23 ± 4	23 ± 6	0.602

Abbreviations: MAP, Mean Arterial Pressure; HR, Heart Rate; $PETCO_2$, Partial Pressure Carbon Dioxide; CBVR, Cerebrovascular Conductance; F_b, Frequency of Breathes

2.3.3. Effect of RIPC or SHAM on CO_2 reactivity

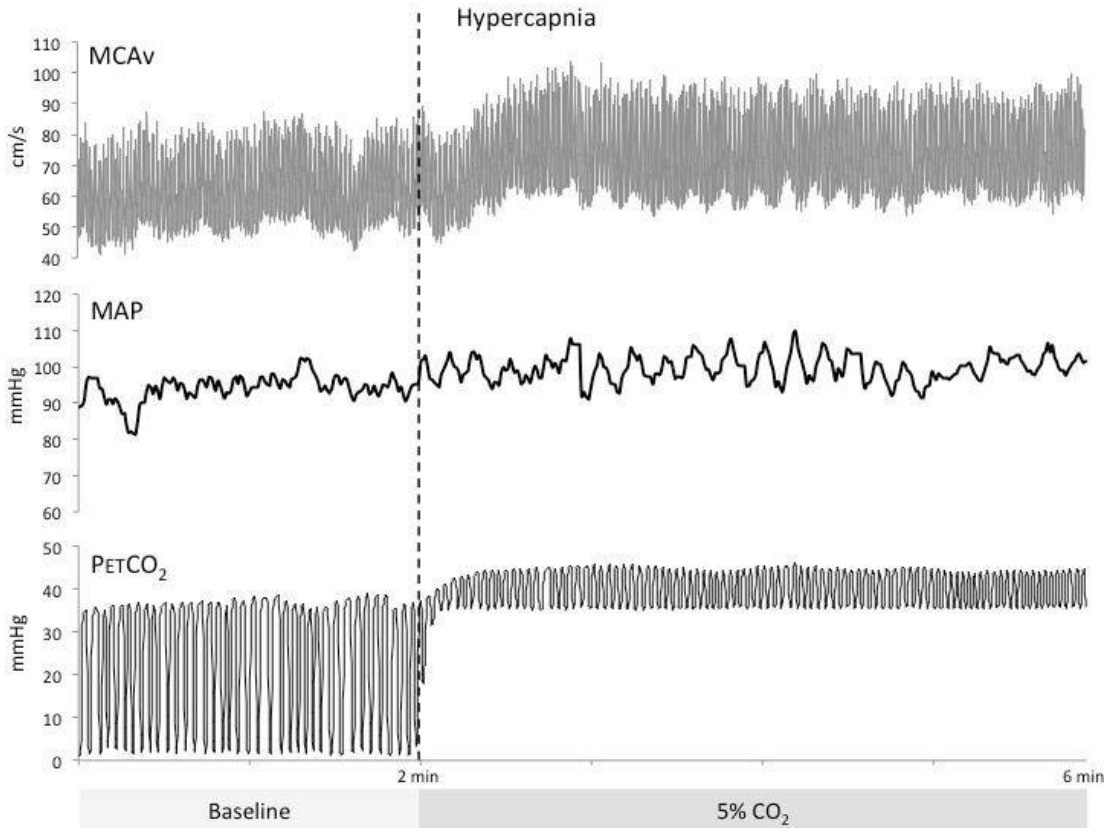

Figure 2.3: An example recording from one representative individual of middle cerebral artery velocity (MCAv), mean arterial pressure (MAP) and end-tidal pressure of carbon dioxide ($PETCO_2$) at baseline and during hypercapnia.

MCAv increased by 18 cm/s (95%CI: 54, 66) during hypercapnia following both the RIPC and SHAM conditions (Figure 2.4), as evidenced by a significant main effect for time (P<0.001). There was no difference in MCAv increases to hypercapnia between conditions (main effect of condition: P=0.959). There was no condition*time interaction (P=0.313).

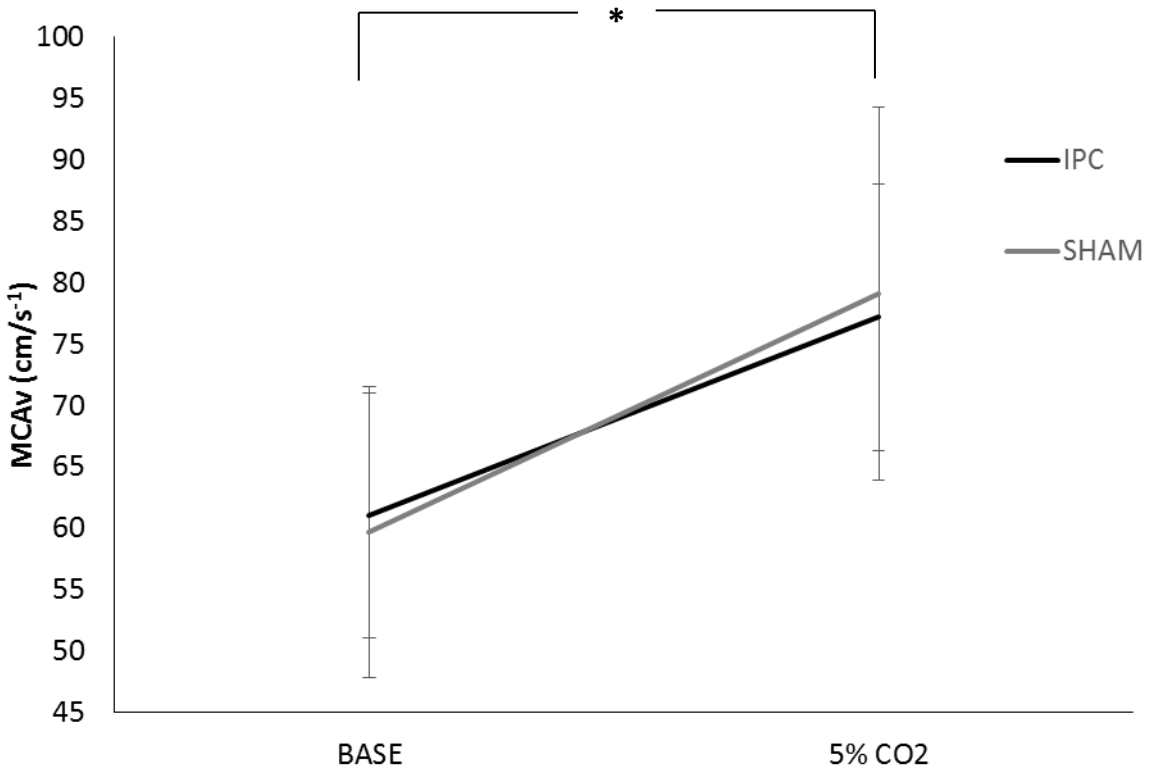

Figure 2.4: Middle cerebral artery velocity (MCAv) when breathing normal air and 5% CO2 (hypercapnia) in RIPC and sham conditions (mean ± SD).

The relative increase in MCAv during hypercapnia when expressed as a relative change from baseline was not different between conditions (P=0.365: Figure 2.5). Similarly, the relative increase in $PETCO_2$ did not differ between conditions (P=0.137) nor MAP (P=0.160).

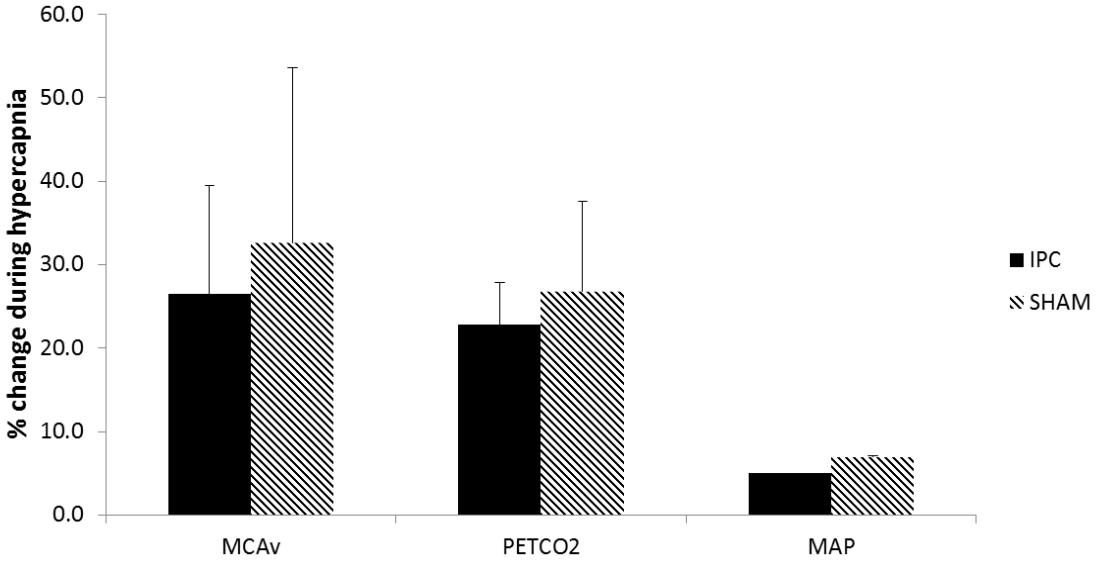

Figure 2.5: Percentage (%) change in middle cerebral artery velocity (MCAv), partial pressure carbon dioxide ($PETCO_2$) and mean arterial pressure (MAP) during hypercapnia in RIPC and sham conditions (mean ± SD)

There was no difference in MCAv and cerebrovascular conductance reactivity slopes between conditions (Table 2.3).

Table 2-3: The reactivity of middle cerebral artery velocity (MCAv) and cerebrovascular conductance per mmHg to an increase in partial pressure carbon dioxide ($PETCO_2$) during hypercapnia (mean ± SD).

	RIPC	SHAM	*P* Values
MCAv **(**cm/s/mmHg **)**	1.86 ± 0.53	2.09 ± 0.90	0.395
CBVC (cm/s/mmHg)	0.02 ± 0.01	0.01 ± 0.01	0.542

Abbreviations: MCAv (middle cerebral artery velocity), CBVC (cerebrovascular conductance).

2.3.4. Squat-stand maneouvers

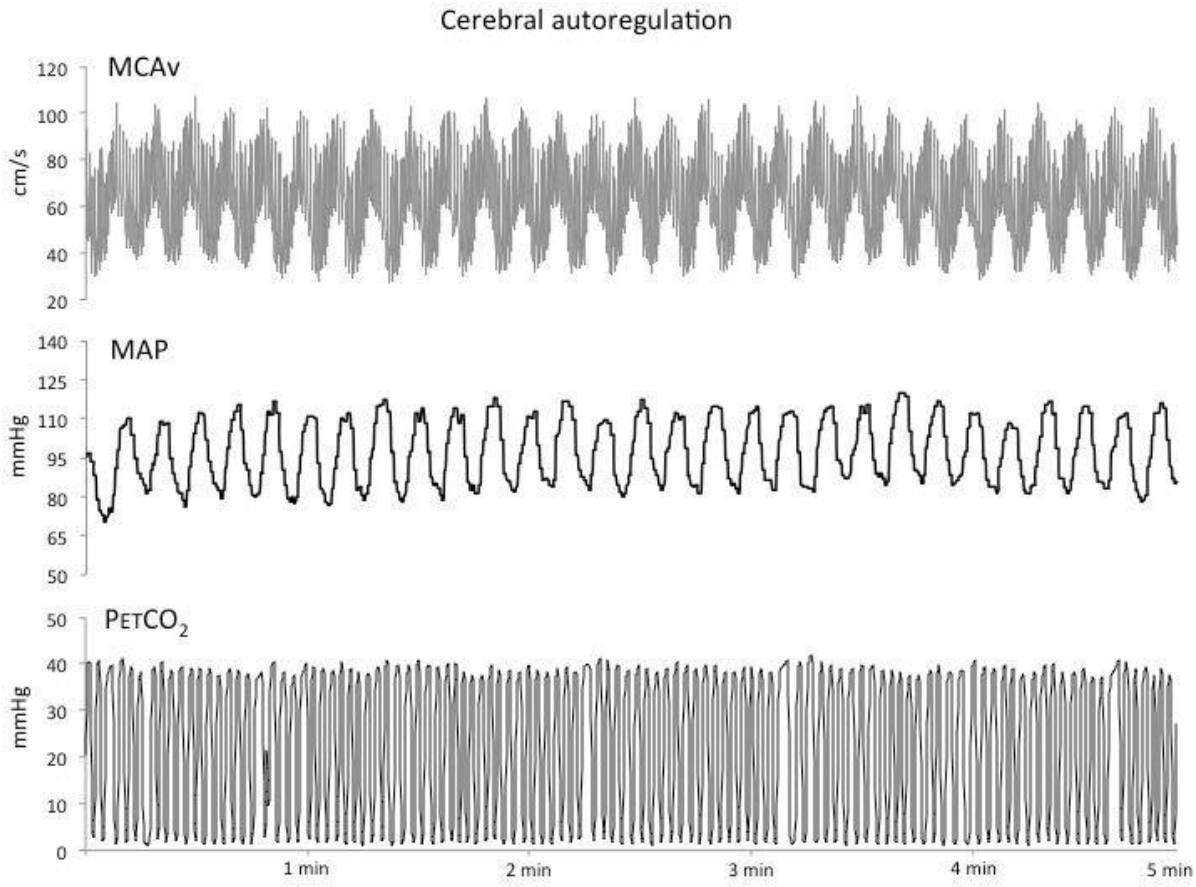

Figure 2.6: An example recording from one representative individual of middle cerebral artery velocity (MCAv), mean arterial pressure (MAP) and end-tidal pressure of carbon dioxide ($PETCO_2$) during the 0.1Hz squat-stand manoeuvres.

Gain at Very Low Frequency (VLF): There was a significant main effect of time (P<0.001) with normalised gain decreasing 0.40 (95%CI: 0.52, 0.28) $cm.s^{-1}.mmHg^{-1}$ under hypercapnic conditions (Figure 2.7). There was no main effect of conditon (P=0.142) nor time*condition interaction (P=0.251). There was no significant main effects or interaction for gain at very low frequency (Table 2.4)

Gain at Low Frequency (LF): There was a significant main effect of time (P<0.001) with normalised gain decreasing by 0.57 (95%CI: 0.74, 0.41) $cm.s^{-1}.mmHg^{-1}$ under hypercapnic conditions (Figure 2.8). No significant main effect for condition (P=0.457) or time*condition

interaction was observed (*P*=0.098). There was a significant main effect of time (*P*=0.018) with low frequency gain decreasing by 0.11 (95%CI: 0.19, 0.02) $cm.s^{-1}.mmHg^{-1}$ under hypercapnic conditions (Table 2.4). There was no significant main effect of condition or interaction for gain at low frequency (Table 2.4)

Gain at High Frequency (HF): There was a significant main effect of time (*P* <0.001) with a normalised gain decreasing by 0.40 (95%CI: 0.55, 0.23) $cm.s^{-1}.mmHg^{-1}$ under hypercapnic conditions. No significant main effect for condition (*P*=0.605) or time*condition interaction was observed (*P*=0.908) (Figure 2.9). There was no significant main effects or interaction for gain at high frequency (Table 2.4)

Table 2-4: Gain determined by transfer function at very low (VLF), low (LF) and high frequency (HF) during repeated squat manoeuvres at 0.10 Hz whilst breathing normal room air and during hypercapnia. Mean ± SD.

	RIPC		**SHAM**		**Two Way ANOVA *P* Values**		
	Normal	*Hypercapnia*	*Normal*	*Hypercapnia*	*Condition*	*Time*	*Int*
VLF ($cm.s^{-1}.mmHg^{-1}$)	0.67 ± 0.15	0.64 ± 0.08	0.68 ± 0.12	0.63 ± 0.15	0.900	0.123	0.793
LF ($cm.s^{-1}.mmHg^{-1}$)	0.90 ± 0.19	0.78 ± 0.13	0.84 ± 0.15	0.75 ± 0.13	0.295	**0.018**	0.724
HF ($cm.s^{-1}.mmHg^{-1}$)	0.73 ± 0.18	0.70 ± 0.10	0.72 ± 0.13	0.70 ± 0.11	0.836	0.491	0.969

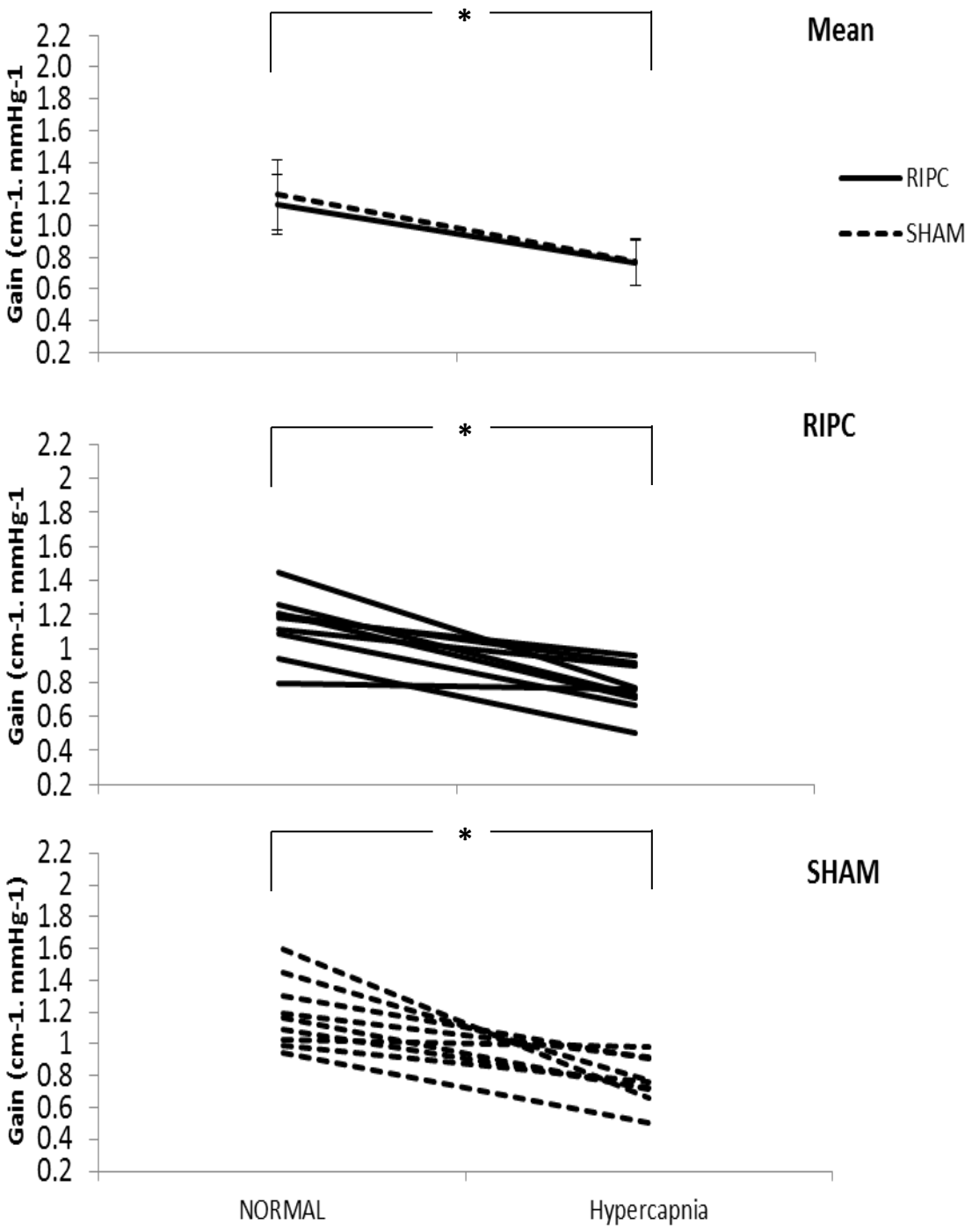

Figure 2.7: Normalised gain in the very low frequency determined by transfer function during repeated squat manoeuvres at 0.10 Hz whilst breathing normal room air and during hypercapnia.

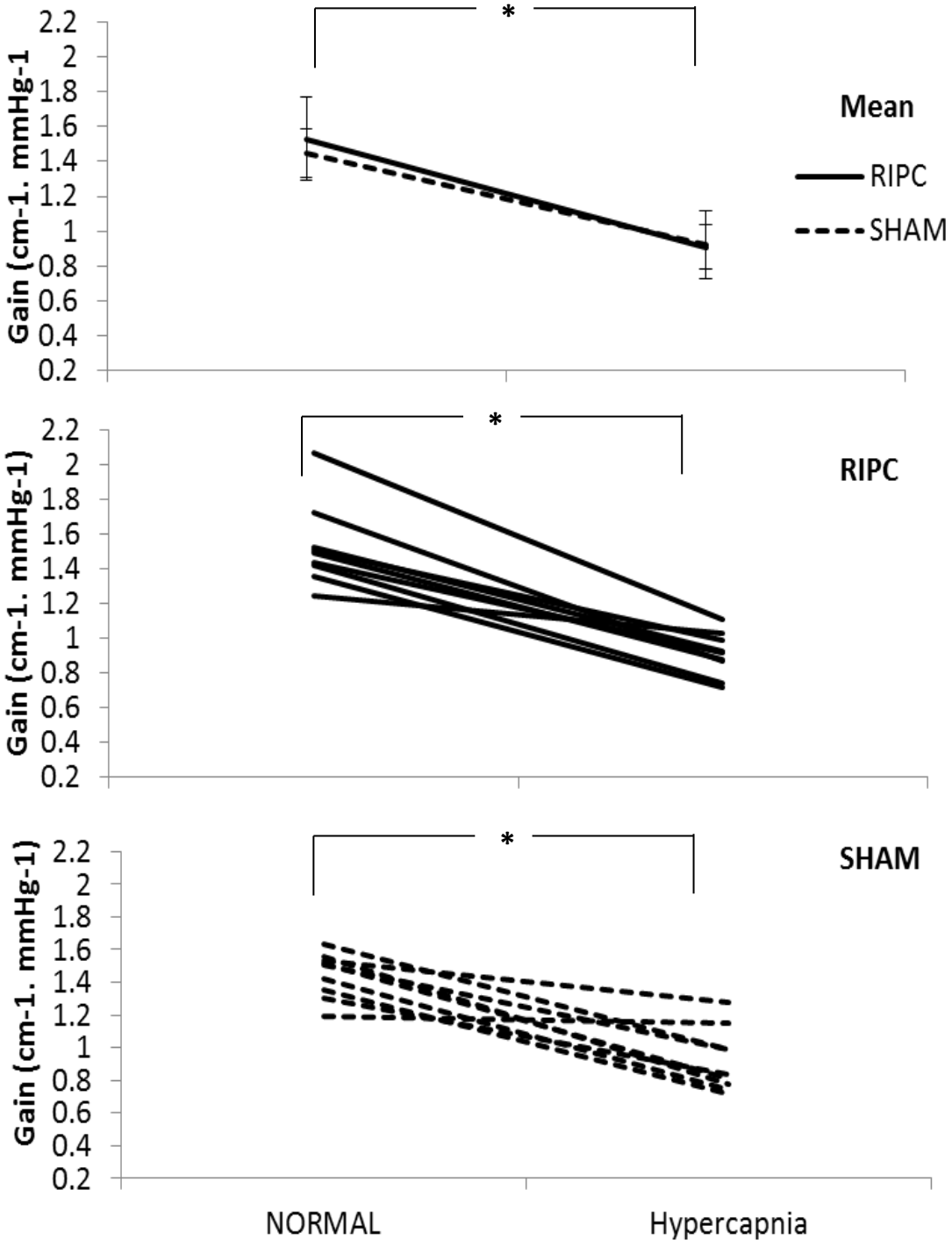

Figure 2.8: Normalised gain in the low frequency determined by transfer function during repeated squat manoeuvres at 0.10 Hz whilst breathing normal room air and during hypercapnia.

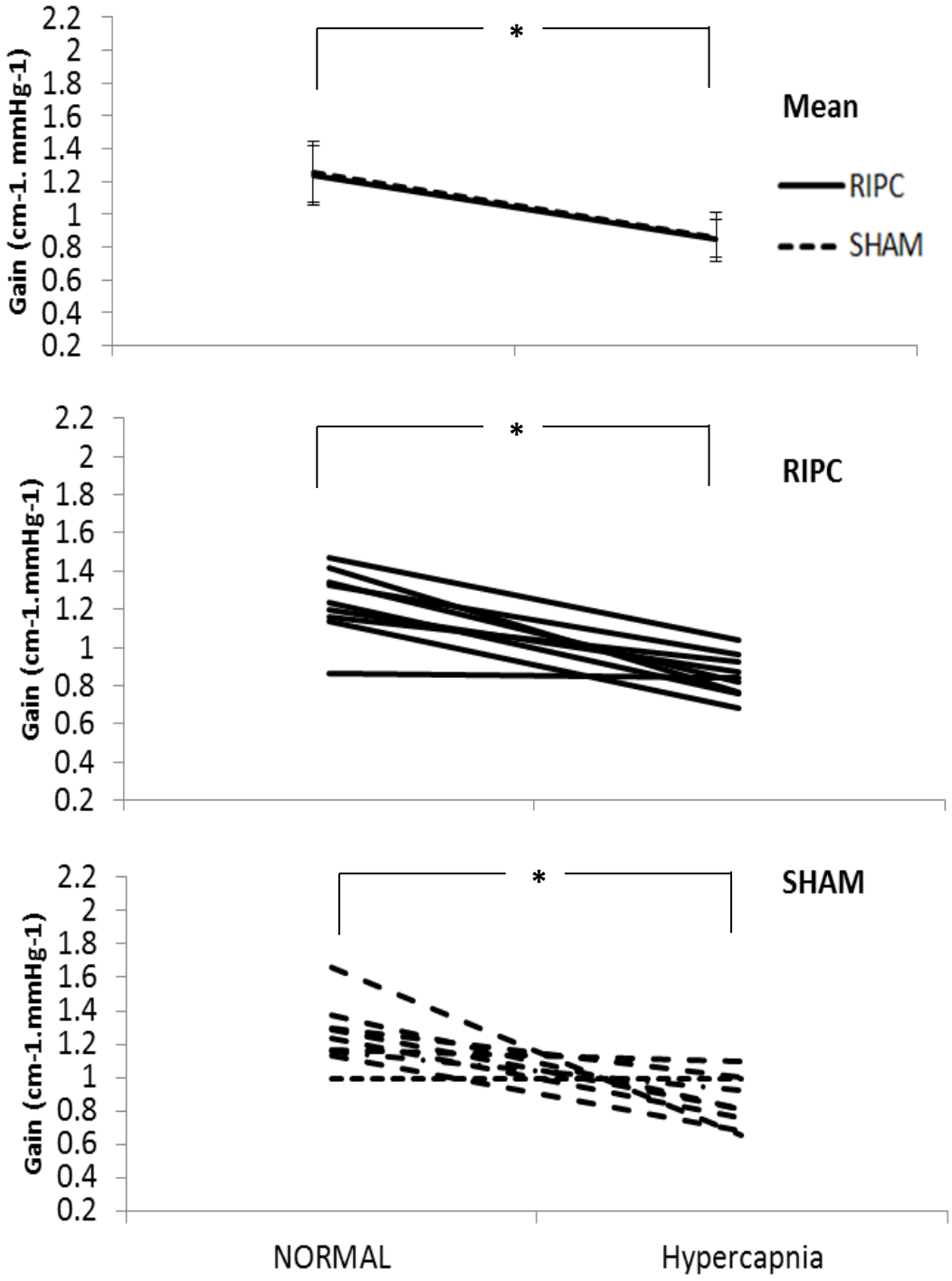

Figure 2.9: Normalised gain in the high frequency determined by transfer function during repeated squat manoeuvres at 0.10 Hz whilst breathing normal room air and during hypercapnia.

Phase at Very Low Frequency (VLF): There was a significant main effect of time (P <0.001) with phase decreasing by 23 (95%CI: 29, 17) radians under hypercapnic conditions (Figure 2.10). No significant main effect for condition (P=0.514) or time*condition interaction was observed (P=0.358).

Phase at Low Frequency (LF): There was a significant main effect of time (P<0.001) with phase decreasing by 10 (95%CI: 14,7) radians under hypercapnic conditions (Figure 2.11). No significant main effect for condition (P=0.655) or time*condition interaction was observed (P=0.966).

Phase at High Frequency (HF): There was no significant main effect for condition (P=0.914), time (P=0.677) or time*condition (P=0.095; Figure 2.11).

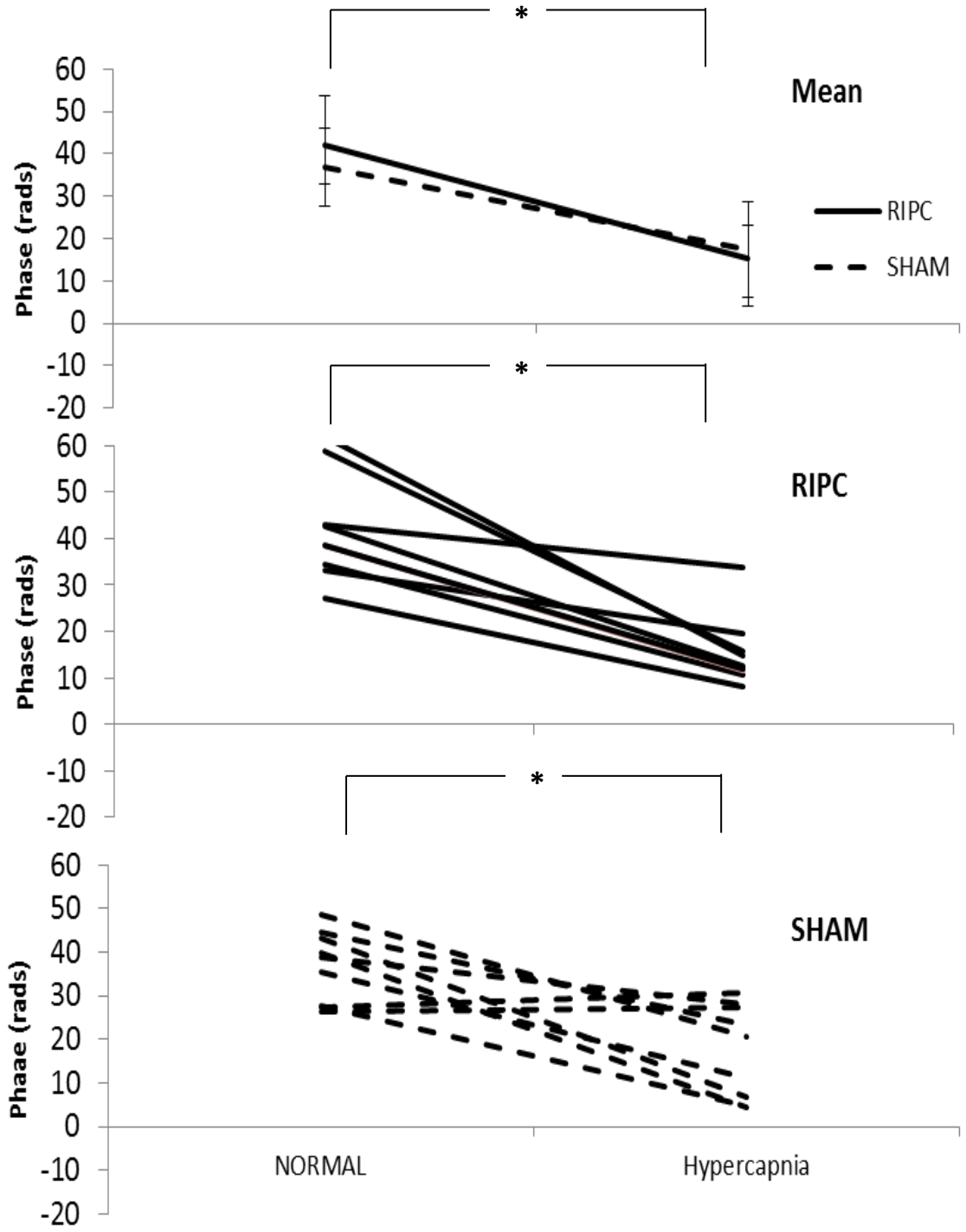

Figure 2.10: Phase in the very low frequency determined by transfer function during repeated squat manoeuvres at 0.10 Hz whilst breathing normal room air and during hypercapnia.

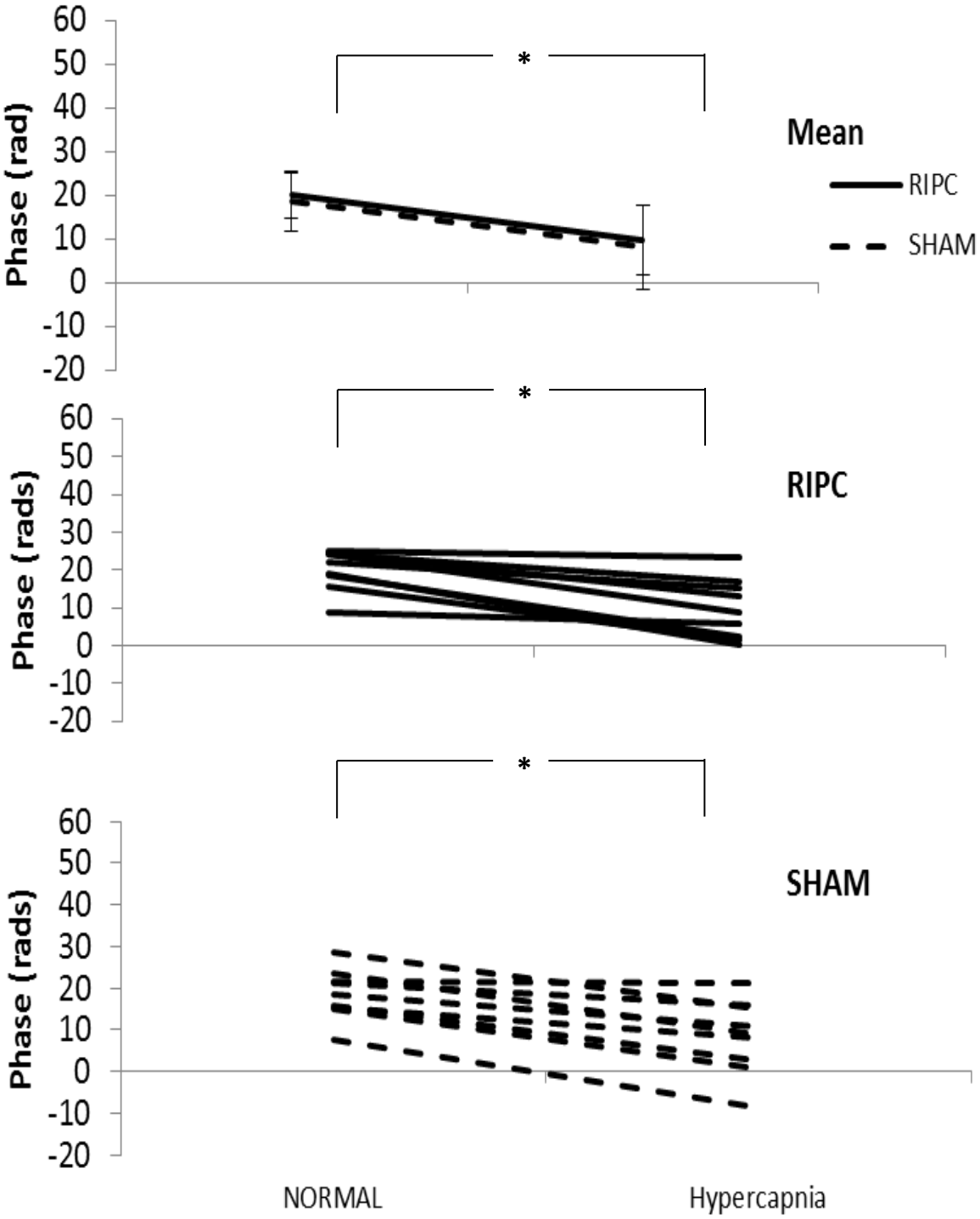

Figure 2.11: Phase in the low frequency determined by transfer function during repeated squat manoeuvres at 0.10 Hz whilst breathing normal room air and during hypercapnia.

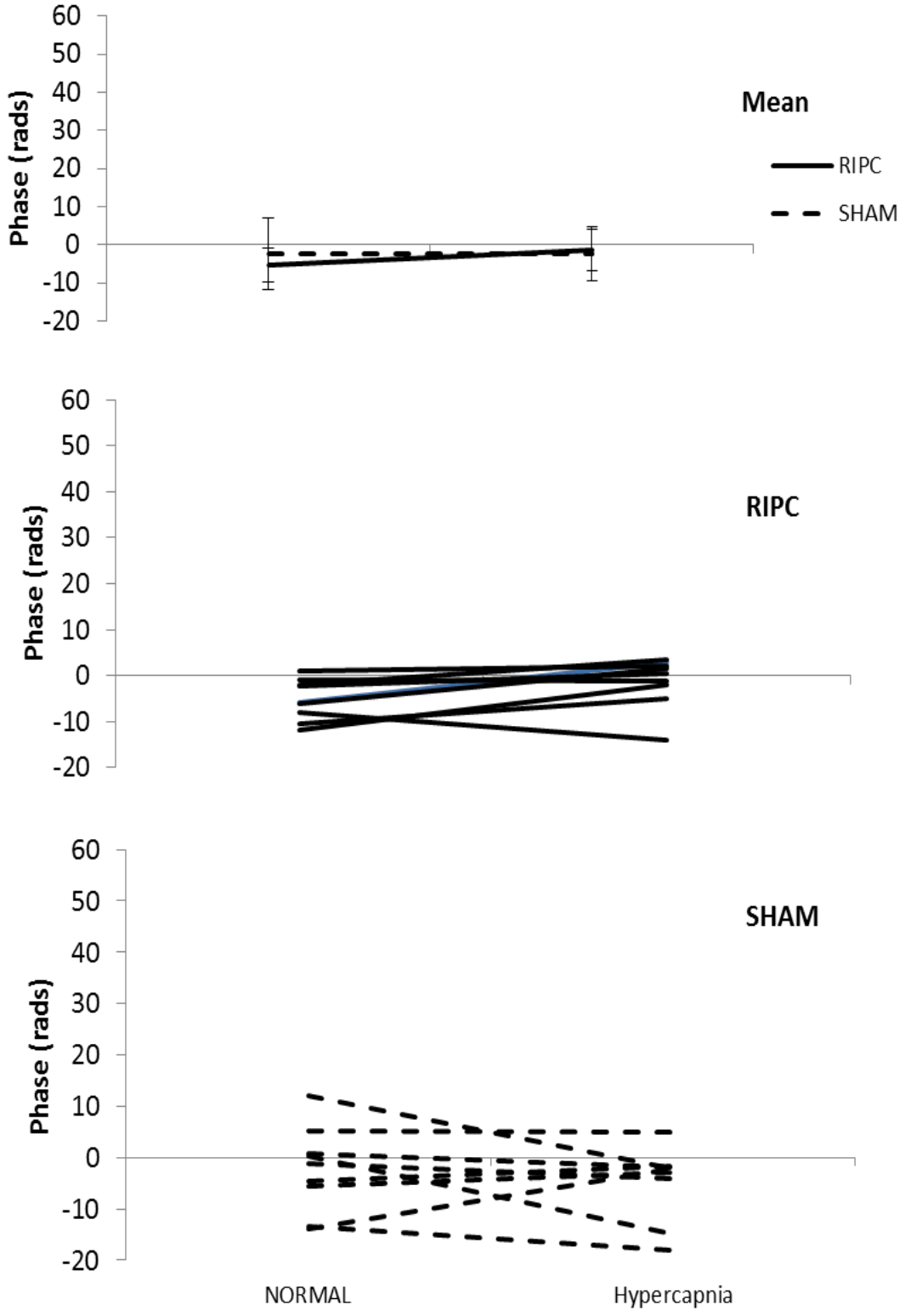

Figure 2.12: Phase in the high frequency determined by transfer function during repeated squat manoeuvres at 0.10 Hz whilst breathing normal room air and during hypercapnia.

Coherence: There was a signficant main effect of time with very low frequency coherance increasing by 0.109. There was no significant main effect of condition or condition*time interaction for very low frequcency coherance (Table 2.5). There was no significant main effects or condition*time interaction in for low and high frequency coherance.

Table 2-5: Coherence determined by transfer function at very low (VLF), low (LF) and high frequency (HF) during repeated squat manoeuvres at 0.10 Hz whilst breathing normal room air and during hypercapnia

	RIPC		**SHAM**		**Two Way ANOVA *P* Values**		
	Normal	*Hypercapnia*	*Normal*	*Hypercapnia*	*Condition*	*Time*	*Int*
VLF	0.63 ± 0.13	0.78 ± 0.06	0.70 ± 0.20	0.77 ± 0.95	0.615	**0.003**	0.430
LF	0.88 ± 0.37	0.82 ± 0.14	0.84 ± 0.08	0.82 ± 0.09	0.765	0.174	0.530
HF	0.82 ± 0.11	0.76 ± 0.12	0.74 ± 0.17	0.72 ± 0.16	0.194	0.267	0.552

2.4. Discussion

The aim of the present study was to determine whether 40 mins of bilateral arm RIPC could enhance cerebrovascular CO_2 reactivity and cerebral autoregulation compared to a SHAM condition. This study also aimed to investigate the impact of RIPC on hypercapnia-induced impairment of cerebral autoregulation. There was no effect of RIPC on cerebrovascular responses to CO_2 reactivity or autoregulation, and RIPC did not attenuate the hypercapnia-induced impairment in cerebral autoregulation. This study is the first to investigate the impact of RIPC on a range of cerebrovascular functional tests in humans. The results of this study suggest that an acute bout of RIPC does not impart immediate cerebrovascular benefits in healthy individuals.

Despite the considerable literature on the effects of RIPC on cardiac and peripheral vascular function in humans, very little research work has been performed on its effect/s on cerebrovascular function, despite stroke and cerebrovascular disease being a leading cause of death worldwide (Roger et al., 2012). This may relate to the difficulties in assessing cerebrovascular function. Whilst, there are a number of tests available for the assessment of peripheral artery function, no such specific test is available to assess cerebrovascular health. The intrinsic mechanisms involved in the maintenance of CBF are highly complex and integrated, and known to be highly sensitive to changes in perfusion pressure (blood pressure) and arterial gases, particularly carbon dioxide. In this context, tests of cerebral autoregulation and CO_2 reactivity were employed in the current study as markers of cerebrovascular function to best gauge the impact of RIPC.

One novel aspect of this study was that both MAP and MCAv, as an index of CBF, were measured during the RIPC and SHAM stimuli to determine the direct impact of RIPC on cerebrovascular control. Previous research studies have demonstrated that acute exposure to RIPC leads to increased blood flow in conduit and resistance arteries in distant areas (such as

the contralateral limb) (Enko et al., 2011) or organs (e.g. the heart) (Zhou et al., 2007, Shimizu et al., 2007) and also to enhanced cutaneous tissue oxygen saturation and arterial capillary blood flow (Kraemer et al., 2011). In contrast to the previous studies, the results of the current study suggest that CBF is not altered during a single episode of RIPC (4 x 5 mins of ischemia and reperfusion). Nevertheless, blood pressure did increase transiently during the RIPC but this increase was also evident in SHAM.

Cerebral autoregulation was assessed using squat-stand manoeuvres at 0.1 Hz (i.e. 6 squats per minute) in the current study. A single episode of RIPC employed immediately prior to the squat-stand manoeuvres did not alter the oscillations between CBF and MAP (transfer function determined gain or phase) when compared to SHAM in the current study. In addition, there was no change in cerebral CO_2 reactivity between conditions. Interestingly, the blood pressure response during hypercapnia appeared to be lower in the RIPC condition, whilst this was not statistically significant, this could suggest a cardio-protective role of RIPC in lowering the pressor response during the hypercapnic stimulus without affecting the magnitude of the cerebral blood flow response. Nonetheless, the apparent lack of impact of an acute bout of RIPC on resting cerebrovascular function may not be surprising as all but one previous study (Moro et al., 2011) has shown that RIPC does not acutely change endothelial function in conduit arteries (Loukogeorgakis et al., 2005, Kharbanda et al., 2002, van den Munckhof et al., 2013). It is likely that a more frequent dose of RIPC (e.g. repeated RIPC) is required to improve CBF and/or cerebrovascular function, given that the only other human RIPC studies measuring cerebrovascular outcomes employed a repeated daily RIPC protocol lasting 300 days (Meng et al., 2012) and 180 days (Meng et al., 2015) and found improvements in cerebral perfusion. Other factors that may explain the absence of an improvement in cerebrovascular function may relate to the group that was assessed. These aforementioned studies included individuals with chronic cerebrovascular dysfunction

(stenosis); therefore it is possible an acute cerebral benefit due to RIPC may be more likely in this cohort due to their lower cardiovascular health a priori. Furthermore, it is important to note that repeated RIPC has improved endothelial function in healthy individuals in conduit, resistance and cutaneous vessels (Jones et al., 2015, Jones et al., 2014, Kimura et al., 2007). Taken together, these results indicate that exposure to a single bout of RIPC does not affect cerebrovascular function in healthy individuals.

This study also aimed to investigate if RIPC could attenuate or prevent hypercapnia-induced impairment of cerebral autoregulation, compared to a SHAM condition. Previous studies have suggested that a sustained period of hypercapnia (inhalation of higher levels of carbon dioxide) impairs cerebral autoregulation (Birch et al., 1995, Zhang et al., 1998), and thus cerebrovascular function. Therefore, the combination of hypercapnia and squat-stand manoeuvres was employed to investigate if RIPC could indeed attenuate cerebrovascular dysfunction. The inhalation of a higher level of CO_2 caused alterations in the oscillations between CBF and MAP shown via a decrease in phase determined via transfer function analysis, which suggests that hypercapnia was successful in causing impaired cerebral autoregulation. Nevertheless, and contrary to the hypothesis, RIPC did not attenuate this impairment compared to SHAM. There are a number of potential reasons for the lack of effect of RIPC on cerebral autoregulation during hypercapnia. Firstly, RIPC is known to show two windows of protection with the first window lasting 2-3 hours after the RIPC stimulus, and a second window appearing 24 hours post RIPC and lasting up to 72 hours (Heusch et al., 2015). The previous studies exploring the impact of RIPC on cerebrovascular dysfunction in animal models (e.g. experimental models of inducing a stroke) have focused on the second window of protection whereby cerebrovascular dysfunction has been assessed 48-72 hours following the RIPC stimulus (Hoyte et al., 2006, Ren et al., 2008). In this study,

assessment of cerebrovascular function was planned approximately 10 minutes after RIPC. Whether differences in the timing and/or efficacy of the window(s) of protection are present between coronary and cerebral vessels is currently unknown. Some, but not all, previous research studies, albeit investigating the impact of RIPC on exercise performance, have suggested that RIPC requires a longer period than used in this current study to offer significant beneficial effects (Bailey et al., 2012). Therefore, it is possible that in the current study cerebrovascular measurements and cerebral dysfunction may have been employed too soon following the RIPC bout.

One final explanation may be related to the dose and timing of the hypercapnia that was employed to cause cerebrovascular dysfunction. It is possible that a higher level of CO_2 (e.g. 6%) (Coverdale et al., 2016) or inhalation for a longer time period may have induced a greater level of cerebrovascular dysfunction. Given that a decrease in gain determined via transfer function was observed during hypercapnia in both RIPC and SHAM conditions, which is usually associated with more efficient cerebral autoregulation (van Beek et al., 2008), it is plausible that a sufficient decrease cerebrovascular dysfunction was not induced. Additionally, this decrease in both gain and phase may outline that rather than inducing a dysfunction to cerebral autoregulation with hypercapnia, an offset to the timing of autoregulation occurred instead.

Conflicting responses in transfer function outputs (i.e. gain and phase) may potentially be as a result of methodological differences between this study and previous studies. Whilst Zhang et al. (1998) employed a high percentage of CO_2 inhalation during autoregulation, this current study started 5% CO_2 four minutes prior to autoregulation, therefore the immediate responses to gas concentration may have already occurred before autoregulation had begun, as opposed to during the assessment of autoregulation (Zhang et al., 1998). Additionally, differences in how cerebral autoregulation was assessed itself may have caused the conflicting response in

transfer function gain, with previous a study adopting the rapid thigh cuff technique (Zhang et al., 1998) as opposed to the more modern squat-stand technique used in this current study. Regardless, hypercapnia was sufficient in altering the cerebro-functional response to autoregulation, and as the response following RIPC was not different, it suggests RRIPC did not affect cerebrovascular function.

2.4.1. Methodological Considerations

As is inherent in the use of TCD, it is assumed that the diameter of the imaged vessel does not change, which may have influenced the findings of the current study, particularly during hypercapnia. Nevertheless, the absolute CO_2 reactivity responses between the IPC and Sham conditions were similar, indicating similar cerebrovascular responses. This suggests that if the MCA dilated, it was likely to have dilated to the same extent in both conditions, and therefore the within-subject comparison between conditions remains robust.

2.4.2. Clinical Perspectives:

Impairment in cerebrovascular function can lead to serious conditions, such as dementia, cognitive impairment and stroke. With recent clinical trials showing that repeated RIPC can reduce stroke patients future susceptibility to strokes (Meng et al., 2012, Meng et al., 2015), it indicates that despite the results from this study, RIPC could have a cerebral/neural impact that offers a non-invasive, simple and inexpensive strategy to improve clinical consequences of stroke in humans. Therefore, the impact of (R)IPC on cerebrovascular function warrants further research.

2.4.3. Future Directions:

Despite RIPC having no impact on cerebrovascular function in this study, there is still much to explore regarding IPC and the cerebrovascular system. Moving forward, future studies should consider the timing of the RIPC stimulus in relation to the timing of examination of cerebrovascular function. RIPC generates two separate windows of protection, therefore future studies should consider examining cerebrovascular function on multiple occasions to more comprehensively examine the impact of RIPC. In addition, it is possible that the level of impairment in cerebrovascular function induced by the hypercapnia was not sufficient enough to determine whether RIPC can attenuate any potential difference. Therefore future studies may also induce a greater state of hypercapnia, either by using a higher CO_2% and/or duration. Healthy individuals were assessed in the current study and it could be argued that no effect of IPC was observed due to the fact their cerebrovascular function cannot be significantly improved on, therefore repeating the current study in a clinical group with vascular dysfunction may 'un-mask' the effect of IPC on cerebrovascular function. Finally, future studies should consider the dosage of the RIPC applied, with repeated bouts potentially providing a more potent and longer lasting effect (Przyklenk and Whittaker, 2011).

Conclusion

In summary, this study reports that an acute bout of bilateral arm RIPC did not alter cerebral blood flow or enhance cerebral CO_2 reactivity or autoregulation, compared to a SHAM condition in healthy individuals. In addition, RIPC did not attenuate the impairment in cerebral autoregulation induced by hypercapnia.

AASLID, R. 1986. The Doppler Principle Applied to Measurement of Blood Flow Velocity in Cerebral Arteries. ***In:*** **AASLID, R. (ed.)** ***Transcranial Doppler Sonography.*** **Vienna: Springer Vienna.**

AASLID, R., MARKWALDER, T. M. & NORNES, H. 1982. Noninvasive transcranial Doppler ultrasound recording of flow velocity in basal cerebral arteries. ***J Neurosurg,*** **57, 769-74.**

AINSLIE, P. N. & DUFFIN, J. 2009. Integration of cerebrovascular CO2 reactivity and chemoreflex control of breathing: mechanisms of regulation, measurement, and interpretation. ***Am J Physiol Regul Integr Comp Physiol,*** **296, R1473-95.**

AINSLIE, P. N., MURRELL, C., PEEBLES, K., SWART, M., SKINNER, M. A., WILLIAMS, M. J. & TAYLOR, R. D. 2007a. Early morning impairment in cerebral autoregulation and cerebrovascular CO2 reactivity in healthy humans: relation to endothelial function. ***Exp Physiol,*** **92, 769-77.**

AINSLIE, P. N., MURRELL, C., PEEBLES, K., SWART, M., SKINNER, M. A., WILLIAMS, M. J. A. & TAYLOR, R. D. 2007b. Early morning impairment in cerebral autoregulation and cerebrovascular CO2 reactivity in healthy humans: relation to endothelial function. ***Experimental Physiology,*** **92, 769-777.**

ARIES, M. J., ELTING, J. W., DE KEYSER, J., KREMER, B. P. & VROOMEN, P. C. 2010. Cerebral autoregulation in stroke: a review of transcranial Doppler studies. ***Stroke,*** **41, 2697-704.**

ARNOLDS, B. J. & VON REUTERN, G. M. 1986. Transcranial Doppler sonography. Examination technique and normal reference values. ***Ultrasound Med Biol,*** **12, 115-23.**

BAILEY, T. G., JONES, H., GREGSON, W., ATKINSON, G., CABLE, N. T. & THIJSSEN, D. H. 2012. Effect of ischemic preconditioning on lactate accumulation and running performance. ***Med Sci Sports Exerc,*** **44, 2084-9.**

BAYLISS, W. M., HILL, L. & GULLAND, G. L. 1895. On Intra-Cranial Pressure and the Cerebral Circulation: Part I. Physiological; Part II. Histological. ***J Physiol,*** **18, 334-62.**

BIRCH, A. A., DIRNHUBER, M. J., HARTLEY-DAVIES, R., IANNOTTI, F. & NEIL-DWYER, G. 1995. Assessment of autoregulation by means of periodic changes in blood pressure. ***Stroke,*** **26, 834-7.**

BOTKER, H. E., KHARBANDA, R., SCHMIDT, M. R., BOTTCHER, M., KALTOFT, A. K., TERKELSEN, C. J., MUNK, K., ANDERSEN, N. H., HANSEN, T. M.,

TRAUTNER, S., LASSEN, J. F., CHRISTIANSEN, E. H., KRUSELL, L. R., KRISTENSEN, S. D., THUESEN, L., NIELSEN, S. S., REHLING, M., SORENSEN, H. T., REDINGTON, A. N. & NIELSEN, T. T. 2010. Remote ischaemic conditioning before hospital admission, as a complement to angioplasty, and effect on myocardial salvage in patients with acute myocardial infarction: a randomised trial. *Lancet,* 375, 727-34.

BOYAJIAN, R. A., SCHWEND, R. B., WOLFE, M. M., BICKERTON, R. E. & OTIS, S. M. 1995. Measurement of anterior and posterior circulation flow contributions to cerebral blood flow. An ultrasound-derived volumetric flow analysis. *J Neuroimaging,* 5, 1-3.

BREVOORD, D., KRANKE, P., KUIJPERS, M., WEBER, N., HOLLMANN, M. & PRECKEL, B. 2012. Remote ischemic conditioning to protect against ischemia-reperfusion injury: a systematic review and meta-analysis. *PLoS One,* 7, e42179.

BRIAN, J. E., JR., FARACI, F. M. & HEISTAD, D. D. 1996. Recent insights into the regulation of cerebral circulation. *Clin Exp Pharmacol Physiol,* 23, 449-57.

CANDILIO, L., MALIK, A., ARITI, C., BARNARD, M., DI SALVO, C., LAWRENCE, D., HAYWARD, M., YAP, J., ROBERTS, N., SHEIKH, A., KOLVEKAR, S., HAUSENLOY, D. J. & YELLON, D. M. 2015. Effect of remote ischaemic preconditioning on clinical outcomes in patients undergoing cardiac bypass surgery: a randomised controlled clinical trial. *Heart,* 101, 185-92.

CLAASSEN, J. A., MEEL-VAN DEN ABEELEN, A. S., SIMPSON, D. M. & PANERAI, R. B. 2016. Transfer function analysis of dynamic cerebral autoregulation: A white paper from the International Cerebral Autoregulation Research Network. *J Cereb Blood Flow Metab,* 36, 665-80.

COVERDALE, N. S., BADROV, M. B. & KEVIN SHOEMAKER, J. 2016. Impact of age on cerebrovascular dilation versus reactivity to hypercapnia. *J Cereb Blood Flow Metab*.

D'ASCENZO, F., CAVALLERO, E., MORETTI, C., OMEDE, P., SCIUTO, F., RAHMAN, I. A., BONSER, R. S., YUNSEOK, J., WAGNER, R., FREIBERGER, T., KUNST, G., MARBER, M. S., THIELMANN, M., JI, B., AMR, Y. M., MODENA, M. G., ZOCCAI, G. B., SHEIBAN, I. & GAITA, F. 2012. Remote ischaemic preconditioning in coronary artery bypass surgery: a meta-analysis. *Heart,* 98, 1267-71.

DICKSON, E. W., LORBAR, M., PORCARO, W. A., FENTON, R. A., REINHARDT, C. P., GYSEMBERGH, A. & PRZYKLENK, K. 1999. Rabbit heart can be

"preconditioned" via transfer of coronary effluent. *Am J Physiol,* 277, H2451-7.

ENKO, K., NAKAMURA, K., YUNOKI, K., MIYOSHI, T., AKAGI, S., YOSHIDA, M., TOH, N., SANGAWA, M., NISHII, N., NAGASE, S., KOHNO, K., MORITA, H., KUSANO, K. F. & ITO, H. 2011. Intermittent arm ischemia induces vasodilatation of the contralateral upper limb. *J Physiol Sci,* 61, 507-13.

EUSER, A. G. & CIPOLLA, M. J. 2007. Cerebral blood flow autoregulation and edema formation during pregnancy in anesthetized rats. *Hypertension,* 49, 334-40.

FIERSTRA, J., SOBCZYK, O., BATTISTI-CHARBONNEY, A., MANDELL, D. M., POUBLANC, J., CRAWLEY, A. P., MIKULIS, D. J., DUFFIN, J. & FISHER, J. A. 2013. Measuring cerebrovascular reactivity: what stimulus to use? *J Physiol,* 591, 5809-21.

FRANCO FOLINO, A. 2007. Cerebral autoregulation and syncope. *Prog Cardiovasc Dis,* 50, 49-80.

HEUSCH, G. 2013. Cardioprotection: chances and challenges of its translation to the clinic. *Lancet,* 381, 166-75.

HEUSCH, G., BØTKER, H. E., PRZYKLENK, K., REDINGTON, A. & YELLON, D. 2015. Remote Ischemic Conditioning. *Journal of the American College of Cardiology,* 65, 177-195.

HOYTE, L. C., PAPADAKIS, M., BARBER, P. A. & BUCHAN, A. M. 2006. Improved regional cerebral blood flow is important for the protection seen in a mouse model of late phase ischemic preconditioning. *Brain Res,* 1121, 231-7.

HUFFMAN, L. C., KOCH, S. E. & BUTLER, K. L. 2008. Coronary effluent from a preconditioned heart activates the JAK-STAT pathway and induces cardioprotection in a donor heart. *Am J Physiol Heart Circ Physiol,* 294, H257-62.

JABS, A., FASOLA, F., MUXEL, S., MUNZEL, T. & GORI, T. 2010. Ischemic and non-ischemic preconditioning: Endothelium-focused translation into clinical practice. *Clin Hemorheol Microcirc,* 45, 185-91.

JACKSON, W. F. 2005. Potassium channels and proliferation of vascular smooth muscle cells. *Circ Res,* 97, 1211-2.

JENKINS, D. P., PUGSLEY, W. B., ALKHULAIFI, A. M., KEMP, M., HOOPER, J. & YELLON, D. M. 1997. Ischaemic preconditioning reduces troponin T release in patients undergoing coronary artery bypass surgery. *Heart,* 77, 314-8.

JENSEN, H. A., LOUKOGEORGAKIS, S., YANNOPOULOS, F., RIMPILAINEN, E., PETZOLD, A., TUOMINEN, H., LEPOLA, P., MACALLISTER, R. J., DEANFIELD, J. E., MAKELA, T., ALESTALO, K., KIVILUOMA, K., ANTTILA,

V., TSANG, V. & JUVONEN, T. 2011. Remote ischemic preconditioning protects the brain against injury after hypothermic circulatory arrest. *Circulation,* 123, 714-21.

JONES, H., HOPKINS, N., BAILEY, T. G., GREEN, D. J., CABLE, N. T. & THIJSSEN, D. H. 2014. Seven-day remote ischemic preconditioning improves local and systemic endothelial function and microcirculation in healthy humans. *Am J Hypertens,* 27, 918-25.

JONES, H., NYAKAYIRU, J., BAILEY, T. G., GREEN, D. J., CABLE, N. T., SPRUNG, V. S., HOPKINS, N. D. & THIJSSEN, D. H. 2015. Impact of eight weeks of repeated ischaemic preconditioning on brachial artery and cutaneous microcirculatory function in healthy males. *Eur J Prev Cardiol,* 22, 1083-7.

KASSAB, M. Y., MAJID, A., FAROOQ, M. U., AZHARY, H., HERSHEY, L. A., BEDNARCZYK, E. M., GRAYBEAL, D. F. & JOHNSON, M. D. 2007. Transcranial Doppler: an introduction for primary care physicians. *J Am Board Fam Med,* 20, 65-71.

KETY, S. S. & SCHMIDT, C. F. 1948. THE EFFECTS OF ALTERED ARTERIAL TENSIONS OF CARBON DIOXIDE AND OXYGEN ON CEREBRAL BLOOD FLOW AND CEREBRAL OXYGEN CONSUMPTION OF NORMAL YOUNG MEN. *J Clin Invest,* 27, 484-92.

KHARBANDA, R. K., MORTENSEN, U. M., WHITE, P. A., KRISTIANSEN, S. B., SCHMIDT, M. R., HOSCHTITZKY, J. A., VOGEL, M., SORENSEN, K., REDINGTON, A. N. & MACALLISTER, R. 2002. Transient limb ischemia induces remote ischemic preconditioning in vivo. *Circulation,* 106, 2881-3.

KHARBANDA, R. K., PETERS, M., WALTON, B., KATTENHORN, M., MULLEN, M., KLEIN, N., VALLANCE, P., DEANFIELD, J. & MACALLISTER, R. 2001. Ischemic preconditioning prevents endothelial injury and systemic neutrophil activation during ischemia-reperfusion in humans in vivo. *Circulation,* 103, 1624-30.

KIMURA, M., UEDA, K., GOTO, C., JITSUIKI, D., NISHIOKA, K., UMEMURA, T., NOMA, K., YOSHIZUMI, M., CHAYAMA, K. & HIGASHI, Y. 2007. Repetition of ischemic preconditioning augments endothelium-dependent vasodilation in humans: role of endothelium-derived nitric oxide and endothelial progenitor cells. *Arterioscler Thromb Vasc Biol,* 27, 1403-10.

KITAZONO, T., FARACI, F. M., TAGUCHI, H. & HEISTAD, D. D. 1995. Role of Potassium Channels in Cerebral Blood Vessels. *Stroke,* 26, 1713-1723.

KLONER, R. A., SHOOK, T., PRZYKLENK, K., DAVIS, V. G., JUNIO, L., MATTHEWS, R. V., BURSTEIN, S., GIBSON, M., POOLE, W. K., CANNON,

C. P. & ET AL. 1995. Previous angina alters in-hospital outcome in TIMI 4. A clinical correlate to preconditioning? *Circulation,* 91, 37-45.

KOCH, S. & GONZALEZ, N. 2013. Preconditioning the human brain: proving the principle in subarachnoid hemorrhage. *Stroke,* 44, 1748-53.

KOLBENSCHLAG, J., SOGORSKI, A., KAPALSCHINSKI, N., HARATI, K., LEHNHARDT, M., DAIGELER, A., HIRSCH, T. & GOERTZ, O. 2016. Remote Ischemic Conditioning improves blood flow and oxygen saturation in pedicled and free surgical flaps. *Plast Reconstr Surg*.

KRAEMER, R., LORENZEN, J., KABBANI, M., HEROLD, C., BUSCHE, M., VOGT, P. M. & KNOBLOCH, K. 2011. Acute effects of remote ischemic preconditioning on cutaneous microcirculation--a controlled prospective cohort study. *BMC Surg,* 11, 32.

LAVI, S., GAITINI, D., MILLOUL, V. & JACOB, G. 2006. Impaired cerebral CO2 vasoreactivity: association with endothelial dysfunction. *Am J Physiol Heart Circ Physiol,* 291, H1856-61.

LIPSITZ, L. A., MUKAI, S., HAMNER, J., GAGNON, M. & BABIKIAN, V. 2000. Dynamic regulation of middle cerebral artery blood flow velocity in aging and hypertension. *Stroke,* 31, 1897-903.

LIU, X.-Q., SHENG, R. & QIN, Z.-H. 2009. The neuroprotective mechanism of brain ischemic preconditioning. *Acta Pharmacol Sin,* 30, 1071-1080.

LOUKOGEORGAKIS, S. P., PANAGIOTIDOU, A. T., BROADHEAD, M. W., DONALD, A., DEANFIELD, J. E. & MACALLISTER, R. J. 2005. Remote ischemic preconditioning provides early and late protection against endothelial ischemia-reperfusion injury in humans: role of the autonomic nervous system. *J Am Coll Cardiol,* 46, 450-6.

MAEDA, H., MATSUMOTO, M., HANDA, N., HOUGAKU, H., OGAWA, S., ITOH, T., TSUKAMOTO, Y. & KAMADA, T. 1994. Reactivity of cerebral blood flow to carbon dioxide in hypertensive patients: evaluation by the transcranial Doppler method. *J Hypertens,* 12, 191-7.

MATSUDA, Y., OGAWA, H., MORITANI, K., MATSUDA, M., NAITO, H., MATSUZAKI, M., IKEE, Y. & KUSUKAWA, R. 1984. Effects of the presence or absence of preceding angina pectoris on left ventricular function after acute myocardial infarction. *Am Heart J,* 108, 955-8.

MELLANDER, S. 1989. Functional aspects of myogenic vascular control. *J Hypertens Suppl,* 7, S21-30; discussion S31.

MENG, R., ASMARO, K., MENG, L., LIU, Y., MA, C., XI, C., LI, G., REN, C., LUO, Y., LING, F., JIA, J., HUA, Y., WANG, X., DING, Y., LO, E. H. & JI, X. 2012. Upper limb ischemic preconditioning prevents recurrent stroke in intracranial arterial stenosis. *Neurology,* 79, 1853-61.

MENG, R., DING, Y., ASMARO, K., BROGAN, D., MENG, L., SUI, M., SHI, J., DUAN, Y., SUN, Z., YU, Y., JIA, J. & JI, X. 2015. Ischemic Conditioning Is Safe and Effective for Octo- and Nonagenarians in Stroke Prevention and Treatment. *Neurotherapeutics,* 12, 667-77.

MICHELSEN, M. M., STOTTRUP, N. B., SCHMIDT, M. R., LOFGREN, B., JENSEN, R. V., TROPAK, M., ST-MICHEL, E. J., REDINGTON, A. N. & BOTKER, H. E. 2012. Exercise-induced cardioprotection is mediated by a bloodborne, transferable factor. *Basic research in cardiology,* 107, 1-9.

MORO, L., PEDONE, C., MONDI, A., NUNZIATA, E. & ANTONELLI INCALZI, R. 2011. Effect of local and remote ischemic preconditioning on endothelial function in young people and healthy or hypertensive elderly people. *Atherosclerosis,* 219, 750-2.

MUNK, K., ANDERSEN, N. H., SCHMIDT, M. R., NIELSEN, S. S., TERKELSEN, C. J., SLOTH, E., BOTKER, H. E., NIELSEN, T. T. & POULSEN, S. H. 2010. Remote Ischemic Conditioning in Patients With Myocardial Infarction Treated With Primary Angioplasty: Impact on Left Ventricular Function Assessed by Comprehensive Echocardiography and Gated Single-Photon Emission CT. *Circ Cardiovasc Imaging,* 3, 656-62.

MURRY, C. E., JENNINGS, R. B. & REIMER, K. A. 1986. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation,* 74, 1124-36.

NAKAGAWA, Y., ITO, H., KITAKAZE, M., KUSUOKA, H., HORI, M., KUZUYA, T., HIGASHINO, Y., FUJII, K. & MINAMINO, T. 1995. Effect of angina pectoris on myocardial protection in patients with reperfused anterior wall myocardial infarction: retrospective clinical evidence of "preconditioning". *J Am Coll Cardiol,* 25, 1076-83.

NOVAK, V., NOVAK, P., SPIES, J. M. & LOW, P. A. 1998. Autoregulation of cerebral blood flow in orthostatic hypotension. *Stroke,* 29, 104-11.

OTTANI, F., GALVANI, M., FERRINI, D., SORBELLO, F., LIMONETTI, P., PANTOLI, D. & RUSTICALI, F. 1995. Prodromal angina limits infarct size. A role for ischemic preconditioning. *Circulation,* 91, 291-7.

PANERAI, R. B. 1998. Assessment of cerebral pressure autoregulation in humans--a review of measurement methods. *Physiol Meas,* 19, 305-38.

PANG, C. Y., YANG, R. Z., ZHONG, A., XU, N., BOYD, B. & FORREST, C. R. 1995. Acute ischaemic preconditioning protects against skeletal muscle infarction in the pig. *Cardiovasc Res,* 29, 782-8.

PAULSON, O. B., STRANDGAARD, S. & EDVINSSON, L. 1990. Cerebral autoregulation. *Cerebrovasc Brain Metab Rev,* 2, 161-92.

PETERS, A., SCHWEIGER, U., PELLERIN, L., HUBOLD, C., OLTMANNS, K. M., CONRAD, M., SCHULTES, B., BORN, J. & FEHM, H. L. 2004. The selfish

brain: competition for energy resources. *Neuroscience & Biobehavioral Reviews,* 28, 143-180.

PICKARD, J. M., BOTKER, H. E., CRIMI, G., DAVIDSON, B., DAVIDSON, S. M., DUTKA, D., FERDINANDY, P., GANSKE, R., GARCIA-DORADO, D., GIRICZ, Z., GOURINE, A. V., HEUSCH, G., KHARBANDA, R., KLEINBONGARD, P., MACALLISTER, R., MCINTYRE, C., MEYBOHM, P., PRUNIER, F., REDINGTON, A., ROBERTSON, N. J., SULEIMAN, M. S., VANEZIS, A., WALSH, S., YELLON, D. M. & HAUSENLOY, D. J. 2015. Remote ischemic conditioning: from experimental observation to clinical application: report from the 8th Biennial Hatter Cardiovascular Institute Workshop. *Basic Res Cardiol,* 110, 453.

PRZYKLENK, K., BAUER, B., OVIZE, M., KLONER, R. A. & WHITTAKER, P. 1993. Regional ischemic 'preconditioning' protects remote virgin myocardium from subsequent sustained coronary occlusion. *Circulation,* 87, 893-9.

PRZYKLENK, K. & WHITTAKER, P. 2011. Remote ischemic preconditioning: current knowledge, unresolved questions, and future priorities. *J Cardiovasc Pharmacol Ther,* 16, 255-9.

PUPPO, C., FARINA, G., LOPEZ, F. L., CARAGNA, E. & BIESTRO, A. 2008. Cerebral CO2 reactivity in severe head injury. A transcranial Doppler study. *Acta Neurochir Suppl,* 102, 171-5.

PURKAYASTHA, S. & SOROND, F. 2012. Transcranial Doppler ultrasound: technique and application. *Semin Neurol,* 32, 411-20.

REGAN, R. E., DUFFIN, J. & FISHER, J. A. 2013. Instability of the middle cerebral artery blood flow in response to CO2. *PLoS One,* 8, e70751.

REN, C., GAO, X., STEINBERG, G. K. & ZHAO, H. 2008. Limb remote-preconditioning protects against focal ischemia in rats and contradicts the dogma of therapeutic time windows for preconditioning. *Neuroscience,* 151, 1099-103.

RENTOUKAS, I., GIANNOPOULOS, G., KAOUKIS, A., KOSSYVAKIS, C., RAISAKIS, K., DRIVA, M., PANAGOPOULOU, V., TSAROUCHAS, K., VAVETSI, S., PYRGAKIS, V. & DEFTEREOS, S. 2010. Cardioprotective role of remote ischemic periconditioning in primary percutaneous coronary intervention: enhancement by opioid action. *JACC Cardiovasc Interv,* 3, 49-55.

ROGER, V. L., GO, A. S., LLOYD-JONES, D. M., BENJAMIN, E. J., BERRY, J. D., BORDEN, W. B., BRAVATA, D. M., DAI, S., FORD, E. S., FOX, C. S., FULLERTON, H. J., GILLESPIE, C., HAILPERN, S. M., HEIT, J. A., HOWARD, V. J., KISSELA, B. M., KITTNER, S. J., LACKLAND, D. T., LICHTMAN, J. H., LISABETH, L. D., MAKUC, D. M., MARCUS, G. M., MARELLI, A., MATCHAR, D. B., MOY, C. S., MOZAFFARIAN, D., MUSSOLINO, M. E.,

NICHOL, G., PAYNTER, N. P., SOLIMAN, E. Z., SORLIE, P. D., SOTOODEHNIA, N., TURAN, T. N., VIRANI, S. S., WONG, N. D., WOO, D. & TURNER, M. B. 2012. Heart disease and stroke statistics--2012 update: a report from the American Heart Association. *Circulation,* 125, e2-e220.

SARKAR, S., GHOSH, S., GHOSH, S. K. & COLLIER, A. 2007. Role of transcranial Doppler ultrasonography in stroke. *Postgrad Med J,* 83, 683-9.

SCHMETTERER, L., FINDL, O., STRENN, K., GRASELLI, U., KASTNER, J., EICHLER, H. G. & WOLZT, M. 1997. Role of NO in the O2 and CO2 responsiveness of cerebral and ocular circulation in humans. *Am J Physiol,* 273, R2005-12.

SCHMIDT, M. R., SMERUP, M., KONSTANTINOV, I. E., SHIMIZU, M., LI, J., CHEUNG, M., WHITE, P. A., KRISTIANSEN, S. B., SORENSEN, K., DZAVIK, V., REDINGTON, A. N. & KHARBANDA, R. K. 2007. Intermittent peripheral tissue ischemia during coronary ischemia reduces myocardial infarction through a KATP-dependent mechanism: first demonstration of remote ischemic perconditioning. *Am J Physiol Heart Circ Physiol,* 292, H1883-90.

SEEGER, J. P., BENDA, N. M., RIKSEN, N. P., VAN DIJK, A. P., BELLERSEN, L., HOPMAN, M. T., CABLE, N. T. & THIJSSEN, D. H. 2016. Heart failure is associated with exaggerated endothelial ischaemia-reperfusion injury and attenuated effect of ischaemic preconditioning. *Eur J Prev Cardiol,* 23, 33-40.

SERRADOR, J. M., SOROND, F. A., VYAS, M., GAGNON, M., ILOPUTAIFE, I. D. & LIPSITZ, L. A. 2005. Cerebral pressure-flow relations in hypertensive elderly humans: transfer gain in different frequency domains. *Journal of Applied Physiology,* 98, 151-159.

SHIMIZU, M., KONSTANTINOV, I. E., KHARBANDA, R. K., CHEUNG, M. H. & REDINGTON, A. N. 2007. Effects of intermittent lower limb ischaemia on coronary blood flow and coronary resistance in pigs. *Acta Physiol (Oxf),* 190, 103-9.

SHIMIZU, M., TROPAK, M., DIAZ, R. J., SUTO, F., SURENDRA, H., KUZMIN, E., LI, J., GROSS, G., WILSON, G. J., CALLAHAN, J. & REDINGTON, A. N. 2009. Transient limb ischaemia remotely preconditions through a humoral mechanism acting directly on the myocardium: evidence suggesting cross-species protection. *Clin Sci (Lond),* 117, 191-200.

SLOTH, A. D., SCHMIDT, M. R., MUNK, K., KHARBANDA, R. K., REDINGTON, A. N., SCHMIDT, M., PEDERSEN, L., SORENSEN, H. T., BOTKER, H. E. & INVESTIGATORS, C. 2014. Improved long-term clinical outcomes in patients with ST-elevation myocardial infarction undergoing remote

ischaemic conditioning as an adjunct to primary percutaneous coronary intervention. *Eur Heart J,* 35, 168-75.

SMITH, J. J., LEE, J. G., HUDETZ, A. G., HILLARD, C. J., BOSNJAK, Z. J. & KAMPINE, J. P. 1997. The role of nitric oxide in the cerebrovascular response to hypercapnia. *Anesth Analg,* 84, 363-9.

SOROND, F. A., SERRADOR, J. M., JONES, R. N., SHAFFER, M. L. & LIPSITZ, L. A. 2009. The sit-to-stand technique for the measurement of dynamic cerebral autoregulation. *Ultrasound Med Biol,* 35, 21-9.

STENZEL-POORE, M. P., STEVENS, S. L., XIONG, Z., LESSOV, N. S., HARRINGTON, C. A., MORI, M., MELLER, R., ROSENZWEIG, H. L., TOBAR, E., SHAW, T. E., CHU, X. & SIMON, R. P. 2003. Effect of ischaemic preconditioning on genomic response to cerebral ischaemia: similarity to neuroprotective strategies in hibernation and hypoxia-tolerant states. *Lancet,* 362, 1028-37.

STRANDGAARD, S. & PAULSON, O. B. 1984. Cerebral autoregulation. *Stroke,* 15, 413-6.

THIELMANN, M., KOTTENBERG, E., KLEINBONGARD, P., WENDT, D., GEDIK, N., PASA, S., PRICE, V., TSAGAKIS, K., NEUHAUSER, M., PETERS, J., JAKOB, H. & HEUSCH, G. 2013. Cardioprotective and prognostic effects of remote ischaemic preconditioning in patients undergoing coronary artery bypass surgery: a single-centre randomised, double-blind, controlled trial. *Lancet,* 382, 597-604.

TODA, N., AYAJIKI, K. & OKAMURA, T. 2009. Cerebral blood flow regulation by nitric oxide in neurological disorders. *Can J Physiol Pharmacol,* 87, 581-94.

TOMIYAMA, Y., BRIAN, J. E., JR. & TODD, M. M. 1999. Cerebral blood flow during hemodilution and hypoxia in rats : role of ATP-sensitive potassium channels. *Stroke,* 30, 1942-7; discussion 1947-8.

TRIEDMAN, J. K. & SAUL, J. P. 1994. Blood pressure modulation by central venous pressure and respiration. Buffering effects of the heart rate reflexes. *Circulation,* 89, 169-79.

VAN BEEK, A. H., CLAASSEN, J. A., RIKKERT, M. G. & JANSEN, R. W. 2008. Cerebral autoregulation: an overview of current concepts and methodology with special focus on the elderly. *J Cereb Blood Flow Metab,* 28, 1071-85.

VAN DEN MUNCKHOF, I., RIKSEN, N., SEEGER, J. P., SCHREUDER, T. H., BORM, G. F., EIJSVOGELS, T. M., HOPMAN, M. T., RONGEN, G. A. & THIJSSEN, D. H. 2013. Aging attenuates the protective effect of ischemic preconditioning against endothelial ischemia-reperfusion injury in humans. *Am J Physiol Heart Circ Physiol,* 304, H1727-32.

VERNIERI, F., TIBUZZI, F., PASQUALETTI, P., ROSATO, N., PASSARELLI, F., ROSSINI, P. M. & SILVESTRINI, M. 2004. Transcranial Doppler and near-infrared spectroscopy can evaluate the hemodynamic effect of carotid artery occlusion. *Stroke,* 35, 64-70.

WIJNHOUD, A. D., KOUDSTAAL, P. J. & DIPPEL, D. W. 2006. Relationships of transcranial blood flow Doppler parameters with major vascular risk factors: TCD study in patients with a recent TIA or nondisabling ischemic stroke. *J Clin Ultrasound,* 34, 70-6.

WILLIE, C. K., COLINO, F. L., BAILEY, D. M., TZENG, Y. C., BINSTED, G., JONES, L. W., HAYKOWSKY, M. J., BELLAPART, J., OGOH, S., SMITH, K. J., SMIRL, J. D., DAY, T. A., LUCAS, S. J., ELLER, L. K. & AINSLIE, P. N. 2011. Utility of transcranial Doppler ultrasound for the integrative assessment of cerebrovascular function. *J Neurosci Methods,* 196, 221-37.

WILLIE, C. K., TZENG, Y. C., FISHER, J. A. & AINSLIE, P. N. 2014. Integrative regulation of human brain blood flow. *J Physiol,* 592, 841-59.

WU, Z. K., TARKKA, M. R., PEHKONEN, E., KAUKINEN, L., HONKONEN, E. L. & KAUKINEN, S. 2000. Beneficial effects of ischemic preconditioning on right ventricular function after coronary artery bypass grafting. *Ann Thorac Surg,* 70, 1551-7.

YOSHIZUMI, T., YANAGA, K., SOEJIMA, Y., MAEDA, T., UCHIYAMA, H. & SUGIMACHI, K. 1998. Amelioration of liver injury by ischaemic preconditioning. *Br J Surg,* 85, 1636-40.

ZHANG, R., ZUCKERMAN, J. H., GILLER, C. A. & LEVINE, B. D. 1998. Transfer function analysis of dynamic cerebral autoregulation in humans. *Am J Physiol,* 274, H233-41.

ZHOU, K., YANG, B., ZHOU, X. M., TAN, C. M., ZHAO, Y., HUANG, C., LIAO, X. B. & XIAO, H. B. 2007. Effects of remote ischemic preconditioning on the flow pattern of the left anterior descending coronary artery in normal subjects. *Int J Cardiol,* 122, 250-1.