

**IS IT FEASIBLE TO MANIPULATE ARTERIAL CARBON DIOXIDE LEVELS TO IMPROVE
IMPAIRED CEREBRAL AUTOREGULATION IN ACUTE HAEMORRHAGIC STROKE?**



**Thesis submitted for the degree of
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by

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Abstract

Title: Is it feasible to manipulate arterial carbon dioxide levels to improve impaired cerebral autoregulation in acute haemorrhagic stroke?

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Background: Cerebral Autoregulation (CA) is usually defined as the tendency of cerebral blood flow (CBF) to remain approximately constant despite changes in arterial blood pressure (BP) within the range of 50 to 170mmHg. Dynamic CA (dCA) can be estimated from the transient response of CBF to rapid changes in BP. Spontaneous acute intracerebral haemorrhage (ICH) presents a devastating cerebral event with high morbidity and mortality. The mainstay of treatment remains BP control, which relies on a functioning CA. CA has been shown to be impaired in acute ICH. Arterial partial pressure of carbon dioxide (PaCO₂) has a strong influence on dCA and other cardio- and cerebrovascular variables. Understanding the dynamic CA response to physiological manoeuvres, such as exercise and changes in respiratory patterns, has often been confounded by simultaneous changes in PaCO₂. Hypercapnia leads to vasodilation of cerebral vessels and overall causes deterioration in CA. Conversely, hypocapnia has a vasoconstrictive effect, improving CA.

Aim: The aim of this thesis is to comprehensively model the cerebral haemodynamic response to the entire physiological range of PaCO₂ in order to safely permit the assessment of feasibility of clinical translation of a CO₂-based intervention into a cohort of acute ICH patients with impaired autoregulation.

Objectives: This thesis objectives are to: i) determine the natural history and prognostic implications of CA impairment in acute ICH; ii) determine if the use of current CO₂ measurement techniques leads to significant differences in CO₂-related systemic and cerebrovascular parameters; iii) determine the most appropriate method of initiating and maintaining hypocapnia; iv) determine if key cerebral haemodynamic parameters including autoregulation index (ARI), arterial BP (BP), heart rate (HR), critical closing pressure (CrCP) and resistance-area product (RAP) follow a logistic non-linear model similar to those described for cerebral blood flow velocity (CBFV); v) investigate sex differences in cerebral haemodynamics across the physiological range of PaCO₂ and vi) determine whether hypocapnia (via hyperventilation) in acute ICH may improve CA and consequently clinical outcome.

Methods: CA was measured in healthy and acute ICH patients by transcranial Doppler ultrasound assessment of middle cerebral artery velocities alongside continuous non-invasive monitoring of BP.

Results: 45 healthy volunteers underwent a multi-step protocol inducing hypo- and hyper-capnia and 12 acute ICH patients underwent a metronome based hypocapnic intervention at <48 hours and 10-14 days. The thesis results demonstrated i) the aforementioned parameters follow a logistic curve relationship; ii) CBFV is lower and dCA is impaired in acute ICH as compared to healthy controls; iii) different EtCO₂ measurement techniques do lead to physiological changes and differences in parameter values; iv) dysautoregulation can be minimised by continuous metronome use during hyperventilation-induced hypocapnia; v) logistic curve parameters are influenced by sex and vi) dCA can be improved in acute ICH using a CA targeted CO₂-based interventional manoeuvre.

Conclusion: This thesis presents a new paradigm for assessment of the interaction of CO₂ and dCA and its potential clinical applications. In addition, original findings include improved understanding of CO₂ focussed physiological measurement protocol design, comprehensive clarification of cerebral haemodynamics in ICH, optimisation of hypocapnia induction, demonstration of novel sex differences during PaCO₂ change and via accumulation of the aforementioned knowledge, the safety and feasibility of a novel CA targeted CO₂-based interventional manoeuvre in acute ICH.

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Dedicated to the Guru Nanak Mission Hospital, Jalandhar.

“Burn worldly love, rub the ashes and make ink of it, make the heart the pen, the intellect the writer, write that which has no end or limit”

Sri Guru Granth Sahib, Guru Nanak

Contributorship

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Chapter 3: This physiological measurement optimisation study is based on healthy control data collected, analysed and prepared for publication by the author. The data analysis software was written and developed by Professor Panerai, Department of Cardiovascular Sciences, University of Leicester. Data analysis was performed by the author using this software, with Professor Panerai reviewing the quality of the data. Professor Panerai and Professor Robinson provided critical review of the study report. This chapter is published in *Physiological Measurement (2017)*:

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The author confirms that all work in this thesis is original and their own work, unless stated otherwise.

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List of Abbreviations

ABP	Arterial blood pressure
AIS	Acute ischaemic stroke
ARI	Autoregulation index
ARMA	Autoregressive moving average
BP	Arterial blood pressure
CA	Cerebral autoregulation
CAA	Cerebral amyloid angiopathy
CBF	Cerebral blood flow
CBFV	Cerebral blood flow velocity
CO ₂	Carbon dioxide
CPP	Cerebral perfusion pressure
CrCP	Critical closing pressure
CT	Computed tomography
CVMR	Cerebral vasomotor reactivity
CVCi	Cerebrovascular conductance
dCA	Dynamic cerebral autoregulation
DNAR	Do not attempt resuscitation order
ECG	Electrocardiogram
EtCO ₂	End-tidal carbon dioxide
FM	Face mask
GCS	Glasgow Coma Score
HR	Heart rate
ICH	Intracerebral haemorrhage
ICP	Intracranial pressure
MAP	Mean arterial blood pressure
MCA	Middle cerebral artery
MFV	Mean flow volume
MRI	Magnetic resonance imaging
mRS	modified Rankin Scale

Mx	Mean flow correlation index
MSE	Mean square error
NIHSS	National Institutes of Health Stroke Scale
NIRS	Near-infrared spectroscopy
NC	Nasal cannulae
PaCO ₂	Arterial partial pressure carbon dioxide
PI	Pulsatility index
PRx	Pressure reactivity
RAP	Resistance-area product
RCT	Randomised controlled trial
sCA	Static cerebral autoregulation
SD	Standard deviation
TBI	Traumatic brain injury
TCD	Transcranial Doppler
TFA	Transfer function analysis

1 Chapter One: Introduction

1.1 Stroke

“It is impossible to cure a severe attack of apoplexy, and difficult to cure a mild one”

Hippocrates

In 1970, the World Health Organisation (WHO) provided a clinical definition of stroke, “rapidly developed clinical signs of focal (or global) disturbance of cerebral function, lasting more than 24 hours or leading to death, with no apparent cause other than of vascular origin”.¹ In an attempt to evolve from a clinical to a radiological definition, in 2013, the American Heart Association provided an updated definition to include ‘silent’ infarction or haemorrhage.² Acute ischaemic stroke (AIS) accounts for 85% of all strokes with acute intracerebral haemorrhage (ICH) contributing the remaining 15%.³

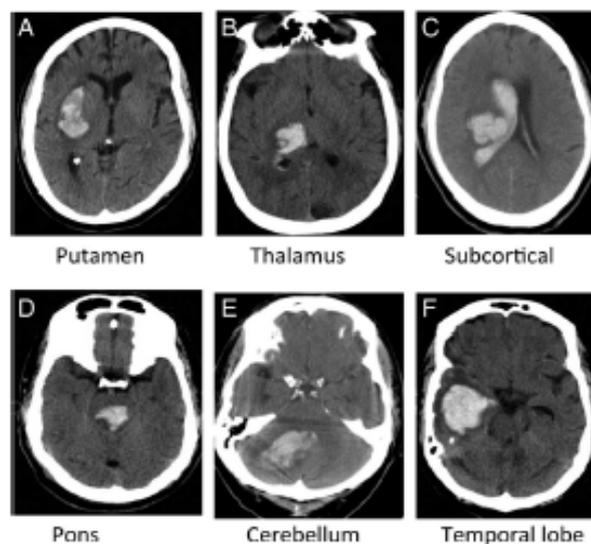
In 2013, stroke was the second most common cause of death worldwide (11.8% of all deaths), after ischaemic heart disease.³ The significant morbidity is demonstrated by its position as the third most common cause of disability (4.5% of DALYs from all cause).³ The absolute number of people suffering stroke worldwide increased by 19% between 1990 and 2010. This increase is largely attributable to increasing population size, the burden of increasing population age and lifestyle changes.³ The average age of stroke disease onset is 73 years of age.³

Despite the advent of new therapies for certain stroke sub-types, the acute ICH stroke sub-type is most often associated with a disabling or fatal outcome. Sadly, the population-based incidence of ICH is rising, particularly in those greater than 75 years of age.⁴ In 2011, these concerns led to the development of a dedicated European research programme for ICH to identify ways to minimise ICH-associated death and disability.⁵

1.2 Intracerebral Haemorrhage

Spontaneous acute ICH is defined as non-traumatic intraparenchymal bleeding.⁶ ICH is common and has a mortality rate of 40% at 1 month.⁶ This is distinct from other forms of intracranial bleeding within the skull vault; dural haematomas and subarachnoid haemorrhages. There are several common risk factors for ICH including hypertension, age, alcohol, illicit drug use (cocaine and amphetamines) and hereditary conditions with amyloid deposits.

Figure 1.1 Typical locations for hypertensive ICH.



Typical locations for hypertensive ICH are putamen (A), thalamus (B), subcortical white matter (C), pons (D) and cerebellum (E). Thalamic and subcortical haemorrhages often extend into ventricles (B and C). CAA, drug abuse or vascular anomaly often causes lobar haemorrhage (F). Reproduced from original reference.⁶

The American Heart Association consensus statement in 2013 defined acute ICH as “non-traumatic intracranial haemorrhage within the ventricles or brain parenchyma”.² This included intraventricular haemorrhage, haemorrhages from arterio-venous malformations and did not include subdural or extradural haemorrhages (Figure 1.1). This ‘tissue’ based definition ultimately acknowledges the advancement in modern neuroimaging delivered in a timely manner during acute stroke recognition.

1.2.1 *Epidemiology and Aetiology*

In the UK, 23,000 (15%) of the 150,000 people who suffer a stroke each year have an acute ICH.⁷ ICH has the highest mortality of all stroke subtypes, with half of sufferers dying within a year. Despite being the most devastating form of stroke, it remains the least treatable form with limited therapeutic options. Importantly, recent data have shown a high case fatality in those over 75 years. Despite a lack of specific targeted therapies, in contrast to AIS, for which intravenous thrombolysis⁸ and mechanical thrombectomy⁹ are effective options, there is demonstrated benefit from timely robust ward based stroke care with careful selection of patients for surgical intervention.² Evidence from population-based studies suggests robust medical care is effective at reducing ICH mortality and morbidity, hence consensus guidelines being directed at the acute stages of ICH care.²

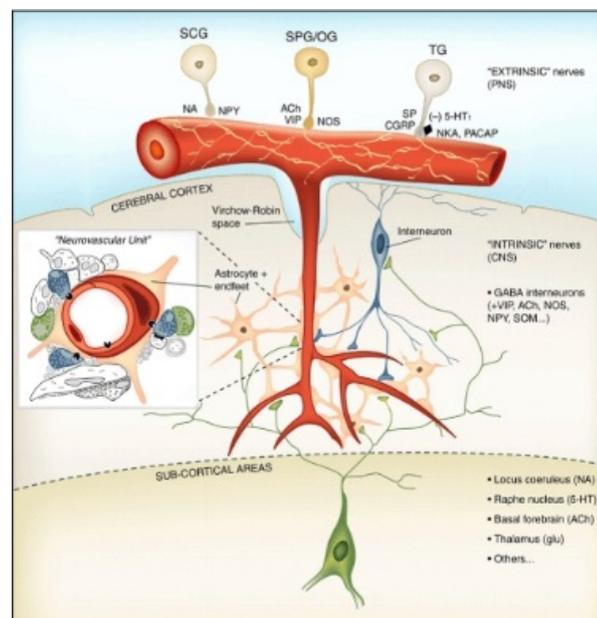
1.2.2 *Pathology*

The most significant cause of primary ICH is chronic hypertension causing cerebrovascular damage to small arteries and arterioles.¹⁰ Cerebral amyloid angiopathy (CAA) is another major cause of primary ICH and importantly a key causative factor for lobar ICH in elderly patient groups. Secondary ICH is largely caused by vascular anomalies including aneurysms, arteriovenous malformations, cavernomas and venous malformations. Generally, ruptured aneurysmal bleeds often lead to interventricular extension and subarachnoid blood.¹⁰

Recently, enlarged perivascular spaces have been found in all patients with spontaneous ICH.¹¹ Perivascular spaces are interstitial fluid cavities surrounding small penetrating vessels and are thought to be pathological. Associations with small vessel disease onset have been demonstrated in imaging studies. The 'neurovascular unit' is widely considered to be a "neuronal-astrocytic-vascular" tripartite unit.¹² This unit responds to released neurotransmitters within the perivascular space which activate receptors (Figure 1.2) on both

vascular and astroglial cells to adjust the cerebrovascular tone of vessels.¹² Unfortunately, current understanding of this neurovascular microanatomy has not permitted explanation of how changes in the tone of brain microvessels that result in an increase or decrease in local brain parenchymal perfusion, largely directed by astroglial signaling, relate to the maintenance of constant cerebral perfusion by controlling of blood volume and intracranial pressure homeostasis.¹²

Figure 1.2 Schematic representation of the “neurovascular unit”.



Schematic representation of the “neurovascular unit” and relationships with cerebral cortex, sub-cortical areas and controlling nerve systems. SCG, superior cervical ganglion; SPG, sphenopalatine ganglion; OG, otic ganglion; TG, trigeminal ganglion. Reproduced from original reference.¹²

1.2.3 Clinical Features

The most common presentation of ICH is sudden acute onset of a focal neurological deficit. This presentation can be accompanied by progressive neurological deterioration with clinical features including headache, vomiting, deteriorating level of consciousness, marked hypertension and sometimes seizures. Hypertension occurs in around 90% of patients with ICH

and seizures occur in 10% although these are not exclusive. Neurological features relate to haematoma location.¹⁰

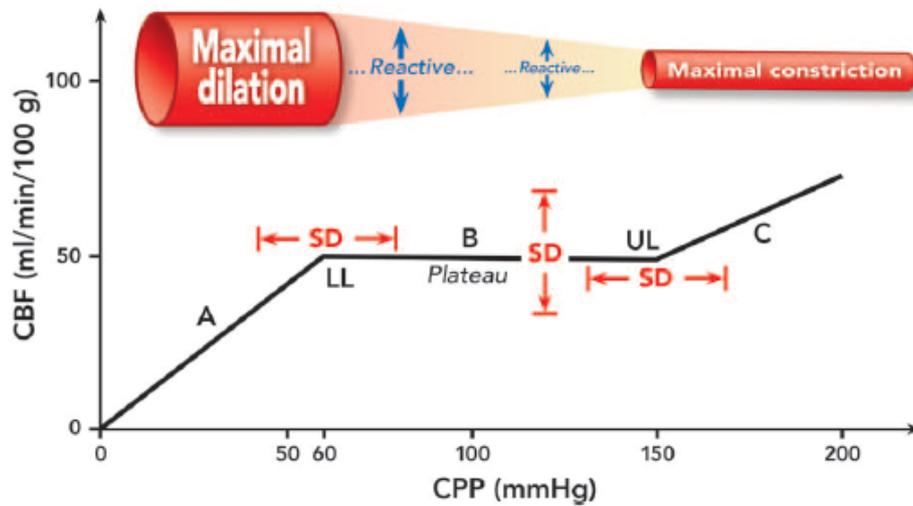
1.2.4 Diagnosis & Imaging

Computed tomography (CT) scanning has permitted rapid recognition of blood products and hence diagnosis of haematomas soon after presentation. Furthermore, volume of haematomas can be calculated using validated methods, such as the ABC/2 score.¹⁰ The ABC/2 score is an approximation for the volume of an ellipsoid where A is the long haemorrhage diameter on axial CT scans, B is the largest diameter 90° to A, and C is the number of CT slices with haemorrhage multiplied by the slice thickness. Magnetic resonance imaging (MRI) provides a more sensitive modality for detecting cavernous malformations than CT imaging.¹⁰

1.2.5 Treatment

The mainstay of acute ICH management relates to BP control. Cerebral autoregulation (CA) aims to maintain cerebral blood flow (CBF) constant over a wide range of cerebral perfusion pressures (CPP) from 60 to 150mmHg. CA can be impaired in acute ICH and those that advocate lowering BP highlight benefits including less oedema formation and lower risk of rebleeding.¹⁰ However, chronic hypertension causes the autoregulatory curve to move to higher pressures (Figure 1.3) which could possibly leave vulnerability to ischaemic change should BP be normalised during this acute neurological state.^{10,13}

Figure 1.3 Cerebral autoregulatory curve.



Relationship between cerebral blood flow (CBF) and cerebral perfusion pressure (CPP) demonstrating static cerebral autoregulation. A, below lower limit (LL); B, plateau portion; C, above upper limit (UL); SD, standard deviation. Cerebrovascular reactivity is also demonstrated at the top of the diagram. Reproduced with permission from original reference.¹³

1.2.6 Blood Pressure Management

Based on strong randomised controlled trial (RCT) evidence (Table 1.1),¹⁴ the recent UK National Clinical Guidelines for stroke concluded that patients with primary ICH presenting within 6 h of onset with a systolic BP above 150 mmHg should be treated urgently using a locally agreed BP lowering protocol to a systolic BP target of 140 mmHg.¹⁵

Table 1.1 Summary of prospective randomised clinical trials assessing the effect of acute BP lowering in ICH. Reproduced from original reference.¹⁴

Study (year of publication)	Patients	No. Subjects	Intervention	Primary outcome	Results
Rapid blood pressure reduction in acute ICH (2008)¹⁶	Supratentorial ICH within 8 hours of symptom onset	42	MAP<110 versus 110-130	Decline NIHSS ≥ 2 points at 48 hours, mRS score ≤ 2 at 90 days, haematoma	No significant differences in early neurological deterioration (p=0.55), haematoma and oedema growth (p=1.0, p=0.35), and clinical outcome at 90 days (p=0.43).
Intensive Blood Pressure Reduction in Acute Cerebral Haemorrhage (INTERACT) (2008)¹⁷	ICH within 6 hours of symptom onset and SBP 150-220	404	SBP<140 versus SBP<180	Proportional change in haematoma volume in 24 hours, mRS score of 3-6 at 90 days	No excess neurological deterioration or other adverse events in intensively treated group, reduced rate of haematoma growth by 8% (p=0.05).
Antihypertensive Treatment in Acute Cerebral Haemorrhage (ATACH) (2010)¹⁸	Supratentorial ICH within 6 hours of symptom onset and SBP ≥ 200	60	IV nicardipine, three tiers of SBP: 170-200 140-170 110-140	Neurological deterioration within 24 hours, serious adverse events within 72 hours	Low rate of serious adverse events and neurological deterioration along all three tiers. No difference in average SBP change between patients with and without neurological deterioration (p=0.47).
Intracerebral Haemorrhage Acutely Decreasing Blood Pressure Trial (ICH-ADAPT) (2013)¹⁹	ICH within 24 hours of symptom onset and SBP ≥ 150	82	IV labetalol, SBP <150 versus <180	Perihaematoma rCBF on CT perfusion, 2 hours after treatment	Peri-haematoma rCBF was not lower among patients randomly assigned to SBP <150 (p=0.18).
INTERACT2 (2016)²⁰	ICH within 6 hours of symptom onset, SBP 150-200	2,794	SBP <140 within 1 hour versus SBP <180	Death or mRS score >2 at 90 days	No significant change in rate of death or major disability. Trend toward improved functional outcome on ordinal analysis. OR 0.87 (95% CI 0.75-1.01, p=0.06).
ATACH-2 (2016)²¹	Supratentorial ICH within 4.5 hours of symptom onset, SBP ≥ 180	1,000	SBP 110-139 versus SBP 140-179	Death or mRS score of 4-6 at 90 days	No difference in the rate of death or severe disability (p=0.72). Higher rate of renal complications in 7 days among treatment arm (p=0.002).

SBP, systolic BP; mRS, modified Rankin Scale; MAP, mean ABP.

The mainstay of general management involves robust stroke ward based nursing care as well as imaging, reversal of anticoagulation and BP control (Table 1.2). In only a small number of cases is surgery warranted.

Table 1.2 General management of patients with spontaneous intracerebral haemorrhage. Reproduced from original reference.²²

Section	Recommendations
General care/nursing care	All patients should be admitted to an acute stroke or intensive care unit. Frequent vital sign checks, neurological assessment and continuous cardiopulmonary monitoring (pulse oximetry, automated BP cuff and ECG telemetry), should be performed.
Airway protection	Ventilation should be considered for patients with impending ventilatory failure, at risk of aspiration, signs of raised intracranial pressure or brainstem dysfunction.
ICP monitoring	Intracranial pressure (ICP) should be monitored in patients with intraventricular haemorrhage, hydrocephalus, signs of transtentorial herniation or Glasgow Coma Score (GCS) ≤ 8 that were presumably caused by haematoma mass effect. Maintenance of ICP of $<20\text{mmHg}$ and CPP of $50\text{-}70\text{mmHg}$ is reasonable.
Blood pressure	In patients with acute ICH presenting within 6 hours of symptom onset, blood pressure should be lowered to SBP $<140\text{mmHg}$ within 1 hour.
VTE prophylaxis	Intermittent pneumatic compression should be used. Graduated compression stockings should be avoided.
Dysphagia screening	Patients should be kept nil by mouth until a formal swallowing assessment is done.
Fever	High grade fever of $>38^\circ\text{C}$ should be treated with paracetamol and/or tepid sponging.
Glucose	Hypoglycaemia and hyperglycaemia should be treated. In treating hyperglycaemia, insulin should be used.
Intravenous fluid	Avoid over-hydration. Avoid dextrose-containing fluid.
Seizure	Patients with clinical seizure(s) should be treated with antiepileptic agent(s). Patients without seizure should not be prophylactically treated with antiepileptic agent(s). Patients with altered conscious level and epileptic discharges detected on electroencephalography should be treated with antiepileptic agents.
DNAR order	Early do not attempt resuscitation (DNAR) order should be avoided in the first few days as it may limit active medical treatment.

1.2.7 Prognosis

Haematoma volume, presentation GCS and radiological evidence of intraventricular extension are markers of a significant likelihood of 30-day morbidity and mortality in ICH patients.¹⁰ European initiatives have called for new treatments aimed at reduction of haematoma volume, restriction of growth and removal of intraventricular haemorrhage in an effort to improve outcome. However, these have not been forthcoming and one-month case fatalities are

alarming high at 25-35% in high income countries and 30-48% in low and middle income countries.^{3,23,24}

1.3 Cerebral Autoregulation

Impairment of cerebral autoregulation (CA) is likely to be a crucial factor in the pathogenesis of cerebrovascular events like acute occlusions (stroke) or global hypoperfusion (intraoperatively). Furthermore, impaired CA has been associated with an array of other clinical pathologies including severe head injury,²⁵ carotid artery disease,²⁶ intracranial hypertension,²⁷ meningitis,^{28,29} heart failure,³⁰ and hepatic encephalopathy.^{31,32} Age,³³ hypertension³⁴ and female sex³⁵ have been linked with CA impairment, though several conflicting studies exist with reference to sex differences and there have been no studies to date assessing carbon dioxide (CO₂) change and CA specifically.³⁶

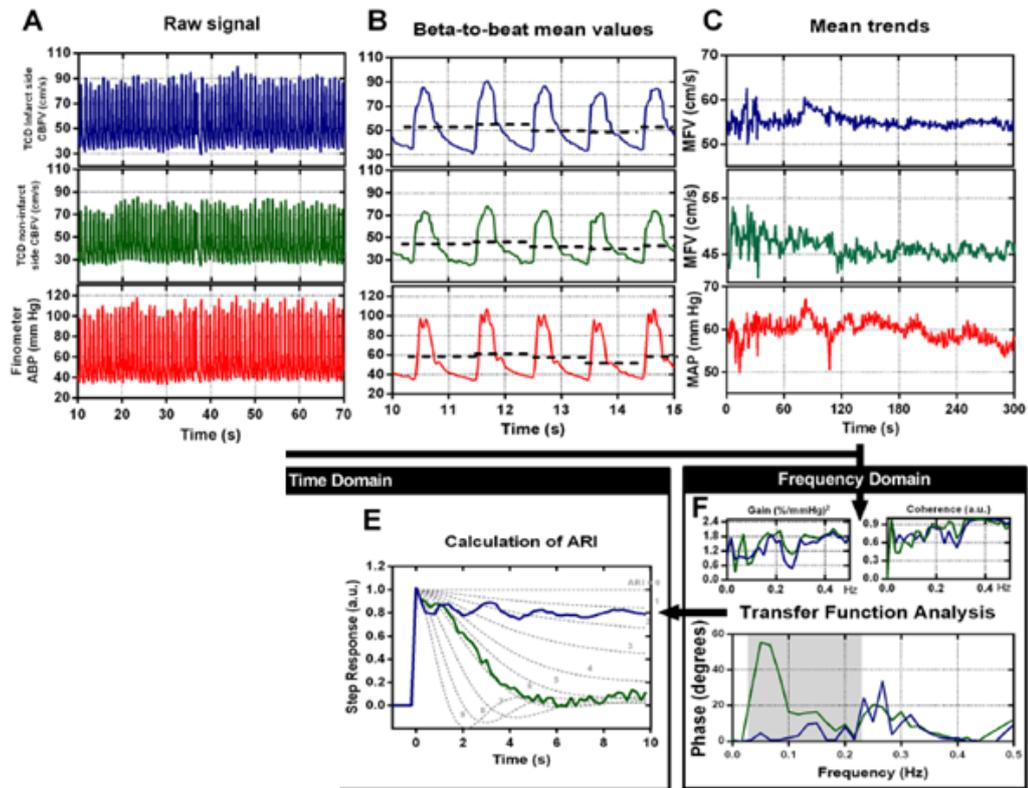
Transcranial Doppler ultrasonography (TCD) measurement, despite some concerns about middle cerebral artery (MCA) diameter change, offers a suitable assessment of MCA cerebral blood flow velocity (CBFV).³⁷

Originally, assessment of CA was traditionally focussed on the assessment of static changes in arterial blood pressure (BP).³⁸ CBFV was used as an estimate of cerebral blood flow (CBF) response to large changes in BP. Unfortunately, feasibility of manoeuvres required to induce such significant BP changes has led to questions of acceptability in vulnerable clinical states like acute stroke or autonomic failure.

Rapidly deflating thigh cuffs during physiological measurement provided a platform for newer dynamic methods of autoregulatory assessment as there was a greater opportunity to assess temporal patterns of change in BP with CBFV than static assessment offered.³⁹ Comparisons of the two methodologies concluded similar results between static and dynamic testing when

assessed using pharmacological agents.⁴⁰ However, in order to maximise the excellent temporal resolution of TCD to record continuous changes in CBFV following transient changes in BP, dynamic methodologies were preferred. Panerai *et al.*⁴¹ provided quantitative analysis of the influence of hypercapnic environments on CBFV. This work was important as it provided an important confirmation that dynamic CA can be assessed from baseline spontaneous fluctuations of BP to characterise CBFV response. Importantly, as well as using induced changes as generated by the thigh cuff,⁴⁰ squat-stand manoeuvres,^{42,43} cold pressor test,⁴⁴ whole-body hypothermia⁴⁵ or lower body negative pressure⁴⁶ to assess dCA, the efficiency of the dCA response can also be reliably assessed by examining CBF responses to hypercapnia and hypocapnia, the latter in particular generated by hyperventilation.⁴⁷ This branch of cerebral haemodynamic assessment, termed CO₂ vasomotor reactivity, is a complementary method of assessment of dCA to step changes in BP.

Figure 1.4 Process of calculating CA.



Demonstration of signal processing methods used: (A) Raw signal from the TCD with ipsilateral (blue) and contralateral (green) CBFV alongside Finometer readings (red). The CA is then calculated in time and frequency domains from (B) mean values of CBFV (termed mean flow volume [MFV] in the figure) and BP (termed mean arterial blood pressure [MAP] in the figure) of the time series (C). Following inverse Fourier transformation of the transfer function (F), step responses to impulses of MAP are calculated. This response is the autoregulation index (ARI) (E). Adapted with permission from original reference.⁴⁸

Since the introduction of servo-controlled arterial volume clamping of the digital artery, beat-to-beat dynamics of the pressure-flow relationship of the CPP (derived from the difference between the mean arterial blood pressure (MAP) and mean cerebral venous pressure) can be more robustly assessed. In this instance the mean cerebral venous pressure is a surrogate for intracranial pressure. The process however of assessing dCA from spontaneous fluctuations of BP requires methodological approaches as shown in Figure 1.4, which include frequency domain (transfer function analysis [TFA]) and time domain analysis. TFA quantifies the dynamic relationship between the input (i.e. BP) and output (i.e. CBFV) recordings, assuming dCA is a linear and stationary system. As a result it provides estimates of the magnitude and the phase

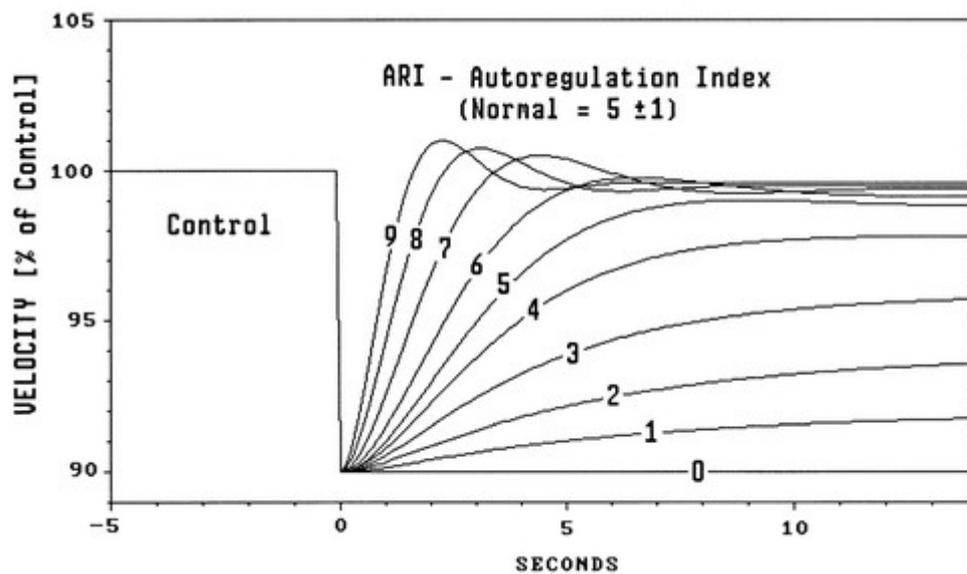
of the relationship between spontaneous or indeed induced changes in BP and CBFV.^{49,50} Gain and phase-shift are two parameters identified from TFA analyses at each frequency.⁵¹ Gain refers specifically to the compression of the relative changes in amplitude of CBFV to arterial blood pressure (BP), whereas phase-shift pertains to the specific time-lag between BP and CBFV. The assessment of coherence function is a key validity step in verifying estimates of gain and phase-shift.^{25,52} The coherence function assessment assumes that the BP-CBFV relationship is linear in nature. Values of coherence above a certain threshold are accepted, the 95% confidence limit of the null hypothesis that input and output are not related forms this threshold, i.e. zero coherence.^{49,50,53} Lastly, two further variables that can be derived from the instantaneous relationship between BP and CBFV are CrCP and RAP. CrCP measures the theoretical pressure at which CBF drops to zero and is a surrogate for cerebrovascular wall tension and transmural pressure, which also involves the contribution of ICP.^{54,55} RAP represents vascular resistance as determined by the slope of the linear ABP-CBF relationship.⁵⁶ Several different CA metrics are derived from time domain analysis including those assessing the linear correlation between slow waves of ABP and ICP (pressure reactivity [PRx]) and of CBFV and ABP (mean flow correlation index [Mx]).^{51,57,58} These parameters have been the focus of studies specifically assessing ICP status and CA in differing forms of haemorrhagic stroke.^{51,58}

1.4 The Autoregulation Index

The autoregulation index (ARI) is a well-established, reproducible parameter⁵⁹⁻⁶² that utilises a quantitative scoring for dCA from 0 to 9; 0 being absent and 9 being perfect autoregulation (Figure 1.5). Alongside simplicity, its validation in a clinical ischaemic stroke and subarachnoid haemorrhage⁶³ setting provides a basis for translation into acute haemorrhagic stroke populations. The perceived benefits of ARI include stability, due to the introduction of the moving-window autoregressive moving average model (ARMA), and hence less sensitivity to change. ARI(t) values can be obtained from an autoregressive moving-average model applied to

a sliding data window and have shown to provide agreement with the well-known effect of CO₂ change on dCA.⁴⁷ The model crucially calculates continuous estimates and facilitates detailed assessment of isolated components of the cerebral autoregulatory response. Crucially, in instances of hypocapnia or hypercapnia, the presence of heightened or dampened autoregulatory response respectively can be assessed with increased confidence.⁶⁴

Figure 1.5 Step responses of the cerebral autoregulatory model to step changes in BP. Reproduced from original reference.⁴⁰



1.5 CA and Stroke

Several studies have assessed CA in acute stroke subtypes.^{48,65-67} However, to date, despite the acceptability of the ARI metric, a limited number of studies have assessed ARI change in acute ischaemic stroke and no studies have assessed ARI in acute ICH. When considering the BP reductions often encountered in acute ICH,^{20,68-70} this is surprising as ARI, as mentioned, has the capability to provide more robust measurements during continuous assessment of spontaneous and induced fluctuations of BP, largely due to the incorporation of all the information pertaining to both gain and phase. In acute ischaemic stroke, preserved CA has been associated with improved three-month functional outcome and worsening ARI in the affected hemisphere is

associated with increased stroke severity.^{67,71} Despite this initial deterioration in CA post-acute stroke, there are data to support a return to 'control' levels in the recovery period post-stroke. However, contradictory findings have been seen in other CA parameters in acute ICH with a 'secondary decline' in CA⁷² noted as well as stable CA during repeated CA⁷³ assessments. Crucially, the relevance of CA in acute stroke has generated controversy with reference to BP lowering to <140mmHg within an hour as mandated following the Intensive Blood Pressure Reduction in Acute Cerebral Haemorrhage Trial (INTERACT2) in acute ICH.²⁰ With reference to acute ischaemic stroke pre-thrombolysis BP, the results of the Enhanced Control of Hypertension and Thrombolysis Stroke Study (ENCHANTED) are awaited and may inform more specific BP targets in this patient population.⁷⁴ However, without concomitant CA evidence during BP lowering in either stroke sub-type, concern exists, mechanistic data from observational studies is necessary to understand potential risks of BP lowering and inform trial design.

1.6 CA and CO₂

The neurologic effects of carbon dioxide (CO₂) change are numerous. The findings of CO₂ increase (hypercapnia) and progressive ascent of the cerebral autoregulatory plateau, a rightward shift of the lower limit and a leftward shift of the upper limit are well recognised (Figure 1.3).¹³ Although noted, the converse relationship of CO₂ decrease and plateau shifting to lower CBFV as well as little change in the lower limit and unclear change in the upper limit is generally poorly understood (Figure 1.3).¹³ Several studies have assessed CO₂ change and dCA both in healthy volunteers and patient populations.^{47,65,75,76} At the University of Leicester, study measurements are recorded using 'in-house' software (PHYSIDAS) developed by Professor Ronney Panerai in conjunction with the Medical Physics Group. This platform affords the ability to assess baseline EtCO₂ (Figure 1.6) and generate change in end-tidal CO₂ (EtCO₂) using subject breathing pattern deliberately synchronised with a metronome (Figure 1.7).

Figure 1.6 Baseline cerebral and peripheral haemodynamic measurements.

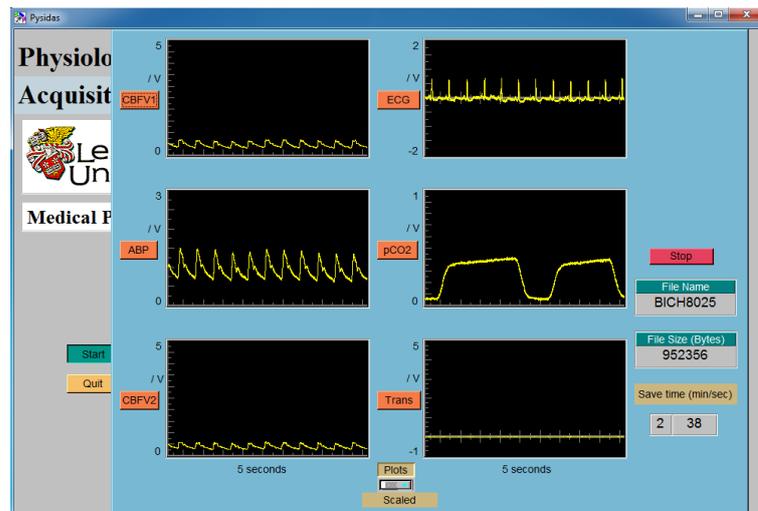
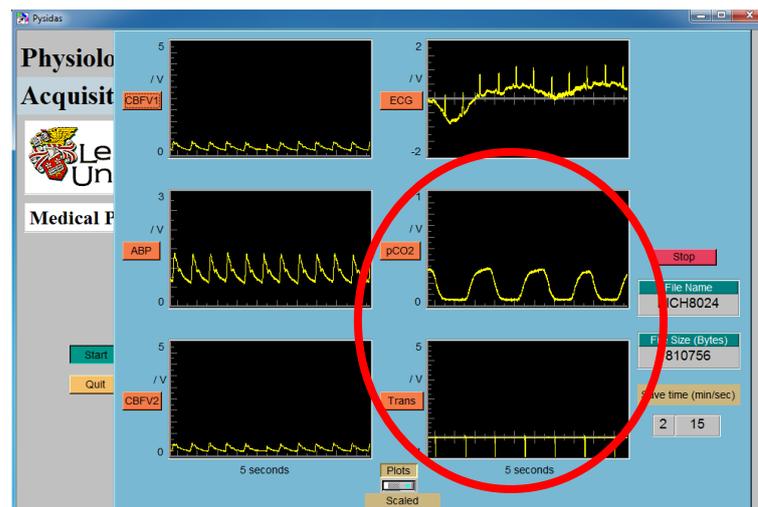


Figure 1.7 Change in EtCO₂ trace in response to initiation of hyperventilation using metronome (circled in red).



Despite some disease states, including metabolic derangements and pulmonary disorders pathologically manifesting a hypocapnic state,⁷⁷ the benefits of induction of hypocapnia are well demonstrated in neurological injury. Despite the inherent risks of vasoconstriction-associated ischaemia, the reduction in intracranial pressure, particularly in haemorrhagic intracranial injury should not be understated. The ‘closed-box’ cranial cavity is able to generate space for evolving oedema and haematoma by potent cerebral vessel vasoconstriction, hence improving neuronal cell function and reducing risk of brain-stem herniation.⁷⁸ Despite prior cerebral haemodynamic

studies demonstrating a level of baseline hypocapnia post-stroke, it remains unclear as to the potential benefits (or indeed feasibility) of accentuated hypocapnia in both a CA and clinical context. Furthermore, the relationship between cerebral haemodynamic parameters outlined and CO₂ change across the physiological range of PaCO₂ is yet to be determined. Central to the possibility of using CO₂ change to derive benefit in acute neurological disease, is the ability to understand the central and peripheral physiological responses within the lab and at the bed-side in order to refine a potential protocol designed to deliver a safe intervention targeting a patient population with whom an impairment of CA is thought to exist but yet to be fully elucidated. International guidelines^{5,79} support development of novel translational strategies to improve outcomes in acute ICH, but to date there have been a paucity of positive pharmacological and non-pharmacological pilot observational and randomised studies.⁸⁰⁻⁸² By understanding the relationship between PaCO₂ change and CA, the presence of CA impairment in acute ICH, and translating this knowledge from bench to bed-side, a possibility exists for a novel intervention designed to improve outcomes non-invasively and non-pharmacologically for a patient cohort with very poor morbidity and mortality.

The aim of the studies reported in this thesis is to comprehensively model the cerebral haemodynamic response to the entire physiological range of PaCO₂ in order to safely permit the assessment of feasibility of clinical translation of a CO₂-based intervention into a cohort of acute ICH patients with impaired autoregulation.

Therefore, this thesis objectives are to: i) determine the natural history and prognostic implications of CA impairment in acute ICH (Chapter 2); ii) assess whether the use of current CO₂ measurement techniques leads to significant differences in CO₂-related systemic and cerebrovascular parameters (Chapter 3); iii) determine the most appropriate method of initiating and maintaining hypocapnia (Chapter 4); iv) determine if key cerebral haemodynamic parameters including autoregulation index (ARI), arterial BP (ABP), heart rate (HR), critical

closing pressure (CrCP) and resistance-area product (RAP) follow a logistic non-linear model similar to those described for CBFV (Chapter 5); v) investigate sex differences in cerebral haemodynamics across the physiological range of PaCO₂ (Chapter 6) and vi) determine whether hypocapnia (via hyperventilation) in acute ICH may improve CA and consequently clinical outcome (Chapter 7).

2 Chapter Two: Cerebral Autoregulation in Haemorrhagic Stroke: A Systematic Review and Meta-Analysis of Transcranial Doppler Studies

2.1 Background and Purpose

Spontaneous acute intracerebral haemorrhage (ICH) is associated with devastating consequences, as evidenced by both high mortality and morbidity.⁷ BP control remains the foremost approach to managing acute ICH.⁸³ However, controversy exists as to whether intensive BP lowering in acute ICH risks cerebral ischaemia.⁶⁹ This is particularly the case if CBF control mechanisms are altered by chronic hypertension or brain injury. CA provides a protective mechanism for the brain parenchyma from extremes of CBF change, with the risk of hyper- or hypoperfusion in response to systemic BP changes.³⁹ A key limitation of large-scale randomised controlled trials has been the inability to provide mechanistic insight into CBF during the acute phase of haemorrhagic stroke.

Importantly, focussed metabolic studies examining BP reduction and risk of perihematoma hypoperfusion have not supported the hypothesis that BP reduction is associated with perihematoma ischaemia, confirming the safety of early intensive BP treatment in acute ICH.⁸⁴ These findings concur with the INTERACT-2 trial, which did not associate rapid BP reduction with worse clinical outcomes.²⁰ The INTERACT-2 trial randomised patients to intensive- (target systolic BP <140mmHg) or guideline-based (systolic BP <180mmHg) BP management. The Antihypertensive Treatment of Acute Cerebral Haemorrhage II (ATACH-II) trial used intravenous nicardipine to test the superiority of very intensive reduction (target systolic BP 100 to 130mmHg) versus standard treatment (target systolic BP 140mmHg to 179mmHg).²¹ The key difference in the BP targets achieved between the two studies is demonstrated by the early profile of systolic BP in the standard-treatment group in ATACH-II being similar to values observed in the intensive-treatment group in INTERACT-2.^{20,21} Primary outcome measures of

death and disability showed no reduction with BP lowering for both studies, but ATACH-II did not confirm the improved functional outcome found in INTERACT-2.^{20,21} However, an important limitation with both studies was the lack of inclusion of individuals with low GCS and large intraparenchymal haematomas, thus providing little data on safety of intensive lowering of systolic BP in individuals with the highest intracranial pressures where CA is likely to be further impaired.^{20,21}

TCD offers repeatable non-invasive bedside investigation with high temporal resolution of the steady-state (static) and dynamic components of CA (4). Dynamic CA (dCA) responds to instantaneous (over seconds) BP and associated cerebral perfusion pressure changes, and prior studies have demonstrated impairment in acute ischaemic stroke.⁵⁰ dCA is often described using transfer function analysis (TFA) parameters⁸⁵; including phase (reflecting speed of autoregulatory response) and gain (associated with damping characteristics of dCA). Furthermore, TCD can provide information regarding the time-course of autoregulation parameters in acute ICH. Unfortunately, despite several TCD studies to date, there remains a lack of consensus as to whether CA is impaired in ICH and whether such impairment relates directly to clinical outcomes. Furthermore, there has been no amalgamation of the evidence of the relationship of CA impairment with time post ICH.

TCD studies are highlighted in this review as they offer the advantage of understanding beat-to-beat dynamics of the relationship between cerebral pressure and flow with attention to fast and slow responses, thus providing mechanistic insights beyond comparable alternative imaging methods. Therefore, this review will focus on TCD studies of CA in the setting of documented acute ICH, and will report impairments in CA and the natural history of CBFV changes following acute ICH.

2.2 Material and Methods

2.2.1 Study Identification

Cochrane Collaboration methodology for meta-analysis reviews modified for observational studies (www.equator-network.org) was used.

2.2.2 Search Strategy

Studies were identified with a search strategy across three English language databases (MEDLINE, EMBASE and CENTRAL) between 1966 and December 2017 accommodating different MeSH terms or subcategories available on each database. References of selected articles were screened for additional relevant articles.

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((("BLOOD FLOW VELOCITY"/ OR "CEREBROVASCULAR CIRCULATION"/ OR HOMEOSTASIS/ OR (autoregulation).ti OR (blood ADJ2 flow).ti OR ((cerebrovascular OR brain).ti AND (circulat*3).ti)) AND ((intracerebral h?emorrhage).ti OR (cerebral h?emorrhage).ti OR "CEREBRAL HEMORRHAGE"/ OR (h?emorrhagic stroke).ti,ab OR (ICH).ti,ab)) AND ("COHORT STUDIES"/ OR "PROSPECTIVE STUDIES"/ OR "FOLLOW-UP STUDIES"/ OR "TIME FACTORS"/ OR "RETROSPECTIVE STUDIES"/ OR (cohort stud*).ti,ab OR ("case control").ti,ab OR "CASE-CONTROL STUDIES"/ OR (temporal*).ti,ab)
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A similar strategy using the closest available terms was used in EMBASE and CENTRAL.

2.2.3 Inclusion and Exclusion Criteria

TCD studies of human CA after ICH were included. Eligibility was assessed by reading abstracts, and, if necessary, whole articles. The effects of impaired CA and CBFV changes on neurological outcome were assessed. Excluded were case reports, non-English language articles, posterior territory stroke studies, and studies with ultrasound contrast agent injection, as well as other perfusion-based studies with CT or MRI.

2.2.4 Data Extraction

The following data were extracted: (1) number of patients and controls; (2) sex; (3) age; (4) acute (<72 hours) vs. chronic phase assessment (>72 hours); (5) method of data analysis and CA parameter used; (6) neurological outcome; (7) cerebral blood velocity (CBFV); (8) partial pressure of carbon dioxide (PaCO₂); (9) BP; (10) haematoma assessment method and size; and (11) main conclusions.

Study quality was assessed using a checklist proposed previously for authors, editors, and reviewers of meta-analyses of observational studies modified for specific use in TCD autoregulation observational studies (Table 2.1)⁵⁰ and based on the Meta-analysis of Observational Studies in Epidemiology: a proposal for reporting (MOOSE) guidelines.⁸⁶ Two independent reviewers (Jatinder S. Minhas, George Ghaly) undertook the methodological quality screening and data extraction of the included studies.

Table 2.1 A modified MOOSE checklist of observational TCD autoregulation studies.^{50,86}

Hypothesis statement in introduction or method section	1
Threshold values of study outcome parameters described in introduction and/or methods	1
Sample size calculation before start of experiment	1
Publication in peer reviewed journal	1
Medical ethics review with informed consent	1
Conflict of interest authors described	1
Patient spectrum/population described sufficiently	1
Confounding factors sufficiently mentioned in introduction, methods or limitations	1
Analysis described sufficiently (mathematical and statistics)	1
Proven reproducibility of data	1
Consecutive patient recruitment	1
Type of intervention/exposure described sufficiently (or with literature reference)	1
Considerations of alternative explanation of observed results in discussion	1
Total Points	13

For the meta-analyses, the variables were compared in acute ICH patients versus control subjects. The software used was Review Manager 5.3 (RevMan 5) provided by the Cochrane Library. The analysis was performed using the fixed effects model; weighted mean difference was used for measurement data and 95% confidence intervals (CI) were used as the effect

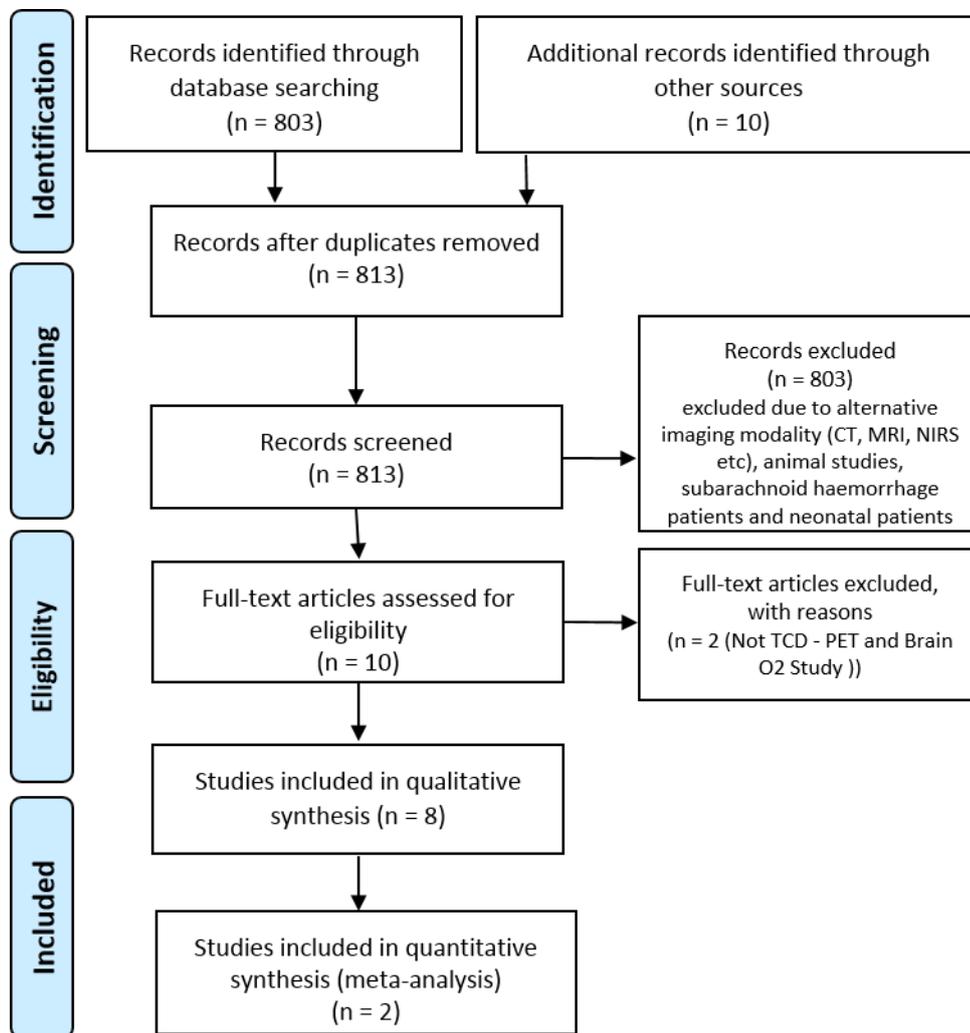
indicator for dichotomous variables. The heterogeneity assumption was checked by the χ^2 -based Q test.

2.3 Results

2.3.1 General Characteristics

Eight hundred and thirteen publications met the search criteria and were evaluated (Figure 2.1).

Figure 2.1 CONSORT diagram detailing search results.



The commonest reasons for exclusion were alternative imaging modality used (e.g. CT, MRI, near-infrared spectroscopy [NIRS]), animal studies, SAH patients, and neonatal haemorrhage. Two further studies were excluded after initially meeting the eligibility criteria, as it was noted

that PET and brain tissue oxygenation measurement, rather than TCD, were used.^{84,87} Overall, the eligibility criteria were met by seven controlled studies and one observational study (Table 2.2). Two studies used the same dataset,^{72,88} but both were included due to the different methods adopted for assessment of CA. Median score on the quality checklist was 10, range 9-11 (Table 2.3). This demonstrated areas of consistent incomplete reporting of previously published minimum acceptable methodological criteria for observational studies.⁵⁰

Table 2.2 Overview of Transcranial Doppler Studies with measurements in acute (<72 hours) and chronic (>72 hours) ICH.

Study	Number ICH Patients and Controls (Male:Female)	Age in Years \pm SD (Control)	Acute (<72 hrs) vs Chronic Phase (>72hrs) Assessment	Method of Data Analysis & CA Parameter	Neurological Severity	CBFV Values (cm/s^{-1})	EtCO ₂ values (mmHg)	BP values (Systolic \pm SD)	Hematoma Assess Method and Size (ABC/2) ($\text{cm}^3 \pm$ SD)	Main Results and Conclusions
Marti-Fabregas 2005	51 patients (30:21)	66.2 \pm 12.4	12 hours of onset (Acute)	PI	NIHSS 17	Affected Mean Velocity 35.6 \pm 16.5, Unaffected 39.6 \pm 18.0	Nil	Nil	51.7 \pm 70.4	TCD parameters, including Pulsatility Index (PI) were correlated with CT data in the acute stage of ICH
Reinhard 2010	26 patients (21:5), 55 controls (44:11)	65 \pm 11, (64 \pm 8)	Acute Day 1 and Chronic Days 3 and 5	Mx	NIHSS 12 \pm 7, GCS 13 \pm 2	Mean MCA CBFV (cm/s) Ipsilateral 43.6 (n = 26; SE = 3.4) 55.8 (n = 21; SE = 3.6; p = 0.0020) 53.6 (n = 22; SE = 3.6; p = 0.0091). Contralateral 47.7 (n=26, SE 3.5), 56.6 (n=21, SE 3.7, p=0.015), 57.9 (n=22, SE 3.7, p=0.0051)	34.9 (n = 26; SE = 0.9) 34.3 (n = 21; SE = 0.98; p = 0.5322) 35.1 (n = 22; SE = 0.96; p = 0.8815)	Mean MAP Finapres (mmHg) 90.9 (n = 26; SE = 4.26) 85.5 (n = 21; SE = 4.57; p = 0.2220) 84.4 (n = 22; SE = 4.51; p = 0.1337)	26 \pm 22	CA preserved in acute ICH, but a secondary decline ipsilateral occurred. This decline was associated with poor clinical status
Kiphuth 2011	61 ICH patients (37:24), 53 IVH patients (28:25)	ICH 62, IVH 70	Within 24 hours (Acute), 5-7 days (Chronic) and 7-9 days (Chronic)	N/A	N/A	N/A	N/A	N/A	ICH 25, IVH 41	Cerebral vasospasm with secondary infarction may occur in patients with spontaneous IVH
Nakagawa 2011	21 patients (8:13), 23 controls (8:15)	66 \pm 15 (65 \pm 9)	<72 hours (Mean Time TCD 34 \pm 14)	TFA	Nil	Ipsilateral 49.8 \pm 22.5/Contralateral 50.6 \pm 24.2 (Control 66.6 \pm 18.9)	Nil	77 \pm 15 (89 \pm 21)	36 \pm 27	dCA may be less effective in patients with spontaneous lobar or basal ganglia ICH compared to healthy controls

Oeinck 2013	26 patients (21:5), 55 controls (44:11)	65±11, (64±8)	Acute Day 1 and Chronic Days 3 and 5	TFA	NIHSS 12±7, GCS 13±2	Mean MCA CBFV (cm/s) Ipsilateral 43.6 (n = 26; SE = 3.4) 55.8 (n = 21; SE = 3.6; p = 0.0020) 53.6 (n = 22; SE = 3.6; p = 0.0091). Contralateral 47.7 (n=26, SE 3.5), 56.6 (n=21, SE 3.7, p=0.015), 57.9 (n=22, SE 3.7, p=0.0051)	34.9 (n = 26; SE = 0.9) 34.3 (n = 21; SE = 0.98; p = 0.5322) 35.1 (n = 22; SE = 0.96; p = 0.8815)	Mean MAP Finapres (mmHg) 90.9 (n = 26; SE = 4.26) 85.5 (n = 21; SE = 4.57; p = 0.2220) 84.4 (n = 22; SE = 4.51; p = 0.1337)	26 ± 22	Dynamic temporal characteristics of CA (phase) are not generally altered in acute ICH. Poorer individual phase values are, however, associated with larger ICH volume, lower blood pressure, and worsened outcome
Ma 2016	43 patients (31:13), 30 controls (21:9)	53.7±10.0 (52.3±8.1)	Acute (Days 1-2), Chronic (4-6, 10-12 and Day 30)	TFA	Admission (7.4±5.0), Discharge (5.1±4.5) NIHSS	D1-2 (49.6±19.1 Affected, 52.3±20.6 Unaffected), D4-6 (56.0±17.2 Affected, 58.4±16.7 Unaffected), D10-12 (60.0±14.5 Affected, 61.2±16.1 Unaffected), D30 (59.3±14.1 Affected, 62.4±13.8 Unaffected) Control 62.9±13.0	D1-2 (34.3±3.7), D4-6 (34.9±2.8), D10-12 (34.8±2.2), D30 (35.2±2.4) Control 35.1±2.5	D1-2 (122.5±15.1), D4-6 (117.2±14.1), D10-12 (113.2±11.9), D30 (107.6±12.1) Control 92.1±11.6	15.3±10.6	dCA is bilaterally impaired lasting at least 10 to 12 days and recovers within a month
Lee 2017	12 patients (11:1), 7 controls (6:1)	59.3±13.1, (61±9.2)	Within 3 days (<72 hours)	Mx	Nil	N/A	N/A	N/A	21.7±28.6	Acute ICH may impair CA and increase CBFV variability ipsilateral to the hematoma
Ma 2017	53 patients (40:13), 30 controls (21:9)	54.3±11.1. (52.3±8.1)	4-6 days after onset	TFA	NIHSS 9 (6-16)	D4-6 (53.6±17.1 Affected, 57.2±14.5 Unaffected) Control 62.9±13.0	D4-6 (34.7±3.3) Control 35.1±2.5	D4-6 (118.6±15.2) Control 92.1±11.6	14.7±10.2	dCA is bilaterally disturbed and larger hematoma volume is likely to independently predict poorer CA status ipsilateral to hematoma

CBFV indicates cerebral blood velocity; BP, blood pressure; CO₂, carbon dioxide; PI, pulsatility index; MCA, middle cerebral artery; TCD, transcranial Doppler; MAP, mean arterial pressure; EtCO₂, end-tidal carbon dioxide; NIHSS, National Institutes of Health Stroke Scale; GCS, Glasgow Coma Score; TFA, Transfer Function Analysis; CA, cerebral autoregulation; Mx, mean flow correlation index; ICH, intracerebral haemorrhage; IVH, intraventricular haemorrhage; SD, standard deviation. All studies examined the MCA and assessments within the acute and sub-acute phase refer to a single TCD assessment.

Table 2.3 Study quality assessment based on modified MOOSE checklist of observational TCD autoregulation studies.^{50,86}

Criterion (as per Aries 2010)	Marti-Fabregas (2005)	Reinhard (2010)	Kiphuth (2011)	Nakagawa (2011)	Oeinck (2013)	Ma (2016)	Lee (2017)	Ma (2017)
Hypothesis statement in introduction or method section	1	1	1	1	1	1	1	1
Threshold values of study outcome parameters described in introduction and/or methods	1	1	1	1	1	1	1	1
Sample size calculation before start of experiment	0	0	0	0	0	0	0	0
Publication in peer reviewed journal	1	1	1	1	1	1	1	1
Medical ethics review with informed consent	1	1	1	1	1	1	1	1
Conflict of interest authors described	0	1	1	1	1	1	1	1
Patient spectrum/population described sufficiently	1	1	1	1	1	1	1	1
Confounding factors sufficiently mentioned in introduction, methods or limitations	1	1	1	1	1	1	1	1
Analysis described sufficiently (mathematical and statistics)	1	1	1	1	1	1	1	1
Proven reproducibility of data	0	0	0	0	1	0	0	0
Consecutive patient recruitment	0	0	1	0	0	1	0	1
Type of intervention/exposure described sufficiently (or with literature reference)	1	1	1	1	1	1	1	1
Considerations of alternative explanation for observed results in discussion	1	1	1	1	1	1	1	1
Total Points	9	10	11	10	11	11	10	11

Patient numbers ranged from 12 to 114. All studies included patients in the ‘acute’ (<72 hours) range, though five studies^{72,73,88-90} also had measurements beyond 72 hours (chronic phase).

Three studies provided measurements beyond five days,^{72,73,90} with the longest measurement interval from initial stroke onset to follow-up being 30 days.⁷³ In all studies, except two, neurological outcome data were provided using the National Institute Health Stroke Scale (NIHSS), with scores ranging from 5 to 17. All studies included an ABC/2 assessment of haematoma volume with a mean range from 14.7 to 51.7 cm³. Two studies reported radiological outcome measures with correlation of blood flow with CT data and assessment of vasospasm.^{89,91} Four studies detailed EtCO₂ values for acute ICH patients. These ranged from 34.3 to 34.9mmHg in the acute phase (<72 hours)^{72,73,88} and 34.7 to 35.2mmHg in the chronic (>72 hours) phase.^{72,73,88,90} Studies provided varying detail for BP recordings, with three providing no data, three providing mean arterial pressure (MAP), and one providing systolic readings only.

2.3.2 Cerebral Haemodynamic Parameters

Six of the seven studies provided CBFV for each hemisphere separately,^{72,73,88,90-92} with three providing comparable healthy control CBFV values.^{73,90,91} All eight studies assessed^{72,73,88-93} cerebral haemodynamic measurements at rest. In one study, providing CBFV data up to 30 days following ICH onset, it was reported that CBFV in both the affected and unaffected hemispheres failed to return to control subject values at all assessed time-points until day 30.⁷³

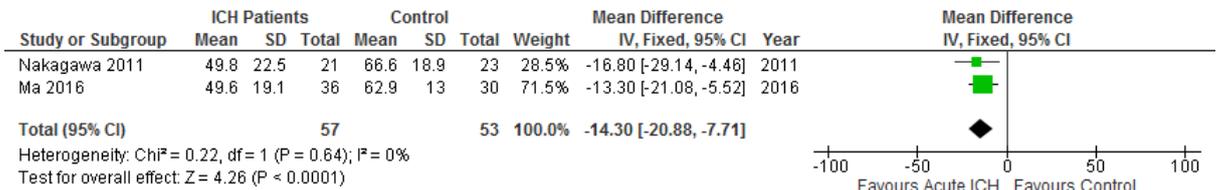
dCA assessed by TFA and mean flow correlation index (Mx), was impaired in the acute (<72 hours) period in most,^{73,88,91,93} but not all studies.⁷² However, this latter study reported that a 'secondary decline' in dCA more than five days post-ICH onset was associated with clinical features likely to be associated with poor prognosis, including haematoma volume and GCS.⁷² A further study found an increase in Pulsatility Index (PI) was associated with larger hematoma volumes, possibly reflecting increased mass effect.⁹²

2.3.3 Meta-analysis of CBFV Data

There was significant heterogeneity in study design and presentation of basic physiological measurements. Therefore, a quantitative meta-analysis was only possible for two acute studies (<72 hours) of CBFV data only,^{73,91} which, compared to controls, demonstrated significantly lower mean CBFV in the ipsilateral (49.7 vs. 64.8 cms^{-1} , $Z=4.26$, $p < 0.0001$) and contralateral hemispheres following ICH (51.5 vs. 64.8 cms^{-1} , $Z=3.44$, $p=0.0006$) (Figure 2.2a and 2.2b). Unfortunately, the studies did not provide data on hand dominance. A more recent study by Ma *et al.* was excluded from meta-analysis as the cohort were of chronic ICH patients assessed at 4 to 6 days post stroke.⁹⁰ However, this study demonstrated lower CBFV and EtCO_2 values in ICH patients compared to controls.

Figure 2.2 Meta-analysis of CBFV (cms^{-1}) in acute ICH (<72 hours).

2.2a



2.2b

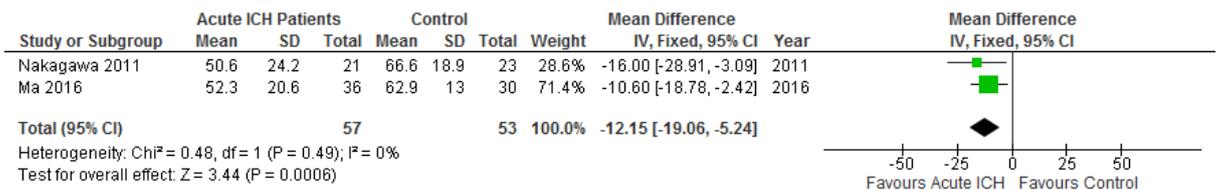
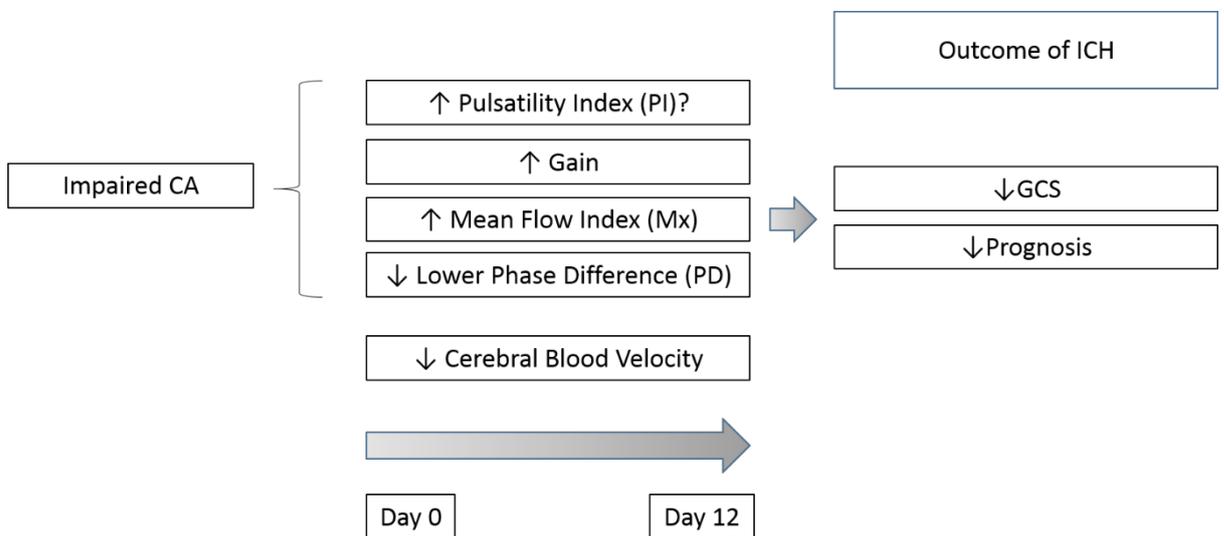


Figure 2.2a demonstrates mean ipsilateral TCD measurements (cms^{-1}) compared to mean control measurements, Figure 2.2b demonstrates mean contralateral measurements (cms^{-1}) compared to mean control measurements. Control values are average values (cms^{-1}) from ipsilateral and contralateral measurements.

2.4 Discussion

This systematic review of cerebral haemodynamics following acute ICH demonstrates impaired dCA, as assessed by TFA and Mx parameters, in both ipsilateral and contralateral hemispheres compared to control subjects. This impairment seems to persist for up to 12 days following ICH onset, though this conclusion is limited by the fact that only two studies investigated changes in the 'chronic' phase beyond 72 hours. Importantly, worsening CA parameters were associated with clinical features likely to be associated with poor prognosis, including haematoma volume and GCS score. The hypothesised relationship between autoregulation, CBF and clinical outcome is summarised in Figure 2.3. This concurs with similar findings in ischaemic stroke, where worsening CA is associated with adverse prognostic features, such as haemorrhagic transformation and cerebral oedema.⁹⁴

Figure 2.3 Hypothesised influences of cerebral autoregulation and cerebral blood flow on the outcome of acute ICH.



There are several limitations with the studies included in this systematic review. First, studies were mainly limited to patients with mild-to-moderate stroke severity, as evidenced by neurological impairment (NIHSS 5-12) and GCS >8. Secondly, these studies used BP instead of cerebral perfusion pressure to calculate dCA parameters, thus neglecting the potential

contribution of intra-cranial pressure. Thirdly, no study formally assessed the effects of BP lowering therapy on dCA, so there are limited data on the impact of intensive BP lowering on cerebral haemodynamics. Fourthly, the review is limited to data reported in the selected articles and does not examine individual patient data in the quantitative or qualitative analyses. This review is limited to TCD studies (as opposed to CT, MRI or NIRS) as they provide a robust assessment of beat-to-beat, dynamic cerebral and peripheral haemodynamic changes associated with the physiological perturbations precipitated by acute ICH. This limits generalisability of the findings to other modalities though does provide important mechanistic insights nonetheless. Therefore, a mechanistic understanding from a cerebral haemodynamic perspective of different targets for intensive BP lowering is lacking. Finally, the included studies generally suffer from methodological heterogeneity, lack of control subjects, and relatively small numbers. Nonetheless, the homogeneity of the evaluated pathology (supratentorial haemorrhage of similar stroke severity) should be regarded as a strength. Overall, the methodological quality of each study was good and assessed using objective criteria. Therefore, there are important findings with respect to key haemodynamic measures.

2.4.1 CBFV

A limited meta-analysis demonstrated lower CBFV values in ICH patients in both the ipsilateral and contralateral hemispheres compared to control subjects, though overall little data exist for CBFV changes following acute ICH. By contrast, studies in acute ischaemic stroke have shown similar lower CBFV values for ipsilateral (43.5 cm s^{-1}) and contralateral (41.1 cm s^{-1}) hemispheres compared to control subjects (49.6 cm s^{-1}).⁶⁵ Previous TCD work comparing flow velocities in small (<25mL) vs. large (>25mL) ICH volumes measured using CT, demonstrated large ICH has consistently depressed ipsilateral mean velocities ($35 \pm 13 \text{ cm/s}$).⁷⁸

2.4.2 Pulsatility Index

Previous studies have shown increased intracranial pressure and decreased cerebral perfusion pressure led to characteristic changes in TCD waveforms – decrease in diastolic velocity and increase in PI.⁹² Importantly, Marti-Fabregas *et al.*⁹² confirmed that PI values obtained using TCD correlated well with CT signs associated with mass effect, volume of haematoma and volume of surrounding oedema, total volume, midline shift and intraventricular extension. This correlation was particularly evident when PI values were significantly raised. Interestingly, these findings highlight a role for TCD as an indirect assessment method of intracranial hypertension and mass effect.

2.4.3 Mean flow correlation index (Mx)

As well as dynamic indices of autoregulation, the time correlation index known as the mean flow correlation index (Mx), which is based on correlation coefficients between BP and CBFV, was also studied. Two studies assessed Mx in acute ICH^{72,93}. Interestingly, the first of the two studies provided negative findings in the acute phase of ICH (<72 hours), demonstrating no difference between Mx values in patients and controls up to Day 5. However, higher Mx values (i.e. poorer autoregulation) were associated with a lower GCS, ventricular extension and lower cerebral perfusion pressure, ultimately leading to a poorer clinical picture at 90 days. These findings suggest some preservation of CA in acute ICH, and certainly conflict with findings of studies with transfer function analysis and PI outcome measures. The second more recent study assessed variability of CBFV using non-linear entropy analyses.⁹³ Ultimately, the authors drew comparisons between Mx and non-linear entropy analyses to assess sensitivity to CA changes. The data suggested that acute ICH does impair CA and increased CBFV variability exists within the ipsilateral hemisphere.⁹³

2.4.4 Transfer Function Analysis

Nakagawa *et al.*⁹¹ assessed patients with lobar or basal ganglia haemorrhage early (<72 hours) post ICH and compared to controls. The patients had higher gains across a wide range of frequency ranges compared to controls. This suggested that dCA is impaired in the early days after ICH. However, data presented by Oeinck *et al.*⁸⁸ (same dataset as Reinhard *et al.*)⁷² showed that phase was not altered in acute ICH but (similar to Mx) there were prognostic associations. This included poorer phase being associated with larger ICH volume, lower BP and worsened outcome. Interestingly, gain was generally higher in acute ICH but not associated with clinical factors for which phase has shown an association. The largest TFA based study in patients with supratentorial ICH included a robust set of serial measurements (days 1 to 2, 4 to 6, 10 to 12, and day 30).⁷³ Phase difference was lower bilaterally and therefore possibly impaired up to days 10 to 12 with a significant recovery by day 30.⁷³ Importantly, phase difference values were associated with clinical status in the acute stage, and impaired phase difference at 4 to 6 days post-ICH onset was an independent predictor for clinical outcome.⁷³ More recently, the bilateral disturbance of dCA was confirmed at the 4-6 day period post ICH with larger haematoma volume being independently predictive of poorer CA status ipsilateral to the haematoma.

2.4.5 Impaired Autoregulation

This review highlights the complex interplay that exists between different markers of autoregulatory impairment. Crucially, there exists a variation of approaches to assessment of autoregulatory impairment leading to difficulties in interpreting the overall picture due to inherent heterogeneity. However, we can summarize that higher gain, higher Mx, and lower phase difference are all suggestive of poorer CA (Figure 2.3), and provide a platform for future work examining CA impairment and consideration for potential interventions targeting CA improvement.

2.4.6 *Future Studies*

Future mechanistic work on blood flow is necessary to examine the nature of the lower CBFV values in acute ICH. A dual modality approach of TCD and arterial spin labelling may provide more information. Significant heterogeneity in outcome measures prevented any further meta-analyses on CA measures or other physiological parameters. Hence basic physiological data is required in future TCD work to ensure that more robust intra-study comparisons can be made, particularly baseline EtCO₂ values, CBFV values for all time points including any controls used. Above all, future studies should include assessment of dCA and its time course following interventions to reduce BP.

2.5 **Conclusion**

There is limited evidence to suggest that dCA is impaired up to 12 days post ICH. There is evidence for an association between reduced TFA phase and Mx values and worsened clinical features, including haematoma volume and GCS values. Furthermore, lower bilateral CBFV values as compared to controls provide a rationale for further studies examining the impact of intensive BP lowering strategies on CBF and its regulatory mechanisms.

3 Chapter Three: PaCO₂ measurement in cerebral haemodynamics: face mask or nasal cannulae?

3.1 Introduction

Continuous recordings of EtCO₂ are increasingly used in physiological and clinical studies as surrogate estimates of PaCO₂. Capnographic estimates of PaCO₂ have been shown to be useful for continuously monitoring the respiratory status of patients in intensive care settings.⁹⁵ In addition to applications in exercise physiology and respiratory diseases, studies of the cerebral circulation require assessment of PaCO₂ changes due to its potent effects on CBF.⁹⁶ Although face masks (FM) are the preferred option to sample respiratory gases, they are often poorly tolerated by patients and can induce changes in breathing frequency due to anxiety and discomfort. As an alternative, nasal cannulae (NC) have been preferred in many settings, despite concerns about their ability to reflect true expired EtCO₂ if subjects occasionally breathe through the mouth.⁹⁷ Given the need to determine if these two approaches are interchangeable or not, we tested the hypothesis that the use of FM versus NC does not lead to significant differences in PaCO₂-related systemic and cerebrovascular parameters.

3.2 Methods

The study was conducted in accordance with the Declaration of Helsinki (2013).⁹⁸ Ethical approval was obtained from the University of Leicester Ethics Committee (Reference: jm591-c033). Healthy volunteers were recruited from University departmental staff, students and their relatives. Participants aged above 18 years were included. Exclusion criteria included physical disease in the upper limb, poor insonation of both temporal bone windows and any significant history of cardiovascular, neurological or respiratory disease. Subjects with mild, controlled hypertension were accepted as representative of active and otherwise healthy older adults.

The research was undertaken in the University of Leicester's Cerebral Haemodynamics in Ageing and Stroke Medicine research laboratory, maintained at a constant ambient temperature of approximately 24°C and free of distraction. For the purposes of the study, participants were asked to refrain from caffeine, alcohol and nicotine in the 12-hour period prior to measurements being undertaken. Beat-to-beat blood pressure BP was recorded continuously using the Finometer® device (FMS, Finapres Measurement Systems, Arnhem, Netherlands), which was attached to the middle finger of the left hand. The servo-correcting mechanism of the Finometer® was switched on and then off prior to measurements. The hand bearing the finger cuff was at the level of the heart to negate any hydrostatic pressure artefact. HR was recorded using a standard 3-lead electrocardiogram (ECG). EtCO₂ was measured throughout the initial resting baseline and hypercapnic phase using the FM connected to a capnograph (Capnocheck Plus). During the second baseline and hypocapnic phase it was measured via NC (Salter Labs). Bilateral insonation of the middle cerebral arteries (MCAs) was performed using transcranial Doppler (TCD) ultrasound (Viasys Companion III; Viasys Healthcare) with a 2MHz probe. This probe was secured in place with a head-frame that was adjusted to ensure comfort at the outset. The MCAs were identified according to two main characteristics: signal depth and velocities.⁹⁹

3.2.1 Experimental Protocol

All measurements were conducted at a single visit. An initial period of 15 minutes of stabilisation preceded a 5-minute baseline recording supine at rest using FM. This was followed by fixed inspiration for a minimum of 90 s (ideally 120 s) of 5% CO₂ in air. After a further period of 5 min of stabilisation, participants performed a 5 min baseline recording using NC, which was followed by a set of hyperventilation measurements. Measurements were continuously recorded at a rate of 500 samples/s in the PHYSIDAS data acquisition system for subsequent off-line analysis. Systolic and diastolic brachial BP readings (OMRON Model 705IT) were performed at each stage

of the protocol (hypercapnia and hypocapnia). These values were then used to calibrate the Finometer recordings.

3.2.2 *Data Analysis*

The data collected corresponded to six individual files for each participant: two at baseline, two hypercapnic and two hypocapnic. Data were initially inspected visually and calibrated to recorded systolic and diastolic OMRON BP. Narrow spikes (<100ms) were removed using linear interpolation and the CBFV recording was then passed through a median filter. All signals were low-pass filtered with a zero-phase Butterworth filter with cut-off frequency of 20Hz. The software was then used to ensure the R-R interval was marked correctly with the ECG trace. This allowed mean BP, HR, EtCO₂ and mean CBFV to be calculated for each cardiac cycle. The critical closing pressure (CrCP) and resistance-area product (RAP) were estimated using the first harmonic method.¹⁰⁰ Dynamic cerebral autoregulation was assessed with the autoregulation index (ARI), derived by transfer function analysis from the CBFV response to a hypothetical step change in BP.³⁹

3.2.3 *Statistical Analysis*

Tests of normality were performed using the Kolmogorov-Smirnov test. The baseline measurements were assessed for differences between values derived for right and left hemispheres using a paired Student's t-test. These were averaged when no significant differences were found. Comparisons were made between FM and NC values using either the Student's t-test or Wilcoxon Signed Rank test as appropriate. Analysis of agreement was performed with Bland-Altman plots. Statistical significance was accepted at $p < 0.05$.

3.3 Results

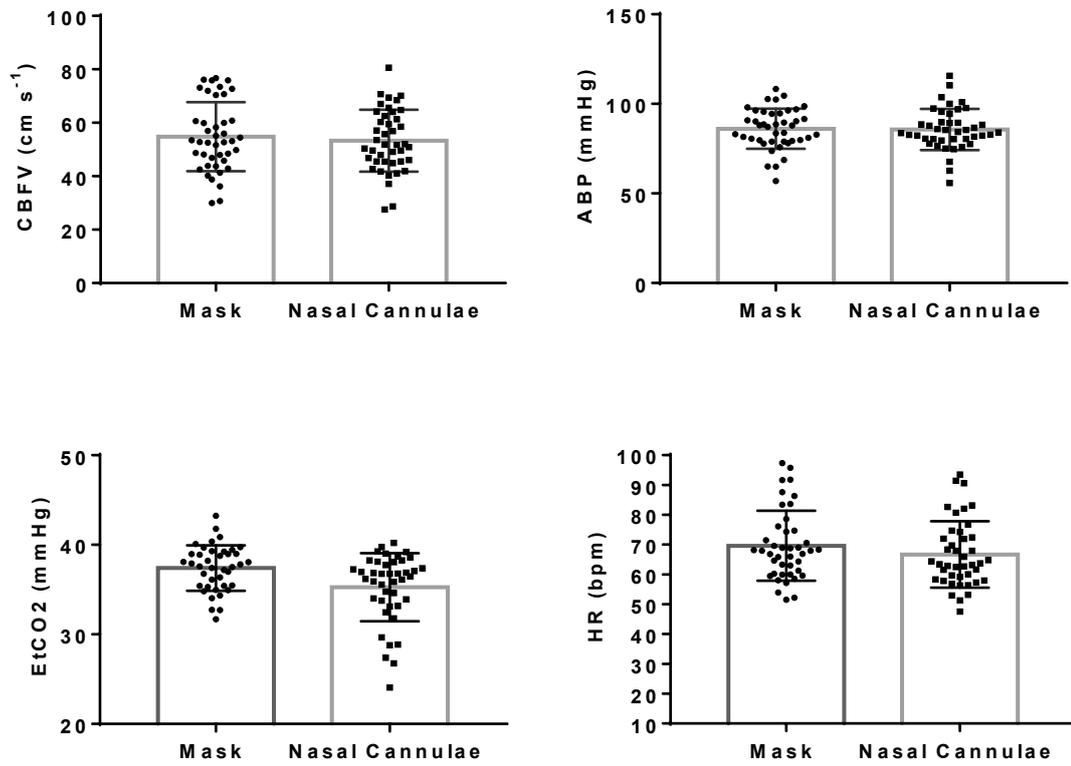
Forty-two subjects were recruited. Baseline systemic and cerebrovascular parameters for FM and NC are given in Table 3.1, showing highly significant differences for baseline EtCO₂ and HR (Figure 3.1). Noteworthy, ARI, CrCP, RAP and CBFV were not different. The differences between FM and NC for EtCO₂ can be better appreciated in the Bland-Altman plot (Figure 3.2) indicating a significant positive bias due to higher values for FM compared to NC. The relatively large 95% limits of agreement should also be noted.

Table 3.1 Peripheral and cerebral haemodynamic parameters recorded with the face mask and nasal cannulae for continuous measurements of EtCO₂ (n=42).

Parameters	Face mask	Nasal cannulae	p-value
CBFV (cm s⁻¹)	54.8±12.9	53.3±11.6	0.094
Mean ABP (mmHg)	86.1±11.2	85.7±11.5	0.793
End-tidal CO₂ (%)	37.4±2.5	35.3±3.8	0.001
Heart rate (beats.min⁻¹)	69.6±11.2	66.7±11.1	0.001
CrCP (mmHg)	34.8±13.0	35.8±14.7	0.693
RAP (mmHg.cm s⁻¹)	1.04±0.35	1.01±0.37	0.856
ARI	5.6±1.6	5.9±2.1	0.593

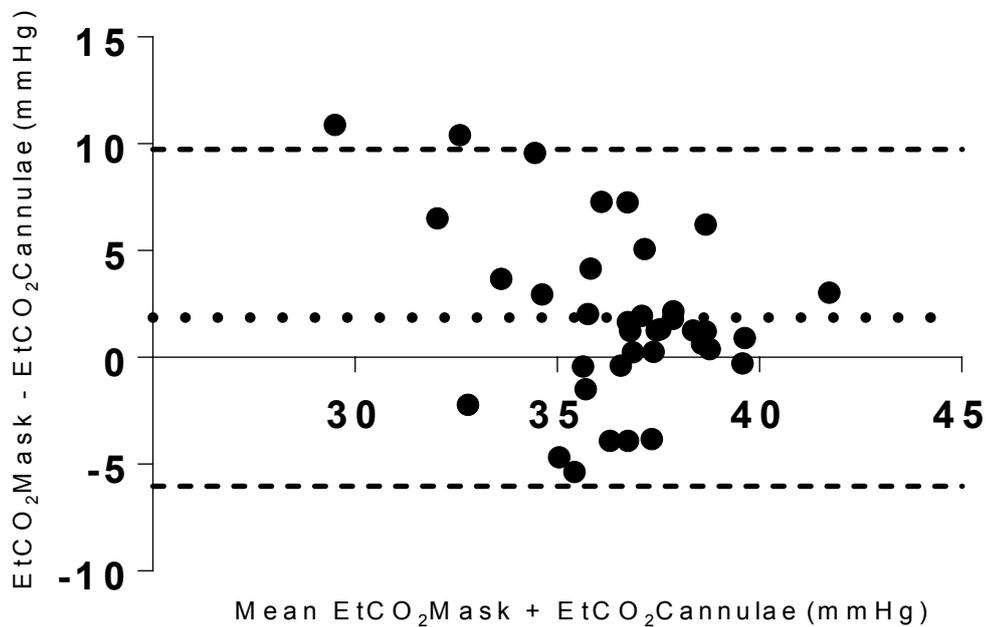
Values are mean (SD). CBFV, cerebral blood velocity; ABP, arterial blood pressure; EtCO₂, end-tidal arterial pressure of carbon dioxide; CrCP, critical closing pressure; RAP, resistance area product; ARI, Autoregulation Index. CBFV, CrCP, RAP and ARI were averaged for the right and left MCAs.

Figure 3.1 Distribution of CBFV, ABP, EtCO₂ and HR for FM and NC.



Bar graphs represent mean ± SD.

Figure 3.2 Bland Altman plot for agreement between EtCO₂ and differing gas sampling method.



Agreement between EtCO₂ (end-tidal carbon dioxide) for measurements performed with either FM (face mask) or NC (nasal cannulae), expressed by a Bland Altman plot, representing the bias (dotted line) and 95% limits of agreement (bias \pm 1.96SD, dashed line).

3.4 Discussion

Continuous recordings of PaCO₂ are essential for assessment of CBF regulatory mechanisms, such as dynamic cerebral autoregulation, CO₂ reactivity and neurovascular coupling. For this purpose, intravascular recordings are usually replaced by non-invasive measurements based on infra-red capnography, which are safer, less costly and much better accepted by study participants. For a relatively large number of subjects, the higher values of EtCO₂ obtained for FM, compared to NC, were to be expected as NC does not provide perfect sampling of all expired CO₂, and mouth breathing can also contribute to missed sampling in some subjects. Despite this difference, it is reassuring that key parameters, such as the mean CBFV and ARI, were not influenced by the CO₂ sampling modality as it allows better comparability between studies.

Accordingly, there is a potential opportunity to use both modalities interchangeably in complex multi-stage protocols where FM might not be a satisfactory option, for example in long duration baseline recordings.

Another important finding in our study was the elevated HR seen with FM, which is likely a sympathetic response to the discomfort or anxiety associated with using the mask. Sympathetic activation is also likely to have an effect on the autonomic nervous system regulation of CBF.¹⁰¹ Although ARI was not different between FM and NC in the healthy population assessed in the present study, it is possible that particular patient sub-groups might be more susceptible to FM-induced sympathetic activation leading to alterations in cerebral autoregulation or neurovascular coupling.⁷⁶

The authors acknowledge two potential study limitations. First, an inability to randomise the order of the experiment, which was largely attributable to concerns that prior hypocapnia (as opposed to hypercapnia) may cause persistent cerebral vasoconstriction, thus affecting MCAV-CO₂ response to hypercapnia. Secondly, possible physiological alterations associated with each CO₂ sampling modality. In particular the heightened anxiety, cognitive stimulation and physical involvement required to maintain adequate respiration with the face mask as compared to the nasal cannulae.

Previous experimental work has determined that biological factors, such as tidal volume and respiratory rate, can impact on sampling accuracy via NC; a “clinically acceptable” upper limit for accuracy being 20 breaths/min.⁹⁷ Despite being at rest and with no participants likely to have achieved respiratory rates at this level, we demonstrated less effective delivery and measurement compared to the FM.

Lastly, previous work has shown that TCD-estimated CBFV and ARI (using TFA) during inhalation of O₂ and CO₂ have acceptable levels of reproducibility.¹⁰² However, further assessments of

these parameters are warranted in diseased states using the most effective means of delivering and measuring EtCO₂ to ensure accuracy of baseline recordings.

4 Chapter Four: Different strategies to initiate and maintain hyperventilation: their effect on continuous estimates of dynamic cerebral autoregulation

4.1 Introduction

As outlined in Chapter 1, CA describes the ability of the cerebrovascular system to maintain a near constant CBF throughout fluctuations in systemic arterial BP.¹⁰³ Between BP values of 60-150mmHg, CBF tends to be independent of changes in mean arterial pressure (MAP) due to vessel diameter modifications, which alter the cerebrovascular resistance.^{103,104} CA can be described in terms of its static or dynamic properties. Static CA (sCA) represents the steady-state relationship between ABP and CBF and is used to describe the response of the cerebrovascular system to sustained changes in BP. Dynamic CA (dCA) describes the regulation of CBF in response to acute BP perturbations and therefore evaluates the temporal aspects of CA as well as the degree of functioning.^{40,105}

A key vasoactive stimulus often used in CA studies is the PaCO₂, which is known to directly influence CA.^{38,47,101,106} Specifically, hypercapnia impairs dCA and hypocapnia augments dCA, as demonstrated in a number of previous studies.^{38,41,47,107,108} Continuous recordings of EtCO₂ are increasingly used in physiological and clinical studies as surrogate estimates of PaCO₂. Capnographic estimates of PaCO₂ have been shown to be useful for continuously monitoring the respiratory status of patients in intensive care settings.^{109,110} In addition to applications in exercise physiology and respiratory diseases, studies of the cerebral circulation require assessment of PaCO₂ changes due to its potent effects on CBF.⁹⁶ Importantly, PaCO₂ changes can be induced using respiratory manoeuvres; the variance in EtCO₂ concentration measured being assumed analogous to that of the arterial concentration. Hypercapnia can be induced using several methods including rebreathing techniques, breath holding and direct inhalation of

CO₂.^{111,112} Conversely, the only method of obtaining hypocapnia in humans is to induce hyperventilation, either mechanically or voluntarily, dependent on the subject population.^{108,113-117} It is therefore pertinent to refine the methods used to induce and maintain hyperventilation in order to obtain the most valid and reproducible results.

A novel analysis approach by Dineen *et al.*⁴⁷ used a moving-window autoregressive moving average (MW-ARMA) model to explore the temporal patterns of dCA during respiratory manoeuvres. On evaluation, an initial decrease in autoregulation was demonstrated during hyperventilation-associated hypocapnia. This dysautoregulation lasted around 30 seconds prior to the anticipated augmentation.⁴⁷ Previously used batch-processing analysis methods calculate a single average for each recording; the presence of this unexpected decrease may confound such estimates, leading to underestimation of indicators of resistance like the cerebrovascular resistance index (CVRI).²⁵ This unexpected finding may go some way to explaining the discrepancies in the existing literature concerning the effects of hypocapnia on dCA.^{100,101} It was theorised that the temporary dysautoregulation observed may relate to changes in mental activation attributable to metronome inception, and the task of achieving and maintaining metronome-synchronised breathing to assess haemodynamic parameters adequately.⁴⁷

We therefore aimed to 1) investigate the effects of different hyperventilation strategies on temporal estimates of dCA, with a view to validating the most effective strategy for inducing hypocapnia (herein referred to as 'induction strategy'); and 2) investigate whether two different levels of hypocapnia (-5mmHg and -10mmHg) had differing effects during maintenance of hypocapnia on basic haemodynamic parameters (herein referred to as 'maintenance protocol').

4.2 Methods

4.2.1 Study Population

The study was conducted in accordance with the Declaration of Helsinki (2013).⁹⁸ Ethical approval was obtained from: 1) Induction strategies: Leicestershire, Northamptonshire and Rutland Research Ethics Committee (09/H0402/79); 2) Maintenance protocol: University of Leicester Ethics Committee (Reference: jm591-c033). In both cases, healthy volunteers were recruited from University departmental staff, students and their relatives. Participants aged above 18 years were included. Exclusion criteria were physical disease in the upper limb, poor insonation of both temporal bone windows and any significant history of cardiovascular, neurological or respiratory disease. All participants provided written, informed consent.

4.2.2 Procedures

The research was undertaken in the University of Leicester's Cerebral Haemodynamics in Ageing and Stroke Medicine (CHiASM) research laboratory, maintained at a constant ambient temperature of approximately 24°C and free of distraction. For the purposes of the study, participants were asked to refrain from caffeine, alcohol and nicotine in the 12-hour period prior to measurements being undertaken. Beat-to-beat BP was recorded continuously using the Finometer[®] device (FMS, Finapres Measurement Systems, Arnhem, Netherlands), which was attached to the middle finger of the left hand. The servo-correcting mechanism of the Finometer[®] was switched on and then off prior to measurements. The hand bearing the finger cuff was at the level of the heart to negate any hydrostatic pressure artefact. HR was recorded using a standard 3-lead ECG.

EtCO₂ was measured throughout using small nasal cannulae (Salter Labs) connected to a capnograph (Capnocheck Plus). Bilateral insonation of the MCAs was performed using TCD

ultrasound (Viasys Companion III; Viasys Healthcare) with a 2MHz probe. This probe was secured in place with a head-frame that was adjusted to ensure comfort at the outset. The MCAs were identified according to two main characteristics: signal depth and velocities.⁹⁹

Measurements were continuously recorded at a rate of 500 samples/s in the PHYSIDAS data acquisition system (Department of Medical Physics, University Hospitals of Leicester). Systolic and diastolic brachial BP readings (OMRON Model 705IT) were performed at each stage of the measurements (normocapnia and hypocapnia) with a minimum of three recordings per individual. These values were then used to calibrate the Finometer recordings.

4.2.3 *Hyperventilation Induction Strategies*

Following a 20-minute stabilisation period, a 5-minute baseline recording was taken of the subject breathing spontaneously at rest. Hyperventilation strategies were then repeated twice in random order, with 5-minute intervals between each to allow stabilisation of all parameters and return to normocapnia. The different hyperventilation induction strategies are detailed below; hyperventilation being maintained for a total of 90 seconds in each strategy:

- Delayed metronome (hereafter, termed delayed): 2 minutes of spontaneous breathing followed by sudden inception of a metronome (KORG Metronome MA-30). The metronome speed was individualised to achieve a respiratory rate 40% greater than the original resting rate.
- Continuously increasing metronome (continuous): Use of a continuous metronome, started at a rate analogous to that of the subject's baseline resting rate. After one minute of baseline recording, the rate was increased gradually over a period of 60 seconds to reach a hyperventilation rate 40% greater than baseline.

- Voluntary (voluntary): Hyperventilation was induced without the use of a metronome. After 2 minutes of spontaneous respiration, subjects were vocally instructed to increase their respiratory rate independent to any other auditory stimulus.

For all the strategies, recordings were extended to include periods of spontaneous respiration for 2 minutes before and 3 minutes after hyperventilation in order to ascertain the temporal patterns of all parameters.

4.2.4 *Hyperventilation Maintenance Protocol*

All measurements were conducted at a single visit. After a period of 5 minutes of stabilisation, participants performed a 5-minute baseline recording and then were asked to hyperventilate in random order, at different respiratory rates to produce incremental reductions in EtCO₂ of 5mmHg and 10mmHg less than normocapnia for that individual. Hyperventilation was sustained for a minimum period of 60s. For hyperventilation, participants were asked to breathe with a metronome creating a respiratory rate of at least 5 breaths per minute above their resting rate for at least 90 s using the delayed metronome technique. Two-minute washout periods of normal respiration were allowed between successive measurements. Each incremental reduction in EtCO₂ was repeated on two occasions during the same session.

4.2.5 *Data Analysis*

Data collected corresponded to individual files for each participant at baseline and during hypocapnia. First, data were inspected visually and calibrated to recorded systolic and diastolic OMRON BP. Narrow spikes (<100ms) were removed using linear interpolation and the CBFV recording was then passed through a median filter. All signals were then low pass filtered with a zero-phase Butterworth filter with cut-off frequency of 20Hz. Automatic detection of the QRS complex of the ECG, to mark the R-R interval was used, but also visual inspection was

undertaken with manual correction whenever necessary. This allowed mean ABP, HR, EtCO₂ and mean CBFV to be calculated for each cardiac cycle. Randomisation was not disclosed until data collection was completed.

4.2.6 *Induction Strategies and Maintenance Protocol*

Baseline files were analysed using a MW-ARMA model as described by Dineen *et al.*⁴⁷ with a time window of 60 seconds and a window shift of 0.6 seconds for each consecutive estimate. This produced multiple estimates of ARI, which were then averaged to produce a single baseline ARI value for each file.

For the hyperventilation strategies, continuous estimates of ARI(t) were produced for each file using the same MW-ARMA model. These were then digitally marked at the point of EtCO₂ increase (signifying the end of hyperventilation) as this proved to be the most recognisable and reproducible point between strategies. Marked files were synchronised at 120 seconds and these data used to produce coherent averages of ARI(t) for each strategy, allowing comparison between the different conditions.

4.2.7 *Statistical Analysis*

Data normality was assessed with the Kolmogorov-Smirnov test. Baseline measurements were assessed for differences between values derived for right and left hemispheres using a paired Student's t-test. These were averaged when no significant differences were found.

For comparison of hyperventilation strategies, each participant acted as their own control, with an average value for each subject used where repeated manoeuvres were available for analysis. Hyperventilation strategies were compared using means of maximal changes in ARI(t) and EtCO₂(t). Two calculations of maximal ARI(t) change were performed. First, the difference

between the initial baseline value and the minimal ARI during hyperventilation initiation was calculated. Additionally, the difference between this ARI trough and the subsequent peak of recovery was obtained. The maximal EtCO₂(t) change was calculated by subtracting the EtCO₂ at maximal hypocapnia from an average baseline, calculated using the 30 seconds prior to each manoeuvre. All values were compared between the three strategies using repeated-measures ANOVA. Where statistical differences occurred, paired t-tests with a Bonferroni adjustment were employed to clarify where differences lay. Values of p<0.05 were considered significant, giving an alpha significance level of p<0.017 following Bonferroni adjustment.

4.3 Results

4.3.1 Induction Strategies

Sixty-one healthy volunteers were recruited to the study (16 and 45 within the induction and maintenance protocol studies, respectively), one subject (from the induction strategies study) had all data removed from analysis as it failed to show a synchronous physiological reaction of BP or CBFV to EtCO₂ changes. Demographic data for all 61 subjects are presented in Table 4.1. There were no significance differences between the male and female demographics.

Table 4.1 Population characteristics and baseline parameter values.

	Induction Protocol			Maintenance Protocol		
	Male	Female	p-value	Male	Female	p-value
Number (n)	7	8	-	19	26	-
Age (years)	37 ± 15 [21, 62]	33 ± 12 [22, 53]	0.57	36 ± 15 [21, 69]	39 ± 14 [21, 69]	0.87
Systolic BP (mmHg)	127 ± 18 [100, 146]	117 ± 15 [100, 144]	0.67	125 ± 12 [106, 146]	118 ± 15 [91, 151]	0.07
Diastolic BP (mmHg)	81 ± 18 [56, 107]	70 ± 12 [57, 96]	0.26	72 ± 8 [54, 90]	70 ± 11 [47, 96]	0.52
HR (bpm)	64 ± 11 [54, 82]	65 ± 9 [57, 79]	0.67	66 ± 13 [49, 101]	72 ± 10 [59, 97]	0.19
RR (min ⁻¹) baseline	13 ± 3 [10, 20]	14 ± 2 [10, 16]	0.34	15 ± 3 [10, 20]	15 ± 3 [10, 20]	0.62
RR (min ⁻¹) -5mmHg				21 ± 1 [20, 24]	21 ± 2 [16, 25]	0.53
RR (min ⁻¹) -10mmHg				28 ± 1 [26, 31]	27 ± 2 [23, 34]	1.00

Values are given as the mean ± standard deviation with the range denoted in brackets. BP, arterial blood pressure; HR, heart rate; RR, respiratory rate.

The mean ARI for the group was calculated as 5.95 ± 1.07 with a range of 4.36 to 7.86 during spontaneous respiration, which is within the normal range.^{40,118} Drifting of the BP signal led to rejection of two files, saturation of ARI was seen in a further two files and two manoeuvres failed to show a synchronous physiological reaction to changes in EtCO₂. As such, the final statistical analysis included a total of 26 recordings for the delayed metronome strategy, 24 recordings for the continuous increasing metronome strategy and 25 recordings for the voluntary strategy.

Coherent averages of ARI(t) and EtCO₂(t) were produced for each strategy (Figure 4.1) and the means of maximal change calculated (Table 4.2). Significant differences were observed between strategies in both the initial ARI(t) decrease and the ARI(t) recovery. The extent of initial decrease was significantly smaller in the continuous metronome strategy compared to the delayed metronome and voluntary strategies (Δ ARI 0.33 ± 1.18 , 2.80 ± 3.33 and 3.69 ± 2.79 ,

respectively, $p < 0.017$). There was no difference in the extent of initial decrease between the delayed metronome and voluntary strategies. The resultant degree of recovery needed to return the ARI to an augmented state was also significantly smaller in the continuous metronome when compared to the voluntary strategy ($\Delta\text{ARI } 1.98 \pm 1.90$ and 4.79 ± 2.94 respectively, $p < 0.017$); however, the difference between the continuous and delayed metronome strategies was borderline after the Bonferroni correction ($p = 0.02$).

Differences were also observed in baseline EtCO_2 and in the extent of induced hypocapnia between strategies. Baseline EtCO_2 was significantly lower in the continuous metronome strategy compared to the delayed metronome and voluntary strategies (EtCO_2 34.5 ± 2.3 , 39.0 ± 1.6 and 38.6 ± 2.0 mmHg, respectively, $p < 0.017$). The degree of induced hypocapnia was similar between the delayed metronome and continuous metronome strategies (ΔEtCO_2 -5.5 ± 3.3 and -4.8 ± 2.3 mmHg, respectively, $p = 0.25$), but significantly greater with the voluntary strategy (ΔEtCO_2 -8.3 ± 3.6 mmHg, $p < 0.017$).

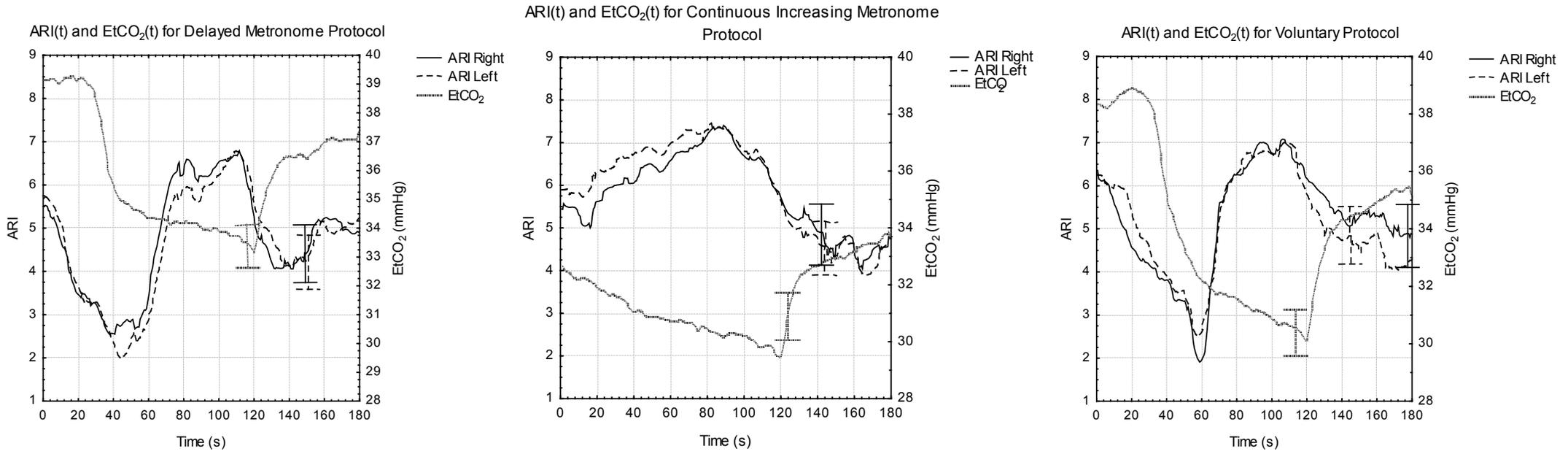
In addition, coherent average graphs were also created for CBFV(t), critical closing pressure (CrCP)(t), resistance-area product (RAP)(t), ABP(t) and HR(t). These graphs allowed visual inspection of the stability of each parameter throughout the strategies (Figure 4.2 – 4.6). Across all parameters, the visual depictions demonstrated greater parameter stability over time with the continuous metronome strategy as compared to the two alternatives. The delayed and voluntary metronome strategies demonstrated more abrupt changes at the beginning and end of the measurement periods.

Table 4.2 Mean values of ARI for each protocol at baseline and showing the means of maximal changes. Baseline EtCO₂ and the extent of induced hypocapnia are also shown.

		Delayed metronome	Continuous metronome	Voluntary	P-value
ARI	Baseline	5.62 ± 1.78	5.66 ± 2.04	6.12 ± 1.74	0.65
	Extent of initial decrease	2.80 ± 3.33	0.33 ± 1.18	3.69 ± 2.79	0.001*
	Degree of recovery	4.08 ± 2.57	1.98 ± 1.90	4.79 ± 2.94	0.01*
EtCO ₂ (mmHg)	Baseline	39.0 ± 1.6	34.5 ± 2.3	38.6 ± 2.0	<0.001*
	Degree of hypocapnic change	5.5 ± 3.3	4.8 ± 2.3	8.3 ± 3.6	<0.001*

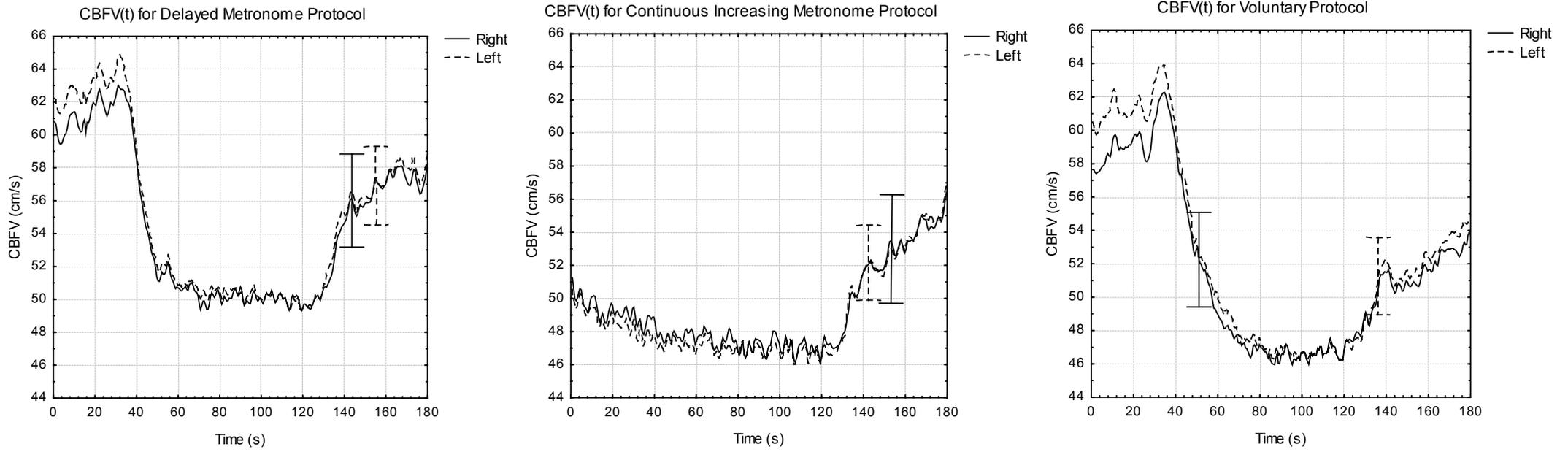
Values are given as the mean ± standard deviation. Significant differences between the protocols are marked using *. ARI, autoregulation index; EtCO₂, end-tidal arterial pressure of carbon dioxide.

Figure 4.1 Coherent average of ARI(t) and EtCO₂(t) for each hyperventilation strategy for induction of hypocapnia.



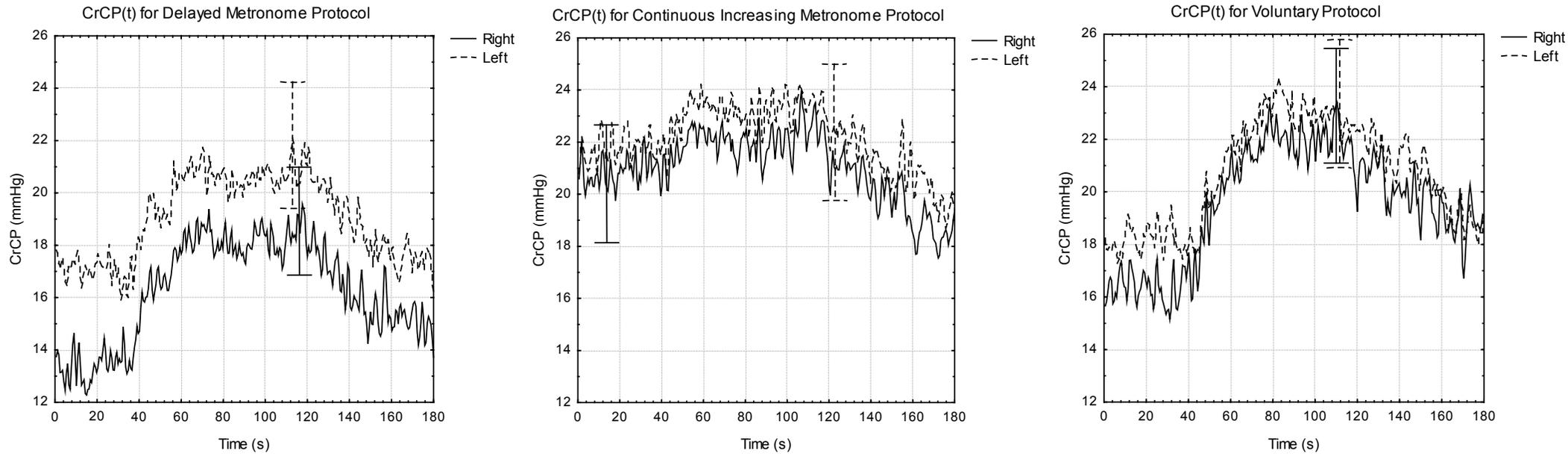
Error bars show \pm one SEM, calculated using the maximum standard deviation and displayed at the point where maximum error occurred

Figure 4.2 Coherent average graphs of CBFV(t) for each hyperventilation strategy for induction of hypocapnia



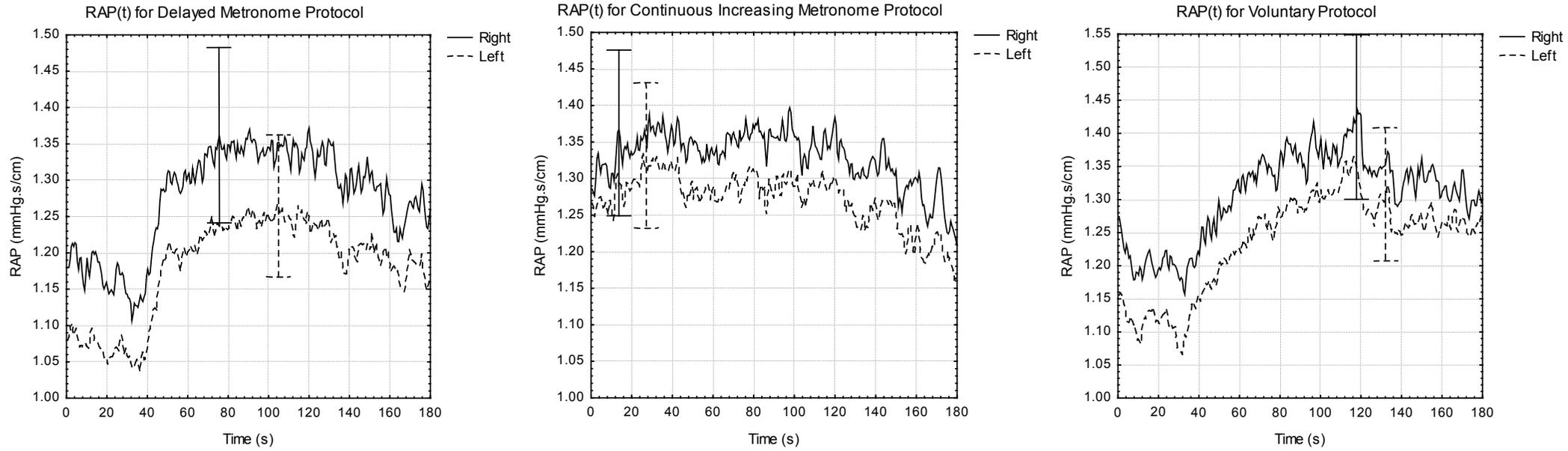
Error bars show \pm one SEM, calculated using the maximum standard deviation and displayed at the point where maximum error occurred

Figure 4.3 Coherent average graphs of critical closing pressure (CrCP)(t) for each protocol.



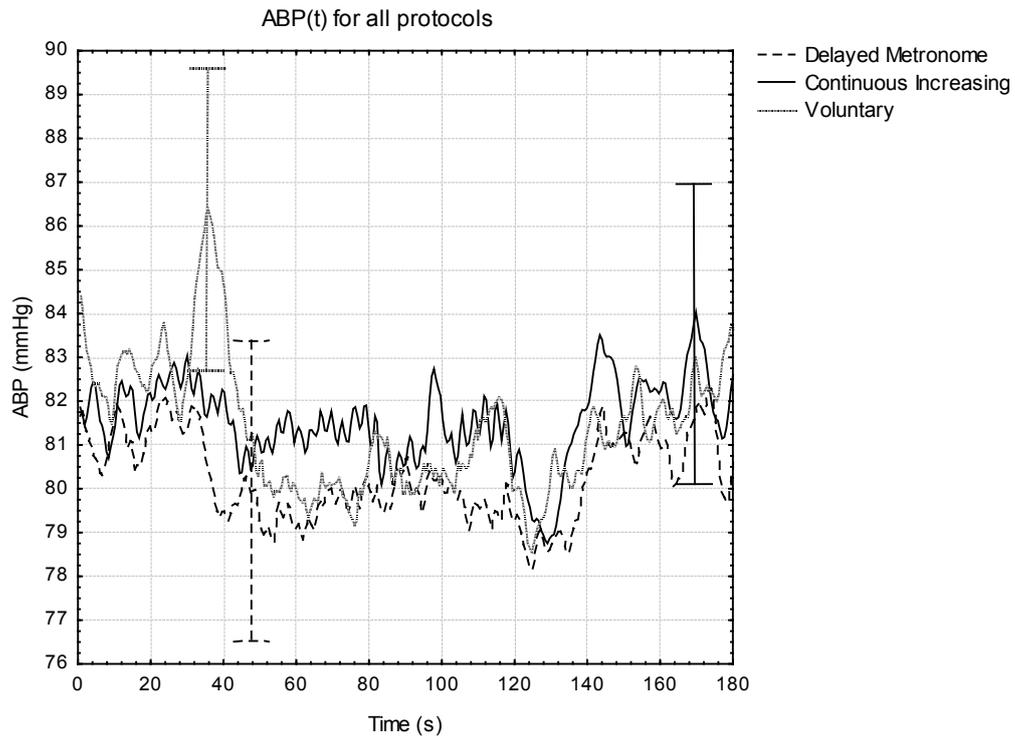
Error bars show \pm one SEM, calculated using the maximum standard deviation and displayed at the point where maximum error occurred

Figure 4.4 Coherent averages of Resistance Area Product (RAP)(t) for each hyperventilation strategy for induction of hypocapnia.



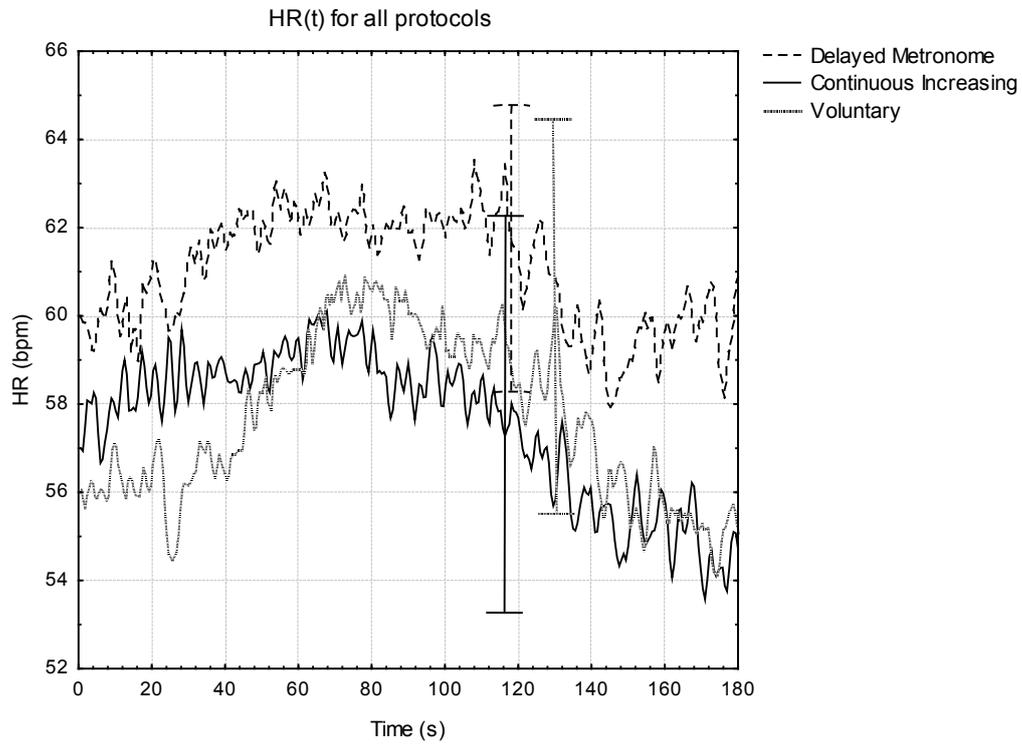
Error bars show \pm one SEM, calculated using the maximum standard deviation and displayed at the point where maximum error occurred

Figure 4.5 Coherent averages of ABP(t) for each hyperventilation strategy for induction of hypocapnia.



Error bars show \pm one SEM, calculated using the maximum standard deviation and displayed at the point where maximum error occurred

Figure 4.6 Coherent averages of HR(t) for each hyperventilation strategy for induction of hypocapnia.

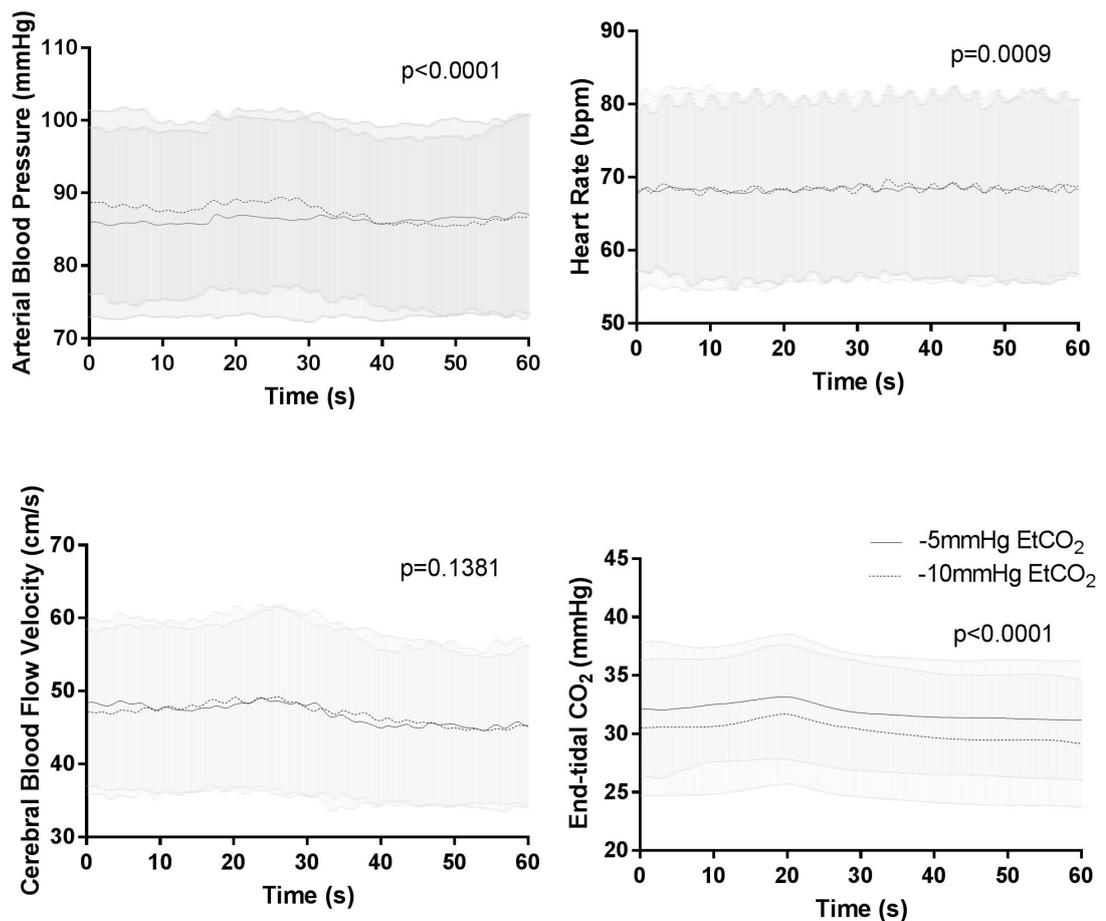


Error bars show \pm one SEM, calculated using the maximum standard deviation and displayed at the point where maximum error

4.3.2 Maintenance Protocol

The maintenance of hypocapnia protocol involved two incremental reductions in EtCO₂ during the same session. These were randomised and involved a significant washout period. As expected, the targeted reductions in EtCO₂ to -5mmHg and -10mmHg were accompanied by increases in respiratory rate (Table 4.1). Interestingly, despite significant differences in EtCO₂ during each incremental lowering ($p < 0.0001$), only CBFV ($p = 0.1381$) remained unchanged, as ABP ($p < 0.0001$) and HR ($p = 0.0009$) both demonstrated significant differences in response to the step change in hyperventilation (Figure 4.7).

Figure 4.7 Coherent averages of ABP(t), HR(t), CBFV(t) and EtCO₂(t) for the maintenance protocol.



Shaded areas show error bars show \pm one standard deviation. Values of $p < 0.05$ were considered significant.

4.4 Discussion

Use of a continuous metronome to induce hypocapnia rather than the sudden inception of an auditory stimulus appeared to reduce the initial decrease in autoregulatory capacity seen previously in work by Dineen *et al.*⁴⁷ This is important as it afforded better estimates of CA, especially when using original batch-processing analysis techniques to calculate mean values of ARI for an entire file. Furthermore, maintenance of EtCO₂ at differing levels of targeted hypocapnia (-5mmHg and -10mmHg) demonstrated no significant effect on central haemodynamic parameters (CBFV), but affected peripheral parameters including HR and ABP. No other studies investigating the effect of hyperventilation protocols on continuous estimates of dCA have been identified.

The coherent averages of ARI(t) (Figure 4.1) clearly demonstrated a reduced initial ARI decrease using the continuous increasing metronome protocol compared to the original delayed metronome. This observation is supported by statistical analysis, which showed an ARI drop of 0.33 ± 1.18 for the continuous metronome protocol and 2.80 ± 3.33 for the delayed metronome ($p < 0.017$). The smaller standard deviation for the continuous metronome protocol also highlighted the greater stability of these measurements compared to those obtained using the original protocol. The minimised initial drop is also reflected in the smaller degree of recovery required to return the ARI to an augmented state. Although not statistically significant, when a single fiducial point is selected, the difference in degree of recovery is clearly visible when examining the complete temporal pattern of the coherent average graphs (Figures 4.1-4.6). In addition, the greater stability demonstrated by the CBFV(t), CrCP(t), RAP(t), ABP(t) and HR(t) coherent averages from the continuous metronome condition provided further evidence of the advantages of this protocol over the original (Figures 4.2-4.6).

Visual inspection shows the augmentation of ARI(t) diminished prior to the cessation of hyperventilation in the continuous metronome condition (Figure 4.1). This differs to the pattern of ARI(t) in both the delayed metronome and voluntary protocols, where dCA remained augmented until hyperventilation was discontinued. On examination of the original data this was found to be a real effect, with the subjects divided into two groups: those who maintained ARI throughout hypocapnia and those who did not. Whilst the continuous metronome protocol appears to predominantly remove what can be thought of as an “alert reaction” in response to hyperventilation induction, it is possible that it induces a less severe but more prolonged mental stress effect. Therefore, rather than producing a sudden initial decrease in ARI, the prolonged mental activation competes with the usual hypocapnic augmentation of dCA. The high metronome rate and long duration of synchronised breathing may have caused a degree of mental and physical fatigue towards the end of measurement that is reflected by a premature ARI decrease in selected individuals. However, the maintenance protocol demonstrated a steady EtCO₂, HR and ABP throughout with little significant variation in trend.

The voluntary protocol was designed to investigate the physiological effect of hypocapnia on dCA without the stress of synchronising breathing to a metronome. For this reason, it was expected that it would cause less of an initial ARI decrease than the delayed metronome protocol. However, on commencement of voluntary hyperventilation, a large decrease in the autoregulatory capacity occurs that is comparable to that of the delayed metronome ($p = 0.30$). This may have occurred for several reasons. The sudden interception of vocal instruction may have startled some participants, creating an ‘alert reaction’ similar to that of the metronome inception. A further possibility is that, without a metronome, participants overcompensated to ensure hyperventilation, meaning mental stimulation and respiratory effort were greater during this protocol. This is supported by a larger EtCO₂ decrease in the voluntary condition compared to the other protocols ($p < 0.017$).

The continuous metronome protocol induced a comparable degree of hypocapnia to that of the delayed metronome protocol supporting implementation of the new protocol as the hypocapnia induced was of a similar magnitude to that obtained with the previous method, making it a suitable alternative for assessment of CVRi.

Baseline EtCO₂ was significantly lower in the continuous metronome protocol than in the delayed metronome or voluntary protocols ($p < 0.017$). In the latter two protocols, baseline EtCO₂ was calculated using the initial period of spontaneous respiration; this was observed to be naturally erratic in most participants, with temporary periods of very low respiratory rate. In contrast, participants synchronised their breathing from the beginning of the continuous metronome recordings and consequently had no opportunity to retain CO₂ through decreases in respiratory rate. This explained the lower values of baseline EtCO₂ for that protocol. These findings apply to the maintenance protocol as the method used was analogous with the delayed protocol and although increments were close to targets of -5mmHg and -10mmHg below baseline, lower values may have been achieved had a continuous protocol been employed prior to maintenance.

4.4.1 Limitations

Individual differences in baseline autoregulatory capacity ought not to have biased comparisons made between protocols as every participant was represented equally between the conditions. In addition, each subject acted as their own control across the protocols, with participants being excluded from the final analysis if data were missing from any of the conditions.

Although small, the sample size for the study had adequate power to detect the Δ ARI differences between protocols. A study by Brodie *et al.* assessed the reproducibility of ARI measurements and determined that in order to achieve 80% statistical power at the $p = 0.05$ significance level, a sample size of 11 was necessary to detect a change in ARI of 2.¹¹⁹ The sample size in this study

was 15 and the difference in the initial Δ ARI between the continuous and delayed metronome condition was +2.47. Whilst it is recognised that the study by Brodie *et al.*¹¹⁹ evaluated ARI calculations based on the traditional batch-processing method, the greater sensitivity of the MW-ARMA model should if anything, increase the power of this study.¹⁰⁰

Synchronising the files to produce coherent averages and the continuous increasing metronome condition was challenging. In the study by Dineen *et al.*⁴⁷, files were synchronised by the transient rise in CBFV signifying the beginning of hyperventilation. However, with the continuous metronome protocol this CBFV peak does not occur, and therefore recordings for all protocols were marked at the end of hyperventilation using the EtCO₂ trace; this being the most reproducible point between files. Marking and synchronisation of data should ideally occur at the point of hyperventilation initiation to increase accuracy and reliability of the averaged data. It may be possible in future work to digitally mark the start of hyperventilation on the traces at time of recording, thus avoiding this problem.

There are also some practical considerations for implementation of the continuous metronome protocol. During the condition, participants were required to breathe in time with a metronome for a total of four minutes. Whilst most subjects were able to synchronise their breathing at baseline, some patients were unable to maintain this throughout the rate increase. The speed of the metronome may also have been stressful for the participant due to technical problems encountered when trying to adjust the metronome speed manually. This problem could be overcome with the use of a programmable digital metronome rather than the use of a hand-held device. This measurement error will have contributed to the shortfall in the difference (2-3mmHg) between target incremental levels (-5mmHg and -10mmHg EtCO₂). However, despite generating an adequate increment, CBFV values did not differ, therefore questioning the value of multiple levels of hypocapnic measurement. This is crucial as studies have previously used multiple increments to assess cerebral haemodynamic change, and although these have

provided valuable information on the relationship of EtCO₂ change on dCA, future studies may not require multiple increments unless significantly greater than 3mmHg.^{101,120} Therefore, there remains a possibility that had the increment been larger; CBFV may have differed, though we can conclude that an increment of 2-3mmHg does not demonstrate significant CBFV change to warrant such increments in the study design.

Another consideration is that whilst some individuals were confident synchronising their breathing to an external stimulus, those who were not, seemed to find it a particular challenge when the stimulus was varying. In this study the problem was relatively minor; however the applicability of the continuous metronome protocol must be tested in other populations. Of particular concern are the elderly and individuals of ill health who may have limited concentration or lower tolerance for sustained hypocapnia. Furthermore, sex-related differences in cerebral haemodynamics have been demonstrated, though underlying mechanisms remain unclear with vessel diameter, hormonal variation and basic metabolic rate all discussed as influencing factors.³⁵ However, female subjects have higher MCA velocities, CO₂ cerebrovascular reactivity and vasomotor range than males.³⁵ This study was not designed to inform the extent to which sex may influence the initiation and maintenance of hyperventilatory interventions, though it is reassuring the baseline demographic data did not demonstrate sex differences.

As with any study of this kind, the limitations of TCD for measuring CA have to be considered. The use of TCD to estimate CBF relies upon the assumption that the MCA has a fixed diameter and therefore any changes in measured CBFV correspond to changes in flow. Numerous studies have assessed MCA diameter change during changes in ABP and EtCO₂. The majority of these studies have demonstrated during direct visualisation of both the MCA and internal carotid artery by MRI assessment that no change in diameter occurs.¹²¹⁻¹²³ However, more recent studies have demonstrated that during high levels of hypercapnia (>45mmHg) there is

sensitivity to CO₂ change and hence diameter change.^{124,125} Importantly, there remains a paucity of data supporting the existence of such changes in hypocapnic conditions. Therefore, for the purposes of this study the changes in CBFV measured were assumed to correspond to changes in CBF.

4.4.2 Future Directions

Before the continuous metronome protocol can be accepted as a suitable method for testing cerebrovascular reactivity to hypocapnia, its use must be tested in different patient populations to establish reliability of results as well as how it can be practically implemented in more vulnerable groups.

It would also be of interest to explore the independent physiological effects of mental stimulation and changing PaCO₂ on dCA. Investigation into the effects of brain activation could be achieved by sudden synchronisation of breathing to an external stimulus set at an identical rate to the participants' resting respiratory rate. The effect of changing PaCO₂ could be assessed using a re-breathing protocol to passively alter the concentration of inspired gases. This approach has been previously used in autoregulation studies but in combination with a MW-ARMA model, it may extend our understanding of the physiological parameters affecting CA.

In conclusion, use of a continuous metronome to induce hypocapnia rather than the sudden inception of an auditory stimulus appears to reduce the initial decrease in autoregulatory capacity seen previously in work by Dineen *et al.*⁴⁷ This is important as it affords better estimates of CA, especially if using original batch-processing analysis techniques to calculate mean values of ARI for an entire recording.

5 Chapter Five: Modelling the cerebral haemodynamic response in the entire physiological range of PaCO₂

5.1 Introduction

As introduced in Chapter 1, CA is usually defined as the tendency of CBF to remain approximately constant despite changes in BP within the range 50 to 170 mmHg.^{103,104} However, outside these limits, CA becomes passive and CBF follows changes in BP. Importantly, this classical relationship, usually referred to as 'static' CA, has been challenged and ultimately the physiological properties of CA remain largely inconclusive.¹²⁶⁻¹²⁸ dCA can be estimated from the transient response of CBF to rapid changes in BP³⁸ and this has been the preferred approach for the assessment of CA in human physiological and clinical studies.^{96,108,129} Understanding the dynamic CA response to physiological manoeuvres, such as exercise and changes in respiratory patterns, has often been confounded by simultaneous changes in the PaCO₂.^{47,116} Hypercapnia leads to vasodilation of cerebral vessels¹³⁰ and overall causes deterioration in CA.³⁸ Conversely, hypocapnia has a vasoconstrictive effect, improving CA.^{47,101,116} Indeed, experimental work has suggested hypercapnia can be used to emulate a state of impaired dCA.⁷⁶ Although these effects of PaCO₂ changes on CBF and dynamic CA are widely accepted, there is a need for a comprehensive quantitative model covering the entire physiological range of PaCO₂, to allow further refinements in the data analysis of physiological and clinical cerebrovascular studies. This is crucial for cerebral haemodynamic parameters as well as systemic haemodynamic parameters as these are often considered significant confounders when assessing blood flow during physiologically vulnerable states like altitude, extremes of exercise and acute neurological emergencies.

Changes in PaCO₂ induced by transient breath-by-breath adjustment demonstrate non-linear effects on CBF.^{114,131} Amongst different potential non-linear models, the logistic function has

been shown to provide a realistic description of the CBFV, responding to changes in PaCO₂.¹⁰⁶ Logistic models have also proved successful to describe the effects of PaCO₂ on cerebrovascular conductance index (CVCi)¹⁰⁶ and cerebrovascular resistance.¹³²

Current understanding of vascular physiology principles has led to models adopting the principle that vascular bed resistance-CO₂ response relationships are sigmoidal.¹³² Although logistic modelling has been applied to parameters like branch pressure or resistance, there is a clear clinical importance in developing such models in health and pathological states to help understand variations in cerebrovascular CO₂ responsiveness.

We used a wide range of PaCO₂ within a multi-step protocol to test for the first time the hypotheses that i) a commonly used index of dCA, the ARI shows a dependence on PaCO₂ following a logistic non-linear model, similar to that described for CBFV; and ii) key cerebral haemodynamic parameters including ABP, HR, CrCP and RAP can also have their dependence on PaCO₂ described by a logistic non-linear model.

5.2 Methods

5.2.1 *Subjects and Measurements*

The study was conducted in accordance with the Declaration of Helsinki (2013).⁹⁸ Ethical approval was obtained from the University of Leicester Ethics Committee (Reference: jm591-c033). Healthy volunteers were recruited from University departmental staff, students and their relatives. Participants aged above 18 years were included. Exclusion criteria were physical disease in the upper limb, poor insonation of both temporal bone windows and any significant history of cardiovascular, neurological or respiratory disease. All participants provided written, informed consent.

The research was undertaken in the University of Leicester's *Cerebral Haemodynamics in Ageing and Stroke Medicine* research laboratory, maintained at a constant ambient temperature of approximately 24°C and free of distraction. For the purposes of the study, participants were asked to refrain from caffeine, alcohol and nicotine in the 12-hour period prior to measurements being undertaken. Beat-to-beat BP was recorded continuously using the Finometer® device (FMS, Finapres Measurement Systems, Arnhem, Netherlands), which was attached to the middle finger of the left hand. The servo-correcting mechanism of the Finometer® was switched on and then off prior to measurements. The hand bearing the finger cuff was at the level of the heart to negate any hydrostatic pressure artefact. HR was recorded using a standard 3-lead electrocardiogram (ECG). EtCO₂ was measured throughout the initial resting baseline and hypercapnic phase using a face-mask connected to a capnograph (Capnocheck Plus). During the second baseline and hypocapnic phase EtCO₂ was measured via nasal prongs (Salter Labs). Bilateral insonation of the middle cerebral arteries (MCAs) was performed using TCD (Viasys Companion III; Viasys Healthcare) with a 2MHz probe. The TCD probes were secured in place with a head-frame that was adjusted to ensure comfort at the outset. The MCAs were identified according to two main characteristics: signal depth and velocities.⁹⁹

5.2.2 *Experimental Protocol*

All measurements were conducted at a single visit. Prior randomisation of the order of hypo- and hypercapnia was conducted using a random number generator. An initial period of 15 minutes of stabilisation preceded a 5-minute baseline recording supine at rest. This was followed by inspiring CO₂ in air, constantly ('fixed inspiration') for a minimum of 90 s (ideally 120 s) with either 5% CO₂ or 8% CO₂ (dependent on randomisation). Each gas inspiration episode was preceded by a 90 s recording to achieve physiological stability before and immediately after the hypercapnia study period. After a further period of 5 min of stabilisation, participants performed a 5 min baseline recording and then were asked to hyperventilate in random order,

as previously described, at different respiratory rates to produce incremental reductions in EtCO₂ of 5mmHg and 10mmHg less than normocapnia for that individual. Hyperventilation was sustained for a minimum period of 90 s and maximum of 120 s. For hyperventilation, participants were asked to breathe with a metronome (KORG Metronome MA-30) creating a respiratory rate of at least 5 breaths per minute above their resting rate for at least 90 s without specific control of amplitude of breathing. Two-minute washout periods of normal respiration were allowed between successive measurements. Each incremental reduction in EtCO₂ was repeated on two occasions during the same session. Measurements were continuously recorded at a rate of 500 samples/s in the PHYSIDAS data acquisition system (Department of Medical Physics, University Hospitals of Leicester). Systolic and diastolic brachial BP readings (OMRON Model 705IT) were performed at each stage of the protocol (normocapnia, hypercapnia and hypocapnia with a minimum of 3 recordings per individual). These values were then used to calibrate the Finometer recordings.

5.2.3 Data Analysis

Data collected corresponded to six individual files for each participant: two at baseline, two hypercapnic and two hypocapnic. First, data were inspected visually and calibrated to recorded systolic and diastolic OMRON BP. Narrow spikes (<100ms) were removed using linear interpolation and the CBFV recording was then passed through a median filter. All signals were then low pass filtered with a zero-phase Butterworth filter with cut-off frequency of 20Hz. Automatic detection of the QRS complex of the ECG, to mark the R-R interval was visually inspected and manually corrected whenever necessary. This allowed mean BP, HR, EtCO₂ and mean CBFV to be calculated for each cardiac cycle. CrCP and RAP were estimated using the first harmonic method.¹⁰⁰ Randomisation was not disclosed until data collection was completed.

Given the non-stationary influence of PaCO₂ on dCA, ARI, proposed by Tiecks *et al.*⁴⁰, was calculated as a function of time (ARI(t)), using a moving-window, autoregressive moving average (ARMA) model, that follows the same structure as the second-order differential equation proposed by Tiecks *et al.*⁴⁰, as described previously.⁴⁷

In short, with V(t) representing beat-to-beat changes in CBFV and dP(t) corresponding changes in BP, normalised by CrCP, the two quantities are linked by:

$$\hat{V}(t) = 1 + dP(t) - K \times x_2(t) \quad [1]$$

where K represents a gain parameter in the second order equation, and x₂(t) is a state variable obtained from the following state equation system representing a second-order equation:

$$x_1(t) = x_1(t-1) + \frac{dP(t-1) - x_2(t-1)}{f \times T} \quad [2]$$

$$x_2(t) = x_2(t-1) + \frac{x_1(t-1) - 2 \times D \times x_2(t-1)}{f \times T} \quad [3]$$

where parameters T and D correspond to the damping and time-constant terms of a second order model, and f is the inverse of the sampling frequency.⁴⁰

From these equations, it is possible to demonstrate that eq. 1 can be expressed as a discrete ARMA model⁴⁷, that is:

$$v(n) = ap(n) + b[p(n-1) - v(n-1)] + c[p(n-2) - v(n-2)] \quad [4]$$

where v(n) and p(n) are discrete samples of V(t) and P(t), respectively, and the coefficients a, b and c are directly related to the original parameters K, D, T above.

The ARMA model was applied for a 60 s time moving window using BP as input and CBFV as output. Using the model coefficients (a, b, c), the CBFV response to a step change in BP was obtained and the corresponding value of ARI was estimated by fitting one of the 10 CBFV step responses proposed by Tiecks *et al.*⁴⁰ The complete time-series of ARI(t) values for each recording was obtained by moving the time-window at 0.6s intervals. Values of ARI(t)=0 represent absence of autoregulation, whilst ARI(t)=9 corresponds to the most efficient CA that can be observed. The ARI(t) time-series was computed for each subject separately for left and right hemispheres for each recording.

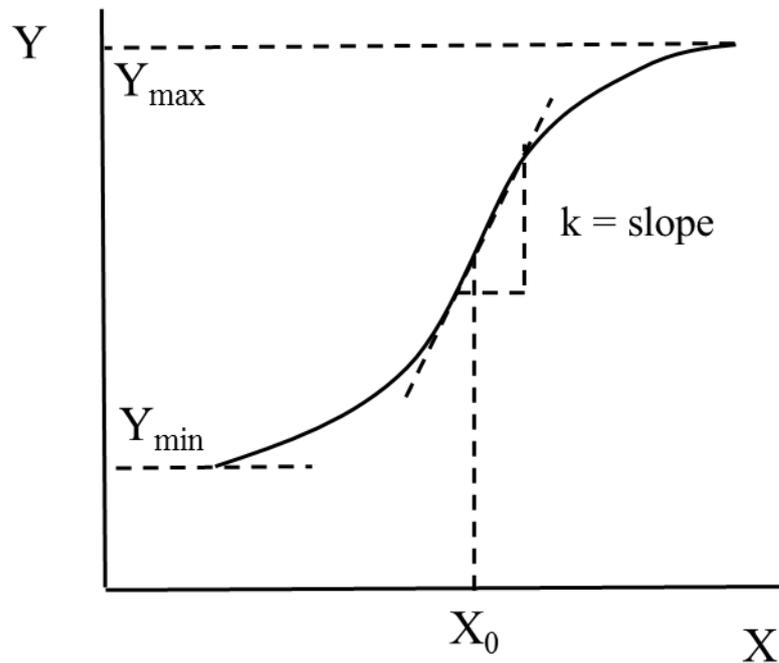
5.2.4 Logistic Model

Following the logistic model for the effects of PaCO₂ on CBFV,¹⁰⁶ a similar model was adopted, and was also extended to test the feasibility of using the logistic relationship to express the influence of CO₂ on dCA and other systemic and cerebrovascular parameters. The logistic model adopted is given by:

$$y = y_{max} + \frac{y_{min} - y_{max}}{1 + e^{k(x-x_0)}} \quad [5]$$

where y can represent CBFV, ARI, BP, HR, CrCP or RAP; x is the EtCO₂ level and k is the exponential coefficient. Fitting the model allows estimation of its four parameters, namely y_{max}, y_{min}, k and x₀ (Figure 5.1).

Figure 5.1 Schematic representation of the four parameter logistic function.



Schematic representation of the four parameter logistic function demonstrating the shape and relationship between parameters: where y can represent either mean CBFV, ABP, HR, CrCP, RAP or ARI(t); x is the EtCO₂ level and k is the exponential gain coefficient. Fitting the model allows estimation of its four parameters, namely y_{\max} , y_{\min} , k and x_0 .

Model parameters were obtained by combining least squares with a recursive bootstrap technique to remove outliers. After estimation of ARI(t) for each of the six different recordings (two each, for baseline, hyperventilation, and CO₂ breathing), each recording was divided into four segments and the mean CBFV, BP, HR, CrCP, RAP, ARI(t) and EtCO₂ of each segment was used to fit equation 1 using 24 sample values (4 segments for 6 recordings) to express the effects of PaCO₂ on these six different parameters. After each stage of error minimisation by least squares, the largest outlier was identified and the process was repeated for a maximum of nine potential outliers. The minimum square error (MSE) and the estimated parameters at each stage were listed and the number of outliers to be removed was chosen under visual inspection, to select model parameters that were stable and a region of MSE that was not critically dependent

on the number of outliers removed. In general, the final number of outliers removed was less than nine. The advantages and limitations of this new approach will be discussed later.

5.2.5 *Statistical Analysis*

The study protocol was tested for differences in each level of CO₂ using one-way ANOVA. Data normality was assessed for skewness and kurtosis. Baseline measurements were assessed for differences between values derived for right and left hemispheres using a paired Student's t-test. These were averaged when no significant differences were found. Repeated measures ANOVA was used to assess for differences between model parameter values for each haemodynamic parameter group.

5.3 Results

Forty-five subjects (19 male) of mean age 37.5 years (range 21 to 71) were included in the analyses. None of the subjects were smokers or had diabetes.

Differences between recordings from the right and left MCA were not significant for any of the bilateral parameters considered, averaged values for the two sides were used in all subsequent analyses. Baseline cerebral haemodynamic parameters are presented in Table 5.1.

Table 5.1 Population characteristics and baseline parameter values.

Parameter	All Subjects (n=45)
Age (years)	37.5 (14.4)
CBFV (cm s ⁻¹)	57.0 (12.6)
Mean ABP (mm Hg)	85.9 (12.4)
EtCO ₂ (mmHg)	37.8 (3.2)
Heart rate (beats/min)	69.4 (11.6)
CrCP (mm Hg)	32.1 (12.4)
RAP (mmHg cm s ⁻¹)	1.03 (0.36)
Brachial systolic BP (mmHg)	119.1 (17.1)
Brachial diastolic BP (mmHg)	70.4 (10.9)
ARI	5.5 (1.6)

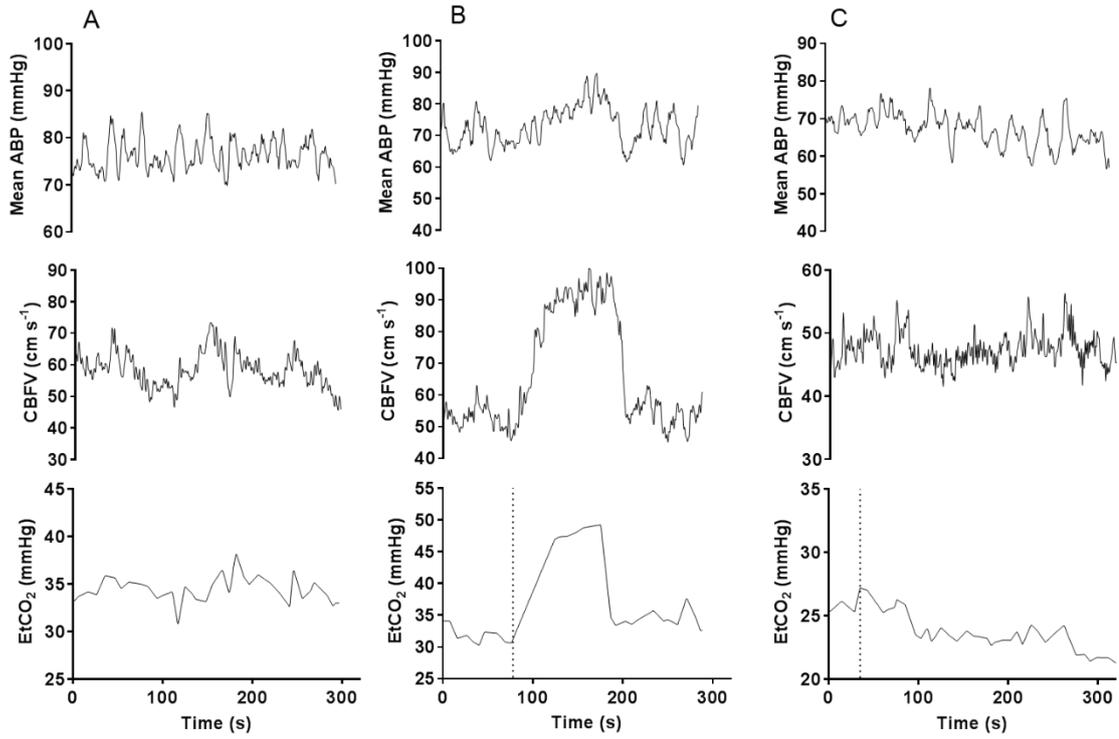
Values are mean (SD). CBFV, cerebral blood velocity; ABP arterial blood pressure; EtCO₂, end-tidal arterial pressure of carbon dioxide; CrCP, critical closing pressure; RAP, resistance area product; ARI, Autoregulation Index. CBFV, CrCP, RAP and ARI were averaged for the right and left MCAs.

5.3.1 *Effect of Hypo- and Hypercapnia on Cerebral Haemodynamics*

Highly significant differences in EtCO₂ resulted from breathing CO₂ in air and hyperventilation (ANOVA $p < 0.0001$) leading to EtCO₂ values of 46.5 (3.7) mmHg (8% CO₂), 42.7 (3.5) mmHg (5% CO₂), 37.8 (3.1) mmHg (baseline), 30.1 (5.7) (-5 mmHg hyperventilation), and 28.5 (5.7) mmHg (-10 mmHg hyperventilation).

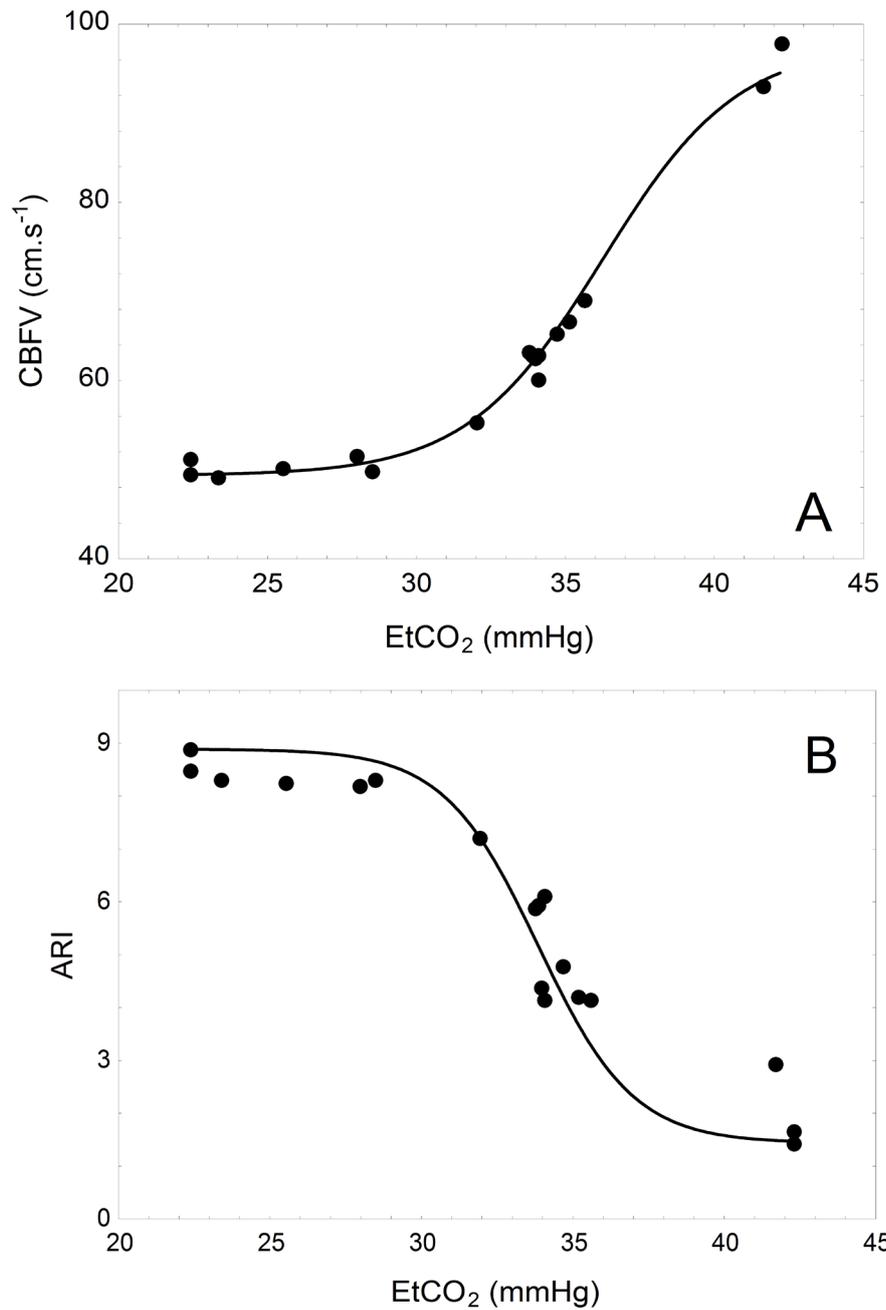
A representative recording is presented in Figure 5.2, showing the temporal patterns of changes in BP, CBFV and EtCO₂ observed during normo-, hypo- and hypercapnia. For the same subject, Figure 5.3 shows the corresponding fitting of the data to the logistic model for both CBFV and ARI. Similar models were obtained for BP, HR, CrCP and RAP.

Figure 5.2 Example recording from healthy volunteer.



Representative recordings from a 21-year-old female study participant. A. Normocapnia, B. Hypercapnia (8% CO₂), C. Hypocapnia (-10mmHg from baseline). Dotted vertical lines represent onset of respective manoeuvres.

Figure 5.3 Logistic model fitting for same healthy volunteer.



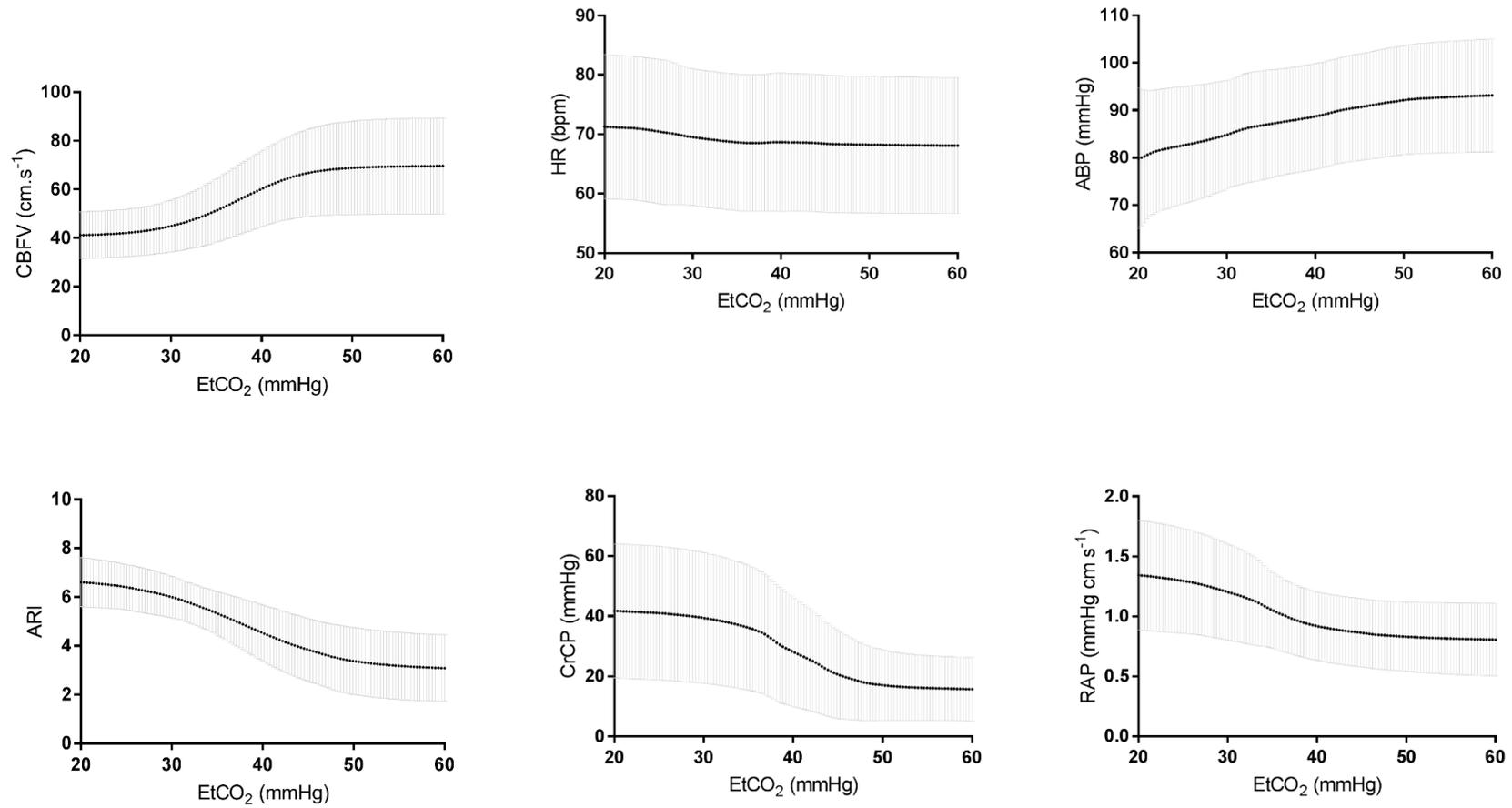
Logistic model fitting for same subject as in Figure 5.1 for (A) CBFV and (B) ARI as a function of EtCO₂. Population values of model parameters are given in Table 5.2, with corresponding population average logistic curves represented in Figure 5.4. In all cases the dependence on EtCO₂ reflects the expected physiological effects of PaCO₂ on each of the parameters modelled, as it will be discussed below.

Table 5.2 Population distribution values of logistic model parameters for CBFV, ARI, HR, ABP, CrCP and RAP as a function of EtCO₂.

Parameters	CBFV (cm.s⁻¹)	ARI	HR (bpm)	ABP (mmHg)	CrCP (mmHg)	RAP (mmHg/cm.s⁻¹)
EtCO _{2min} (mmHg)	25.9 (5.6)	25.9 (5.6)	25.9 (5.6)	25.9 (5.6)	25.9 (5.6)	25.9 (5.6)
EtCO _{2max} (mmHg)	47.9 (3.5)	47.9 (3.5)	47.9 (3.5)	47.9 (3.5)	47.9 (3.5)	47.9 (3.5)
Parameter _{min}	41.2 (9.3)	6.9 (1.0)	71.3 (12.3)	79.9 (16.2)	43.4 (16.1)	1.5 (0.5)
Parameter _{max}	70.5 (19.2)	2.9 (1.4)	67.6 (11.9)	93.1 (11.4)	23.3 (19.1)	0.8 (0.3)
k coefficient (mmHg ⁻¹)	0.4 (0.2)	0.3 (0.2)	1.00 (0.8)	0.7 (0.7)	0.4 (0.4)	0.5 (0.6)
x ₀ coefficient (mmHg)	36.5 (3.6)	36.5 (4.9)	33.1 (7.1)	34.5 (7.4)	38.4 (4.3)	34.0 (6.8)
MSE (variable units)	1.3 (0.5)	0.7 (0.3)	1.4 (0.8)	2.3 (1.3)	2.8 (1.1)	0.2 (0.1)

Values are mean (SD) (n=45). CBFV, cerebral blood velocity; ABP, arterial blood pressure; EtCO_{2min}, minimum values of end-tidal carbon dioxide during hypocapnia; EtCO_{2max}, maximum values of end-tidal carbon dioxide during hypercapnia; CrCP, critical closing pressure; RAP, resistance area product; ARI, Autoregulation Index; k coefficient: exponential gain coefficient; x₀ coefficient: EtCO₂ level corresponding to peak derivative of the logistic curve; MSE: mean square error, same units as dependent variables. CBFV, CrCP, RAP and ARI were averaged for the right and left MCAs.

Figure 5.4 Population average logistic model curves for the dependence of CBFV, ARI, HR, ABP, CrCP and RAP. Corresponding shaded areas represent the ± 1 SEM boundaries.



The model parameters k and x_0 were different when assessed for between group differences for all haemodynamic parameters (Table 5.2). The model parameter k was different between haemodynamic parameter groups ($p < 0.0001$) with HR demonstrating the largest value (SD) of 1.00 mmHg^{-1} (0.8) (Table 5.2). The model parameter x_0 was different between haemodynamic parameter groups ($p = 0.004$) with CrCP demonstrating the largest value (SD) of 38.4 mmHg (4.3). Eight outliers or less were removed for ABP (Median 6, IQR 4-7) and HR (Median 6, IQR 4-7).

5.4 Discussion

5.4.1 Main Findings

To our knowledge this is the first study to date to describe the effects of PaCO_2 on CBFV and dCA, ABP, HR, CrCP and RAP, and the first to demonstrate a logistic model relationship between ARI and EtCO_2 , across a wide physiological range.

5.4.2 Effects of CO_2 on CA

For many pharmacological agents, regression of the stimulus on organ response is non-linear.¹³³ The dependence of CBF, usually estimated from non-invasive measurements of CBFV with TCD ultrasonography, on PaCO_2 has been previously quantified as exponential¹³⁰ or logistic curves, using EtCO_2 as the independent variable.¹⁰⁶ The demonstration that ARI, a widely used index of dCA, decreases with EtCO_2 rises, also following a logistic curve, is of considerable relevance. Above all, the possibility of using the 4-parameter logistic curve to provide a complete representation of CA dependence on PaCO_2 (Figures 5.3 and 5.4) can be seen as an entirely new paradigm for the simultaneous assessment of dCA and CO_2 reactivity in individuals or populations. This new approach could provide a much more robust 'fingerprint' to characterise CBF regulatory mechanisms, than the use of separate indices that are plagued by issues of reliability due to the interaction of multiple co-factors and poor reproducibility. Since the logistic

curve is derived from six different 5-min recordings, it provides a much broader assessment of the response of CBF regulatory mechanisms. The implications of this new approach for clinical studies will be discussed below.

Previous studies have mainly concentrated on the effects of PaCO₂ on CBF or CBFV, reporting non-linear relationships including sigmoidal curves as in our case.^{96,106} On the other hand, Ainslie *et al.*¹⁰¹ reported on the effects of PaCO₂ on TFA measures of dynamic CO₂ using a protocol similar to ours, that is two hypercapnic and two hypocapnic levels. Nevertheless, parameters of gain and phase, often associated with dCA performance did not show a consistent relationship with EtCO₂ as we found for ARI. One possible explanation is the reduced sensitivity of using separate measures of gain and phase, and the fact that ARI incorporates all the information obtained with TFA thus providing a more robust measure of dCA.⁸⁵ Although other studies have described changes in dCA with different levels of PaCO₂, direct comparisons are hindered by the use of different protocols (e.g. thigh cuffs instead of spontaneous fluctuations in BP), or only 2-point comparisons (usually normocapnia to hypercapnia), which does not allow for identification of the nature of the entire dependence of dCA on EtCO₂.^{38,41,47,76,108}

Accordingly, it is important to determine that any differences in cerebral haemodynamic responses that are observed between different physiological conditions or between healthy and disease states are not confounded by differences in PaCO₂.^{96,134} In a healthy control population for example, Ogoh *et al.*¹⁰⁸ demonstrated that hypoxia disrupts dCA, but hypocapnia augments the dCA response. Furthermore, in our own previous work in an acute ischaemic stroke population⁶⁵, measures of cerebrovascular reactivity and neurovascular coupling were impaired compared to controls, though dCA was not. However, baseline hypocapnia in the stroke population may have confounded the effect size. Therefore, there is significant merit in describing the complete relationship between dCA across a physiological range of PaCO₂,

including both hypo- and hypercapnia that could be used to establish comparisons between individuals with different levels of PaCO₂.

Currently, there are no studies to date that have demonstrated PaCO₂ stimulus-response curves for CBFV, ARI, ABP, HR, CrCP, RAP, and ARI(t). Other associated work has demonstrated a sigmoidal relationship between PaCO₂ and vascular resistance using blood oxygenation level dependent (BOLD) imaging as a surrogate for CBF as well as speed of response to hypercapnic stimulus.^{132,135} The 'model branch pressure' reported by Duffin *et al.*¹³² also has the potential to be represented by a logistic model as demonstrated within this study. With reference to RAP and CrCP, previous work^{100,101,136} has shown RAP decreases with PaCO₂ and CrCP increases with this particular study providing no data on associated HR changes though highlighting a relatively static ABP.¹³⁶ Prior work has shown that RAP increases significantly with hypocapnia with similar findings as in our study population.¹³⁷

Hypercapnia leads to vasodilation of the cerebral microcirculation, whilst hypocapnia has the opposite effect. These major effects explain the directional changes reflected by the logistic curves of CBFV, ARI, CrCP and RAP (Figure 5.4). On the other hand, the increase in BP with EtCO₂ has been explained by the increased sympathetic activity induced by hypercapnia^{101,106,136} The small reduction in HR across the range of EtCO₂ values represented in Figure 5.4 though, might be more controversial. Ainslie *et al.*¹⁰¹ reported HR following a U-shaped curve when EtCO₂ changed from hypocapnia to hypercapnia. As in our case, their mean BP increased with hypercapnia. With an intact baroreceptor reflex, this increase in BP would be expected to lead to a reduction in HR, as in our case, but it is possible that in their study, increased sympathetic activity in hypercapnia dominated over the reduction in HR induced by the baroreflex. Further work is needed to improve our understanding of the effects of PaCO₂ on heart rate.

We provide a novel evolution from original logistic relationship studies.^{106,130} This study provides a wider range of PaCO₂ and more participants than Markwalder *et al.*¹³⁰ originally used for corrective velocity experiments on PaCO₂ values in 31 individuals, and the study of Claassen *et al.*¹⁰⁶ demonstrating modified logistic function of CBFV to transient changes in PaCO₂ in 10 subjects.

5.4.3 Clinical Perspectives

An important outcome from this study is the potential to improve comparability of dCA estimates for different patients with different PaCO₂ readings. The clinical necessity of this previous limitation was demonstrated by Salinet *et al.*⁶⁵ following examination of the effects of cerebral ischaemia on neurovascular coupling. They found no difference between groups (patients vs. controls p=0.07). They noted PaCO₂ levels were lower in the stroke population, and concluded that if both groups were normalised to the same PaCO₂, then CA would be significantly impaired in the stroke group. This study provides a meaningful opportunity to consider the extent to which “corrections” could be applied to healthy and potentially diseased populations on the basis of the “dose-response” nature of EtCO₂ and dCA. Our study also provides an example of how it would be possible to progress towards CO₂-adjusted estimates of ARI in future work. For this purpose, further studies involving larger number of individuals are needed to assess the effects of sex, ethnicity and other potential co-factors. This would provide an evolution from the standard CO₂ reactivity test (based on only two arbitrary points taken from the entire curve). Finally, the reproducibility of such a marker in patient populations does require validation, particularly as extremes of physiological variability have been shown to alter reproducibility.¹⁰² However, previous studies in stroke patient populations⁶⁵ have shown less extreme variation in EtCO₂ values and hence ARI (i.e. a trend towards hypocapnia). Instead of simply comparing values of ARI at single operating points, determined by stable PaCO₂ values,

the approach we are proposing, of comparing the entire ARI curve as a function of EtCO₂ (Figure 5.3), might provide a much more robust and general endeavour.

5.4.4 *Limitations of the Study*

Several potential limitations must be considered in this chapter. First, with reference to TCD studies, changes in CBF can be accurately expressed by CBFV, as long as the diameter of the MCA remains constant. This assumption is usually acceptable at normocapnia or mild hypercapnia, but at moderate levels of hypercapnia, as we achieved in our subjects, it is likely that CBFV underestimated CBF, with hypocapnia leading to overestimation.^{124,125} Nevertheless, estimates of ARI (and TFA phase) are independent of the amplitude of CBFV and hence would not be distorted by MCA dilation. However, studies have shown cerebrovascular resistance to be an independent factor to PaCO₂ in altering pressure-flow dynamics and further studies are needed to assess this.¹³⁸ Secondly, based on previous studies^{41,108} we have used a maximum of 8% CO₂ in air. Higher levels of CO₂ in air, for example 10%, could also be considered in future pilot studies to determine if the tail end of the ARI logistic curve (Figures 5.3 and 5.4) can be reduced even further. Informed by previous work¹⁰¹ a washout period of 2 min. was adopted as standard for each individual. It remains unclear though, whether cerebral perfusion baselines were re-established in all individuals following hyperventilation. The randomisation procedure for hypocapnia may have led to an overestimation of dCA at the -5mmHg level if the individual was randomised to have the -10 mmHg as the previous manoeuvre, as it proved difficult in some instances to fully establish a baseline due to the significant change in CBFV. Furthermore, some individuals found the 60 to 120 s period during inhalation of the 8% CO₂ gas difficult and therefore the likelihood of mask leakage was more apparent due to increased anxiety and movement.

Thirdly, the use of a logistic curve model to represent the effects of PaCO₂ on CBFV, ABP, HR, CrCP, RAP and ARI should be regarded as a convenient and simplistic approximation to the true mathematical relationships, likely to be distinct for each of the dependent variables considered. The logistic model takes into account the expected behaviour and limited variation of physiological variables, thus showing gradual saturation on both extremes of PaCO₂. Moreover, the fact that each curve is defined by four parameters (eq. 5) provides simplicity, on one hand, but adequate flexibility on the other. Therefore, if for example, the relationship tended to be more linear, this would be expressed by lower values of the parameter k (eq. 5). Finally, the relatively low values of MSE obtained in each case (Table 5.2), also demonstrate the appropriateness of using logistic curve models to describe the effects of PaCO₂ on systemic and cerebral haemodynamics.

Fourthly, estimation of logistic curve parameters is not a straightforward procedure. For the case of expressing the ARI dependence on EtCO₂ using a sigmoid curve, the problem is worsened by the high variability of ARI estimates, mainly when using a 60 s moving window coupled to an ARMA model.⁴⁷ The choice of breaking down each of the six recordings into four data segments of equal duration, aimed to achieve a compromise between obtaining relatively robust mean values over each of these segments, and having enough degrees of freedom ($6 \times 4 = 24$) to be able to estimate the four main parameters of the logistic model. Noteworthy, this was an empirical choice and more work is needed to assess the sensitivity of parameter estimates to other alternatives. We found the combination of least squares with the bootstrap removal of outliers a fairly robust approach to this problem, as shown by the relatively small model errors (Table 5.2). Nevertheless improvements in this area, and further validation studies, are warranted to achieve new methods for the unsupervised estimation of logistic curve parameters in the presence of low signal-to-noise ratio measurements, as is the case for ARI and CrCP.¹³⁹

Importantly, although we have elected to use the ARI index to describe the dependence of dCA on PaCO₂, due to its widespread use in the literature, this is by no means the only option available and alternative indices, such as TFA phase or the Mx index could be equally employed for this purpose, as long as there are enough data points to describe the logistic curve across the physiological range of PaCO₂. Mx is a mean flow index, based on continuous correlation of slow and spontaneous fluctuations of CBFV and cerebral perfusion pressure offering information on cerebral pressure reactivity.⁵⁸ With different indices though, it is likely that the scatter of the four parameters describing the logistic model (Table 5.2) would be different, thus affecting future use of these data for calculation of adequate sample sizes.

Finally, future studies using larger number of subjects might be able to provide a better characterization of the dependence of the logistic model due to additional co-factors, such as aging, posture or autonomic nervous system function. In addition, the lack of consideration for menstrual phase may be considered relevant, however, 36% of female participants were above the age of 50 therefore may have been post-menopausal, suggesting a likely lack of influence on the results.

5.5 Conclusions

Expressing the influence of PaCO₂ on CBF and its regulatory mechanisms, with a logistic curve reflecting the dependence of ARI as a function of EtCO₂, represents a new paradigm for the simultaneous assessment of dCA and CO₂ vasoreactivity. This new approach has considerable potential to improve the sensitivity and specificity of dCA assessment in clinical studies of cerebrovascular conditions, but further studies are needed involving older individuals and to establish the reliability of this approach.

6 Chapter Six: Sex differences in cerebral haemodynamics across the physiological range of PaCO₂

6.1 Introduction

As previously defined, CA is defined as the homeostatic maintenance of cerebral blood flow (CBF) despite changes in BP within the range 50 to 170 mmHg.^{103,104} CA can be obtained from the transient response of CBF to rapid BP changes.³⁸ CA is a highly sensitive mechanism and in the instance PaCO₂ is raised (hypercapnia) a vasodilatory effect on cerebral vessels ensues¹³⁰ precipitating deterioration in CA.³⁸ Conversely, low PaCO₂ (hypocapnia) despite causing a potent vasoconstrictive effect, improves CA.

Previous work has demonstrated females have a stronger vasodilatory response to changes in PaCO₂ than males.¹⁴⁰ However the mechanisms and physiological significance of such changes remains unclear. Unfortunately, studies to date have focussed solely on hypercapnic conditions with no reported concomitant assessment of other key cerebral haemodynamic variables being reported. However, a good understanding of baseline sex differences has been provided by larger datasets. These have shown differences in CBFV and CrCP at baseline in a middle-aged cohort of 129 healthy volunteers.¹⁴¹

In the previous chapter, the logistic curve relationship of key cerebral haemodynamic variables: CBFV, ARI, HR, ABP, CrCP and RAP was demonstrated, providing a new paradigm for the simultaneous assessment of dCA and CO₂ vasoreactivity.¹²⁰ This feasibility model holds importance as it offers the opportunity to determine that any differences in cerebral haemodynamic responses that are observed between different physiological conditions or between healthy and disease states are not confounded by differences in PaCO₂.^{96,120,134} Furthermore, it is also highly relevant to investigate the potential influences of phenotypes, for

example the contributions of sex to this relationship. Sex-related differences have been shown in CBFV and CrCP, with both being significantly higher in females during normative studies.¹⁴¹ Accordingly, the present study aims to examine the relationship that exists between dCA and PaCO₂, taking into account the influences of sex within the context of a multi-level CO₂ protocol. In summary, we tested the hypotheses that i) sex differences exist in a widely used index of dCA, the autoregulation index (ARI) on dependence across the physiological range of PaCO₂ and ii) CBFV, HR, ABP, CrCP and RAP dependencies on PaCO₂ are influenced by sex.

6.2 Materials and Methods

6.2.1 *Subjects and Measurements*

All study procedures were conducted in accordance with the Declaration of Helsinki (2013).⁹⁸ The University of Leicester Ethics Committee (Reference: jm591-c033) provided ethical approvals for this study. Healthy volunteers were recruited from University departmental staff, students and their relatives. Healthy volunteers 18 years and over were included. Individuals with physical disease in the upper limb, poor insonation of both temporal bone windows and any significant history of cardiovascular, neurological or respiratory disease were excluded. Written informed consent was obtained from all participants prior to any study activities being conducted. The dataset for this study, as detailed in Chapter 5, is the same as that used to describe the feasibility of modelling cerebrovascular variables using a logistic model.¹²⁰

As described in Chapter 5, the University of Leicester's Cerebral Haemodynamics in Ageing and Stroke Medicine research laboratory was the setting for the study, a quiet area maintained at approximately 24°C. Study participants were requested to avoid exercise, caffeine, alcohol and nicotine in the 12-hour period before study measurements. HR was recorded using a standard 3-lead ECG. Beat-to-beat BP was recorded continuously using the Finometer[®] device (FMS, Finapres Measurement Systems, Arnhem, Netherlands), which was attached to the middle

finger of the left hand. The hand bearing the finger cuff was at the level of the heart to negate any hydrostatic pressure artefact. EtCO₂ was measured throughout the initial resting baseline and hypercapnic phase using a face-mask connected to a capnograph (Capnograph Plus). During the second baseline and hypocapnic phase EtCO₂ was measured via nasal prongs (Salter Labs). Bilateral insonation of the MCAs was performed using TCD ultrasound with a 2MHz probe (Viasys Companion III; Viasys Healthcare). A head-frame was used to secure the probes in position and adjusted to ensure maximum comfort for study participants.

6.2.2 *Experimental Protocol*

All measurements were conducted at a single visit with randomisation of the order of hypo- and hypercapnia, determined in advance using a random number generator. Initially, a stabilisation period of 15 minutes of measurement whilst resting and supine was undertaken. This was followed by CO₂ inspiration in air, constantly ('fixed inspiration') for a minimum of 90 s with a randomized order of 5% CO₂ or 8% CO₂. A steady 90 s recording was conducted before each gas inspiration episode to ensure systemic haemodynamic stability before and immediately after the hypercapnic challenge. After a further stabilisation period of 5 minutes, participants performed a further resting supine recording prior to hyperventilation in random order, at different respiratory rates, generating incremental reductions in EtCO₂ of 5mmHg and 10mmHg less than normocapnia for that individual. Hyperventilation was sustained for a minimum period of 90 s. For hyperventilation, participants were asked to breathe with a metronome (KORG Metronome MA-30) creating a respiratory rate of at least 5 breaths per minute above their resting rate for at least 90 s. Two-minute washout periods of normal respiration were allowed between successive measurements. Each incremental reduction in EtCO₂ was repeated on two occasions during the same session. Measurements were continuously recorded at a rate of 500 samples/s in the PHYSIDAS data acquisition system (Department of Medical Physics, University Hospitals of Leicester). Systolic and diastolic brachial BP readings (OMRON Model 705IT) were

performed at each stage of the protocol (normocapnia, hypercapnia and hypocapnia with a minimum of 3 recordings per individual). These values were then used to calibrate the Finometer recordings. As mentioned earlier, this protocol was used to generate the dataset to describe the feasibility of modelling cerebrovascular variables using a logistic model, as detailed in Chapter 5.¹²⁰

6.2.3 Data Analysis

Six individual files were generated for each participant: 2 at baseline, 2 hypercapnic and 2 hypocapnic. Data were visually inspected and calibrated to recorded OMRON systolic and diastolic BP values. After linear interpolation associated removal of narrow spikes (<100ms) the CBFV recording was passed through a median filter. Then signals were low pass filtered with a zero-phase Butterworth filter with a 20Hz frequency cut-off. The QRS complex of the electrocardiogram (ECG) was automatically detected and marking of the R-R interval was manually checked. This permitted mean BP, HR, EtCO₂ and mean CBFV to be calculated for each cardiac cycle. The first harmonic method was used to estimate CrCP and RAP.¹⁰⁰

The dCA parameter, ARI, as described by Tiecks *et al.*⁴⁰, was calculated as a function of time (ARI(t)), using a moving-window, autoregressive moving average (ARMA) model, that follows the same structure as the second-order differential equation proposed by Tiecks *et al.*,⁴⁰ as described previously.⁴⁷ The complete time-series of ARI(t) values for each recording was obtained by moving the time-window at 0.6s intervals. Values of ARI(t)=0 represent absence of autoregulation, whilst ARI(t)=9 corresponds to the most efficient CA that can be observed. The ARI(t) time-series was computed for each subject separately for left and right hemispheres for each recording.

6.2.4 Logistic Model

Each variable was modelled using the logistic equation previously described by Minhas *et al.*¹²⁰:

$$y = y_{max} + \frac{y_{min} - y_{max}}{1 + e^{k(x-x_0)}} \quad [1]$$

where y can represent either mean CBFV, ARI, HR, ABP, CrCP or RAP, and x is the EtCO₂ level.

Fitting the model allows estimation of its four parameters, namely y_{max} , y_{min} , k and x_0 .

Logistic model parameters were estimated by combining least squares with a recursive bootstrap technique to remove outliers. After each stage of error minimisation by least squares, the largest outlier was identified and the process repeated for a maximum of nine potential outliers. The minimum square error (MSE) and the logistic model estimated parameters at each stage were listed and the number of outliers to be removed was chosen under visual inspection, to select model parameters that were stable and a region of MSE that was not critically dependent on the number of outliers removed. In general, the final number of outliers removed was less than nine.

6.2.5 Statistical Analysis

Statistical analyses were performed using GraphPad Prism 7.0. Baseline measurements were assessed for differences between values derived for right and left hemispheres using a paired Student's t-test. These were averaged when no significant differences were found.

Data were analysed for differences in six parameters: minimum and maximum EtCO₂, y_{max} , y_{min} , k and x_0 and MSE (Eq. 1). Differences in variables were assessed using a Student's t-test and Mann Whitney U test, as appropriate. Significance was taken at the $p < 0.05$ level.

6.3 Results

Forty-five subjects (19 male; 26 female) of mean age 37.5 years (range 21 to 71) were included in the analyses. None of the subjects were smokers or had diabetes.

6.3.1 Cerebral Haemodynamic Parameters at Baseline

Baseline cerebral haemodynamic parameters are presented in Table 6.1. CBFV was significantly higher in females when compared to males ($p=0.004$), despite lower values of EtCO_2 ($p=0.005$). RAP was lower in females compared to males ($p=0.005$). Furthermore, brachial systolic BP was higher in males as compared to females ($p=0.037$). The ARI index did not show sex differences at baseline (Table 6.1).

Table 6.1 Population characteristics and baseline parameter values presented by sex and for the entire study cohort.

Parameters	All Subjects (n=45)	Sex (n=45)		p-value
		Male (n=19)	Female (n=26)	
Age (years)	37.5 (14.4)	36.1 (15.3)	38.5 (14.0)	0.483 ^a
CBFV (cm.s-1)	57.0 (12.6)	50.9 (10.4)	61.5 (12.3)	0.004 ^b
Mean Arterial BP (mm Hg)	85.9 (12.4)	87.8 (9.9)	84.4 (13.9)	0.593 ^a
End-tidal CO ₂ (mmHg)	37.8 (3.2)	39.2 (2.8)	36.9 (3.0)	0.005 ^a
Heart rate (beats/min)	69.4 (11.6)	66.2 (13.3)	71.6 (9.9)	0.124 ^b
CrCP (mm Hg)	32.1 (12.4)	30.7 (9.5)	32.9 (14.6)	0.576 ^b
RAP (mmHg cm.s-1)	1.03 (0.36)	1.16 (0.23)	0.94 (0.40)	0.005 ^a
Brachial systolic BP (mmHg)	119.1 (17.1)	125.2 (8.0)	114.6 (12.4)	0.037 ^b
Brachial diastolic BP (mmHg)	70.4 (10.9)	72.4 (8.0)	69.0 (12.6)	0.298 ^b
ARI	5.5 (1.6)	5.7 (1.6)	5.4 (1.6)	0.362 ^a

Values are mean (SD). CBFV, cerebral blood velocity; ABP, arterial blood pressure; EtCO_2 , end-tidal arterial pressure of carbon dioxide; CrCP, critical closing pressure; RAP, resistance area product; ARI, Autoregulation Index. CBFV, CrCP, RAP and ARI were averaged for the right and left MCAs. ^aMann-Whitney *U* Test and ^bt-test.

6.3.2 Effect of Sex on Cerebral Haemodynamics

Differences in logistic model parameters for CBFV, ARI, HR, ABP, CrCP and RAP can be observed in graphical form in Figure. 6.1. A-F, containing the mean and largest SE value separated by sex.

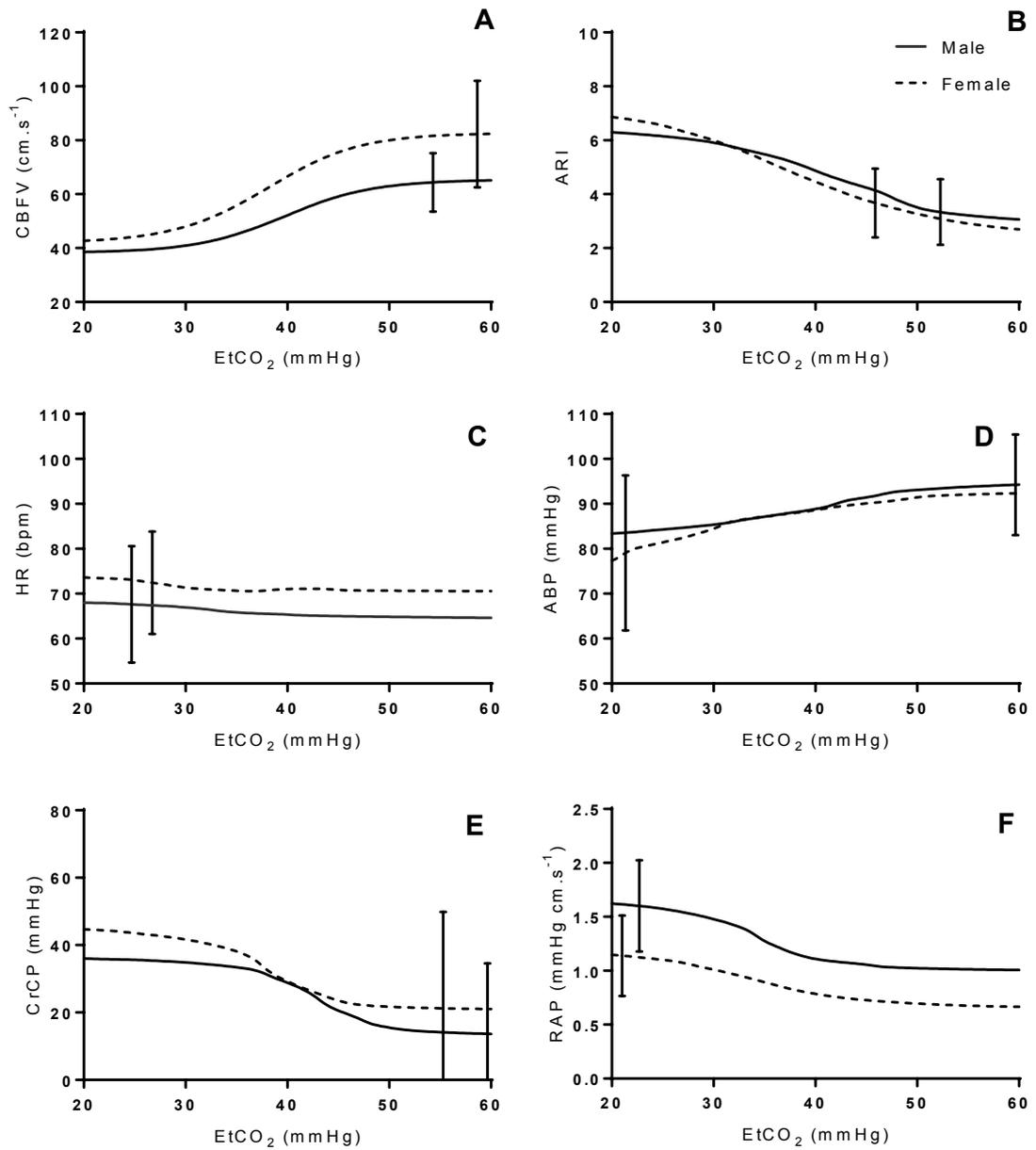
Maximum CBFV attained during hypercapnia was significantly higher in females than males ($p=0.001$) (Table 6.2). Overall, the corresponding sub-plot in Figure 6.1.A indicates the trend for CBFV to be higher in females compared to males over the entire physiological range of PaCO_2 . Maximum ARI attained during hypocapnia was higher in females than males ($p=0.036$) (Table 6.2), with the mean logistic curves crossing over around an EtCO_2 value of 33 mmHg (Figure 6.1.B) and then decreasing in parallel. The influence of sex on the CBFV dependence on EtCO_2 demonstrates an upward parallel shift of the curve for females (Figure 6.1.A). For the logistic models of ARI (Figure 6.1.B), significantly higher values were achieved by females during hypocapnia in comparison with males ($p=0.036$). Minimum and maximum CrCP attained during hypocapnia ($p=0.04$) and hypercapnia ($p=0.005$) were significantly higher in females than males (Table 6.2). This finding follows from the logistic curves in Figure 6.1.E showing similar values of CrCP for males and females around normocapnic values of EtCO_2 , but elevated values of CrCP for females for both hypocapnia and hypercapnia. Minimum and maximum RAP attained during hypocapnia ($p<0.001$) and hypercapnia ($p<0.0001$) were significantly lower in females than males (Table 6.2). Similarly to that observed with CBFV, there was a parallel shift of the curve, with the curve for males remaining above the corresponding curve for females over the entire range of EtCO_2 . CrCP and RAP demonstrated opposing sex differences in relation to dependence on EtCO_2 . CrCP had a parallel upward shift with females having significantly higher values of CrCP throughout the physiological range of PaCO_2 (Figure 6.1.E). In comparison, RAP demonstrated a downward parallel shift with males having significantly higher values of RAP throughout the physiological range of PaCO_2 (Figure 6.1.F).

Table 6.2 Population distribution values of logistic model parameters for CBFV, ARI, HR, ABP, CrCP and RAP as a function of EtCO₂ by sex.

Parameters	CBFV (cm.s ⁻¹)		ARI		HR (bpm)		ABP (mmHg)		CrCP (mmHg)		RAP (mmHg cm.s ⁻¹)	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
EtCO _{2min} (mmHg)	27.6 (5.4)	24.8 (5.5)	27.6 (5.4)	24.8 (5.5)	27.6 (5.4)	24.8 (5.5)	27.6 (5.4)	24.8 (5.5)	27.6 (5.4)	24.8 (5.5)	27.6 (5.4)	24.8 (5.5)
EtCO _{2max} (mmHg)	47.5 (3.8)	48.0 (3.2)	47.5 (3.8)	48.0 (3.2)	47.5 (3.8)	48.0 (3.2)	47.5 (3.8)	48.0 (3.2)	47.5 (3.8)	48.0 (3.2)	47.5 (3.8)	48.0 (3.2)
Variable _{min}	38.1 (8.4)	41.8 (10.3)	6.7 (0.9)	7.4^a (2.2)	68.8 (12.7)	74.4 (11.9)	80.7 (11.4)	73.8 (19.8)	37.7 (8.9)	47.3^b (18.9)	1.7 (0.4)	1.3^c (0.4)
Variable _{max}	65.5 (10.9)	82.8^d	2.6 (1.1)	2.2 (1.2)	63.6 (12.4)	70.3 (10.3)	95.6 (11.4)	92.8 (12.7)	12.3 (20.6)	19.7^e (28.3)	1.0 (0.2)	0.6^f (0.3)
k coeff (mmHg ⁻¹)	0.3 (0.2)	0.3 (0.1)	0.4 (0.4)	0.2 (0.2)	0.7 (0.9)	1.4^g (0.7)	0.6 (0.8)	0.9 (0.9)	0.9 (0.8)	0.8 (0.7)	0.6 (0.5)	0.6 (0.5)
x ₀ coeff (mmHg)	39.5 (3.2)	37.9 (3.2)	40.4 (6.2)	38.9 (6.3)	33.6 (8.4)	32.7 (6.6)	36.0 (8.4)	34.7 (10.1)	39.5 (6.0)	35.3 (6.0)	35.5 (4.7)	32.9 (7.8)
MSE (variable units)	2.2 (0.9)	2.7 ^h (0.8)	1.1 (0.3)	1.1 (0.2)	2.2 (0.9)	2.5 (1.3)	3.6 (1.5)	6.1 ⁱ (2.9)	6.5 (3.0)	6.3 (4.0)	0.1 (0.1)	0.2 (0.1)

Values are mean (SD) (Male = 19, Female = 26). CBFV, cerebral blood velocity; ABP, arterial blood pressure; HR, heart rate; EtCO_{2min}, minimum values of end-tidal carbon dioxide during hypocapnia; EtCO_{2max}, maximum values of end-tidal carbon dioxide during hypercapnia; CrCP, critical closing pressure; RAP, resistance area product; ARI, Autoregulation Index; k coefficient: exponential gain coefficient; x₀ coefficient: EtCO₂ level corresponding to peak derivative of the logistic curve; MSE: mean square error, same units as dependent variables. CBFV, CrCP, RAP and ARI were averaged for the right and left MCAs. Statistically significant differences shown in bold in the table and correspond to superscripted values in legend (p<0.05). ^ap<0.05, ^bp<0.05, ^cp<0.001, ^dp<0.001, ^ep<0.01, ^fp<0.0001 and ^gp<0.01.

Figure 6.1 Population average logistic model curves for the dependence of A) CBFV, B) ARI, C) HR, D) ABP, E) CrCP and F) RAP on EtCO₂.



Error bars represent the largest ± 1 SEM at the point of occurrence.

6.3.3 *Effect of Sex on Peripheral Haemodynamics*

The k coefficient within the logistic model was significantly different for HR, with higher values for females ($p=0.002$) (Table 6.2). In Figure 6.1.C this difference is manifested as a slightly more accentuated curvature, with the logistic curve for females staying constantly above the corresponding curve for males. Both curves do not show a large variation with EtCO_2 (Figure 6.1.C-D). As indicated by the curves in Figure 6.1.D, there was a very similar dependency of BP with EtCO_2 for males and females without any significant difference in the logistic model parameters. The influence of sex on the HR dependence on EtCO_2 demonstrates an upward parallel shift of the curve for females. At baseline, males had a significantly higher systolic BP; this is less apparent when the relationship between BP and EtCO_2 is modelled. For all models, the corresponding mean square errors were relatively small and only showed significant differences for sex for CBFV and ABP (Table 6.2), for which baseline differences were also demonstrated (Table 6.1).

6.4 Discussion

6.4.1 *Main Findings*

To our knowledge this is the first study to date to describe the sex differences in the effects of PaCO_2 on CBFV and dCA.

6.4.2 *Influences of Sex*

In agreement with several previous reports¹⁴¹⁻¹⁴⁶ we found that CBFV was higher in females compared to males. Similar findings in children and young adolescents^{147,148} suggest that the main reason for this difference might be the smaller diameter of the MCA, or other large intracranial arteries, in females, rather than hormonal differences with males.¹⁴⁹ One advantage of our study is the use of a two-parameter model to describe the instantaneous pressure-

velocity curves of the cerebral circulation to examine the separate contributions of CrCP and RAP.¹⁰⁰ The curves in Figure 6.1.A suggest that the higher CBFV in females could be associated with the lower values of RAP (Figure 6.1.F), rather than due to the greater vasoconstriction suggested by the CrCP curves at hypocapnia and hypercapnia (Figure 6.1.E). The parallel shift of the RAP curve seems to suggest the presence of structural factors, such as reduced arterial diameters in females that are not PaCO₂-dependent. On the other hand, the morphology of the CrCP curve observed in females is intriguing, as it suggests some degree of upwards parallel shift, combined with a shift to the left as reflected by a (non-significant) difference of more than 4 mmHg in the x0 parameter. Given the well-known relatively high variability of CrCP estimates, it is possible to speculate that with a larger number of subjects the difference in x0 might become significant. These considerations are important mainly in connection to the interpretation of the influence of sex on the ARI index or other variables reflecting the efficiency of dynamic CA.

In considering the influence of sex on dCA, the literature is much less clear. Some studies are not comparable to ours, as they were based on assessment of static CA, using only two measurement points, either in children¹⁴⁸ or astronauts after space flight.¹⁴⁵ The large-scale study of Deegan *et al.*,¹⁴⁶ using data from the MOBILIZE Boston database, reported superior dCA and CO₂ reactivity in elderly women compared to men of similar age (mean ~ 78 years). However, males also showed a much higher incidence of diabetes that could have reduced CA effectiveness in this group. Based on their findings, one would expect in a cohort of older females that the ARI logistic curves for females and males in Figure 6.1.B would show greater separation, though the study population in this study included younger females. However, it is possible that the lack of separation at normocapnia could be due to the much smaller statistical power of our study, or the average age difference between the two populations (~40 years). Of note, Ortega-Gutierrez *et al.*¹⁵⁰ in a group of similar size and age as ours also failed to detect an

effect of sex on dCA, as assessed by phase from TFA. Despite the lack of differences in ARI over the entire physiological range of EtCO₂, the ARI was significantly different for the minimum values of EtCO₂ observed in hypocapnia, suggesting a more efficient dCA in females in this range of EtCO₂ (Table 6.2). When this finding is put together with the more hypocapnic status of females at rest (Table 6.1), and the trend of the CrCP logistic curve to shift to the left (Figure 6.1.E), it is possible to argue, on teleological grounds that the more hypocapnic operating point in females might bring physiological advantages, by providing a more efficient CA. This argument was also proposed to explain the frequent finding that acute stroke patients can also be more hypocapnic compared to control subjects.¹⁵¹ Further studies are needed using the ARI logistic curve approach, involving older populations and larger sample sizes to increase statistical power.

6.4.3 Clinical Perspectives and Significance

Further studies involving larger number of individuals are needed to confirm and extend the effects of sex and other potential co-factors. Furthermore, the reproducibility of these sex differences in patient populations does require validation, particularly as extremes of physiological variability have been shown to alter reproducibility.¹⁰² Importantly, with significant differences in CrCP, concern over vasoconstriction differences is relevant when considering interventional manoeuvres like hypocapnia.¹¹⁷

6.4.4 Limitations of the Study

Several potential limitations must be considered in this study. Firstly, when considering TCD studies, the changes in CBF are only acceptable as CBFV if the diameter of the MCA remains constant. Unfortunately, at moderate levels of hypercapnia, as demonstrated within this protocol, it is likely that CBFV underestimated CBF, with hypocapnia causing the converse issue of overestimation. Fortunately, dCA measures are independent of the amplitude of CBFV and

thus are not affected by changes associated with dilation of the insonated vessel. Prior work has demonstrated cerebrovascular resistance does not confound PaCO₂ in altering pressure-flow dynamics and further work is required to understand this dynamic further.¹³⁸ Based on previous studies^{41,108} this protocol used a maximum of 8% CO₂ in air.

Although we have elected to use the ARI index, based on widespread use in the published literature, there are a multitude of alternative indices, such as TFA phase or the Mx index that could be assessed using a similar model for sex differences. In our case, a priori information to allow calculation of sample sizes was not available, but the differences due to sex that we found indicate that the sub-group sizes we enrolled were adequate to avoid the likelihood of an alpha error. Nevertheless, future studies, using larger number of subjects, might be able to provide a better characterisation of the dependence of the logistic model due to additional co-factors, such as ageing, posture or autonomic nervous system function.

The lack of consideration for menstrual phase may be considered relevant, however, with 36% of female participants older than 50 years, there is a reduced likelihood of influence on the results as some may have been post-menopausal.

6.4.5 Conclusions

The use of a logistic model to describe the dependence of peripheral and cerebral haemodynamic variables on EtCO₂, over its entire physiological range is a powerful tool to provide a comprehensive assessment of the influences of sex on the cerebral circulation. The demonstration that the logistic curve parameters are influenced by sex highlights the need to take into account phenotypic differences between participants in both physiological and clinical studies. This new approach has considerable potential to improve the sensitivity and specificity of dCA assessment in clinical studies of cerebrovascular conditions, but further studies are needed involving older individuals and to establish the generalisability of this approach.

7 Chapter Seven: Feasibility of Improving Cerebral Autoregulation in Acute Intracerebral Haemorrhage (BREATHE-ICH) Study: An Experimental Interventional Study

7.1 Introduction

CA has been shown to be impaired³⁸ in acute ischaemic stroke (AIS), head injury, in dementia or following premature birth but importantly is associated with worse patient outcomes.¹⁴¹ An ARI can be assigned between 0 and 9 (0 being poor and 9 being the most efficient CA observed) to gauge how good the control over CBF is at a given time.⁴⁰ As discussed in Chapter 2, dCA is a measure of the response of CBF to rapid changes in BP, and several key studies have shown impaired dCA post-acute ICH.^{72,73,88,90,91} Recent studies have demonstrated that dCA impairment lasts up to 12 days,^{73,90} and that CA is bilaterally disturbed after supratentorial ICH.⁹⁰ Importantly the most recent study showed larger hematoma volume is likely to independently predict poorer CA status ipsilateral to haematoma.⁹⁰

Spontaneous acute ICH is associated with both high mortality and morbidity.⁷ There is a relative paucity of management options for acute ICH compared to AIS with BP control the foremost approach. However, controversy exists as to whether intensive BP lowering in acute ICH risks cerebral ischaemia. This is particularly the case if CBF control mechanisms are altered by chronic hypertension or indeed ICH itself. CA provides a protective mechanism for the brain parenchyma from extremes of CBF change, with the risk of hyper- or hypoperfusion in response to systemic BP changes. A key limitation of large-scale randomised controlled trials has been the inability to provide mechanistic insight into CBF during the acute phase of haemorrhagic stroke.²⁰ Lower

mean CBF, in combination with impaired CA, may have implications for more intensive BP lowering and warrants further studies examining such strategies on CBF regulatory mechanisms.

Hypocapnia has been historically used in the management of acute brain injury to reduce intracranial pressure (ICP) and to improve outcome in specific circumstances.^{77,152} This is usually weighed against the risk of hypoperfusion and subsequent neuronal ischaemia, which could worsen outcome. PaCO₂ is a balance between production and removal.⁷⁷ In order to generate hypocapnia, this usually involves a hyperventilatory manoeuvre often in the intubated patient. The use of hypocapnia in acute brain injury has been primarily focussed around the scientific principles of the Monro-Kellie doctrine describing the brain as a 'closed box' with a fixed volume.¹⁵² Raised ICP (sustained >20mmHg) can cause secondary brain injury by impairing cerebral perfusion, through direct pressure or by brainstem herniation.⁷⁷

Hypocapnia can be induced to lower ICP by decreasing the CBF via cerebral arterial vasoconstriction. The effects can be potent: CBF decreases by approximately 3% per mmHg change in PaCO₂ (range, 60 to 20mmHg PaCO₂) in patients with traumatic brain injury (TBI).^{152,153} Despite advances in our understanding of PaCO₂ changes in TBI and other circumstances associated with raised ICP, little is understood about the use of hyperventilation in acute stroke. Hyperventilation has classically been advocated in acute stroke for two reasons: first to reduce intracranial pressure, and secondly to restore homeostasis to penumbral zones around ischaemic tissue by inducing inverse steal and correcting acidosis.⁷⁷ These physiological changes are weighed against both potential vasoconstriction leading to hypocapnia-induced brain ischaemia, as well as cerebral hyperaemia due to subsequent normalisation of PaCO₂.⁷⁷ Lastly, cerebral ischaemic lesions noted on MRI in patients with acute ICH also raise the possibility of harms associated with BP lowering which may be more relevant than issues of perihematoma oedema.

Hitherto, randomised studies have only been conducted in TBI patients, with benefits noted in sustained ICP below 25mmHg, but with a less favourable outcome in those with moderate motor impairment on their Glasgow Coma Score.^{154,155} However, this population is very different to acute stroke patient populations, which form the focus of this study.

This method of manipulating dCA may improve CA in acute ICH; offering a novel non-pharmacological intervention, particularly as hypocapnia (induced by hyperventilation) has been used as a neuroprotective mechanism improving impaired dCA in rats with subarachnoid haemorrhage,¹⁵⁶ patients with liver failure,³² patients undergoing isoflurane anaesthesia¹⁵⁷ and acute bacterial meningitis.²⁹

Importantly, hypocapnia decreases CBF but expands the plateau region of the cerebral autoregulatory curve, hence improving CA and subsequently the ability to keep CBF constant for a wide range of perfusion pressures.^{39,104} Manning *et al.*⁸³ demonstrated systolic BP variability confers a poor outcome in acute ICH and concluded that peaks in systolic BP are likely to confer harm, as opposed to smooth and sustained early intensive lowering (to 140mmHg) which is likely to be beneficial. However, with a growing body of evidence highlighting impaired CA as a marker of poor prognostic outcomes in acute ICH, this relationship is likely to hold significant importance in possible alternative interventions for this devastating neurological state. The argument therefore for assessing the feasibility of hypocapnia for improving CA in acute ICH rests on mild-to-moderate hypocapnia possibly being clinically beneficial than methods that are designed to alter the magnitude of CBF.

As demonstrated in Chapter 2, dynamic cerebral autoregulation (dCA) characterised by multiple different indices has been shown to be impaired in acute intracerebral haemorrhage (ICH).^{72,73,84,88,90} Of clinical importance is the association between impaired dCA and poor prognostic markers including lower GCS values and larger haematoma volumes.⁹⁰ dCA pertains

to the relationship between CBF and CPP, therefore demonstrating the importance of consideration for dCA functionality during blood pressure management strategies in acute stroke. However, PaCO₂ is a powerful modulating factor of cerebral vasomotor tone and deviation of PaCO₂ is increasingly noted during acute illness.^{110,161} During acute stroke, patients have been noted to be mildly hypocapnic, perhaps either generating a protectionary hypocapnic state to preserve some autoregulatory function or indeed responding to cerebrovascular dysfunction, which generates hypocapnia inadvertently. Despite well documented autoregulatory benefits from hypocapnia in healthy volunteers,^{47,120} there is a clear lack of understanding as to whether accentuated hypocapnia in acute stroke may indeed be acceptable or potentially beneficial. International guidelines advocate the use of capnography during interventional stroke procedures for ischaemic stroke,¹⁵ however; widespread usage in acute ICH is limited to intensive care settings where ICP monitoring is in usage.

As demonstrated in Chapter 2, prior work has shown that dCA may be transiently impaired post ICH and correlates with unfavourable outcomes.⁹⁰ During temporal assessment of dCA, there was bilateral disturbance with poor phase difference values noted ipsilateral to the haematoma on days 4-6 post ICH and lasted for up to 14 days. However, more acute disturbance of dCA was also seen <48 hours and later during the recovery period.⁷² As a consequence of this lack of corroboration of findings, further work confirming these findings during the acute and sub-acute period is necessary.

Previous studies have demonstrated a favourable¹⁵² and unfavourable outcome¹⁶² associated with hypocapnia during acute stroke, though the latter involved retrospective data collection during anaesthesia for acute endovascular intervention. There have been no studies evolving our understanding of impaired dCA in acute ICH by applying our knowledge of the ability of hypocapnia to improve dCA. Furthermore, there have been no studies to date assessing dCA in acute ICH using the ARI metric.⁴⁸ ARI affords strength of reproducibility and prior clinical

correlation with ipsilateral ischaemic stroke disease and moderate to severe stroke severity.⁶⁷ Accepted thresholds of impairment have been tested in healthy volunteers¹⁴¹ and heart failure patients.¹⁶³

Therefore the study assessed if: i) ICH sufferers tolerate the hyperventilation manoeuvre; ii) hyperventilation manoeuvre leads to hypocapnia in the ICH population; and iii) it is feasible to improve dCA, whilst maintaining safe levels of CBF. The study investigated for the first time whether a CA-targeted intervention involving a simple breathing exercise in survivors of acute ICH is safe, feasible and effective in improving CA and subsequently clinical outcomes. The intention was to assess whether a simple bedside intervention could be used in the design a larger randomised controlled trial.

7.2 Methods and Analysis

7.2.1 Sample Selection

Acute ICH patients, recruited within 48 hours of onset, able to comply with a respiratory manoeuvre (hyperventilation).

Each stroke patient was required to participate for up to 14 days post stroke symptom onset, during which up to a total of two assessments were made. The first assessment was recorded whilst the participant was an in-patient under the care of the University Hospitals of Leicester NHS Trust Stroke Service (acute: within 48 hours of symptom onset); the next assessment was undertaken 10-14 days post stroke onset (sub-acute).

The sample size included was based on the investigator's pilot study, which included 45 healthy volunteers who underwent the hyperventilation manoeuvre outlined in the 'intervention' section below.¹²⁰

Formal sample size calculations have been previously conducted. In order to detect a change of 1 unit in autoregulation Index (ARI) and to calculate the required sample size, the technique as previously described by others was followed.¹¹⁹ For this study a minimum sample of 40 patients provided 80% power at the 5% significance level to detect a difference in ARI of 1 unit and a minimum sample size of 11 patients to detect a difference of 2 units of ARI.

The inclusion and exclusion criteria were determined based on the clear need for the recruited individual to be able to engage with the instructions associated with the manoeuvre to ensure as reliable a physiological measurement as possible.

7.2.2 Inclusion Criteria

- Clinical diagnosis of haemorrhagic stroke within 48 hours of onset (for patients waking with a stroke, time of onset was taken to be the time when the patient was last asymptomatic)
- Able and willing to give informed consent
- Male or female, aged 18 years or above
- Able (in the Investigator's opinion) and willing to comply with all study requirements
- Willing to allow his or her General Practitioner (GP) to be notified of participation in the study

7.2.3 Exclusion Criteria

- Male or Female, aged under 18 years
- Significant previous airways disease (formal diagnosis of moderate or severe airways disease and having treatment for this respiratory condition – via inhalers or specialist input)

- Unable (in the Investigator's opinion) or unwilling to comply with any study requirements
- Female participants who were pregnant, lactating or planning pregnancy during the course of the study
- Clinical diagnosis of stroke greater than 48 hours from onset
- Having had a resolved transient ischaemic attack (TIA) (i.e. neurological symptoms completely resolved upon hospital presentation)
- Co-morbidity with anticipated life expectancy less than 3 months

Patients with acute ICH meeting the inclusion criteria were considered for enrolment between October 2017 and July 2018. Patients were recruited within a strict 48 hour window post-acute ICH and had to be competent to comply with a respiratory manoeuvre, as judged by the investigator. Those with significant co-morbidity including respiratory disease managed with specialist respiratory follow-up were excluded.¹¹⁷

7.2.4 *Intervention*

The level of impairment of dCA was compared with extent of CT acute ICH findings. dCA measurements were measured as below with a hyperventilation protocol. This involved repeated sustained periods of 90-seconds of hyperventilation at 5mmHg below baseline EtCO₂ regulated using a metronome. The patients received a demonstration from the conducting physician clarifying the expected relationship of their breathing with the metronome beat. Two-minute washout periods of normal respiration were allowed between successive measurements. Each incremental reduction in EtCO₂ was repeated on two occasions during the same session. Patients underwent repeated assessments of hypocapnia with intervening periods of washout, i.e. two repetitions of the hypocapnic intervention with interval 5-minute

baselines. This provided the opportunity to refine the compliance with the metronome and ensure the manoeuvre was successfully achieved on at least one occasion per assessment period. The methodological design was based on successful demonstration of the expected deviation in EtCO₂ during pilot work (Chapter 5).

Participants were monitored throughout the process and any unexpected physiological changes were recorded on an adverse event form. These were gathered and discussed at monthly supervisor meetings (to replicate a data safety monitoring process). Furthermore, individuals will be compared to age-matched controls from the Leicester Cerebral Haemodynamics Database¹⁴¹ and the pilot study (Chapter 5) to ensure physiological parameters are within acceptable limits.¹²⁰ Furthermore, in preparation for the study, lower limits of acceptable EtCO₂ (29 mmHg) and CBFV (33 cm/s) were calculated as within 1 SD from the mean hypocapnia level for cohort of healthy volunteer values (Chapter 5) and based on previous acceptable limits in TBI studies.¹⁵⁸ This helped protect individuals from risk of ischaemia associated with hypocapnia-induced vasoconstriction.

7.2.5 Interpretation

Important classical outcomes associated with this before and after interventional study included death and disability at 14 days and the proportion of recruited individuals able to comply with the full measurement protocol. Furthermore, the data were assessed for quality during the analysis protocol and rejected data files recorded with associated reasoning. Lastly, the percentage change in CBFV at baseline and in response to a hypocapnic manoeuvre in the acute (<48 hours) and sub-acute phase (10 to 14 days) were recorded alongside ARI. Importantly this study was designed to test the feasibility of a CA-targeted intervention using hyperventilation. The inclusion of clinical outcomes was not necessarily the main objective of the study but highly relevant as an early indicator of feasibility to support design of robust larger trials.

7.2.6 *Assessment of Haemodynamic Function*

The methodological setup for this study was based on refinements to existing physiological measurement processes following experiments described in Chapters 3 (gas sampling method), 4 (hypocapnia induction and maintenance processes), 5 (safety limits for hypocapnic interventions). As mentioned, this refinement permitted the robust methodological development of the intervention described above. For all subjects, all assessments were undertaken in a dedicated cardiovascular research laboratory at Leicester Royal Infirmary (LRI), which is at a controlled temperature (20-24°C) and is free from distraction. In the instance the patient was unable to be transferred to the laboratory, the assessments were undertaken in the hyperacute stroke bay (HASU). The subject lay supine on an examination couch. Baseline casual BP were calculated as a mean of three supine brachial BP readings using a validated UA767 BP monitor. Beat-to-beat non-invasive BP were recorded continuously using the Finometer cuff device attached to the middle finger of the non-dominant hand (non-hemiparetic hand in stroke patients). R-R interval was recorded using a 3-lead ECG. Respiratory rate was recorded, and end-tidal partial pressure of carbon dioxide (EtCO₂) monitored using small nasal cannulae placed at the base of the nose (Salter Labs, ref 4000) attached to a capnograph (Capnograph Plus) to monitor respiration. Simultaneous bilateral insonation of the middle cerebral arteries (MCAs) was performed with the subject lying supine on a couch using transcranial Doppler ultrasound (TCD, DWL Dopplerbox 10.5.1 software version) to measure cerebral blood velocity (CBFV) as the most widely accepted surrogate of CBF in dCA studies³⁹. Using 2MHz probes, supported by a custom-made frame, the vessel was located via the temporal bone window, and identified as the MCA by the waveform, its depth, velocity, and the direction of flow. All parameters were simultaneously recorded onto a computer data acquisition system (PHYSIDAS), for subsequent off-line analysis. The physiological measurement setup is demonstrated in Figure 7.1.

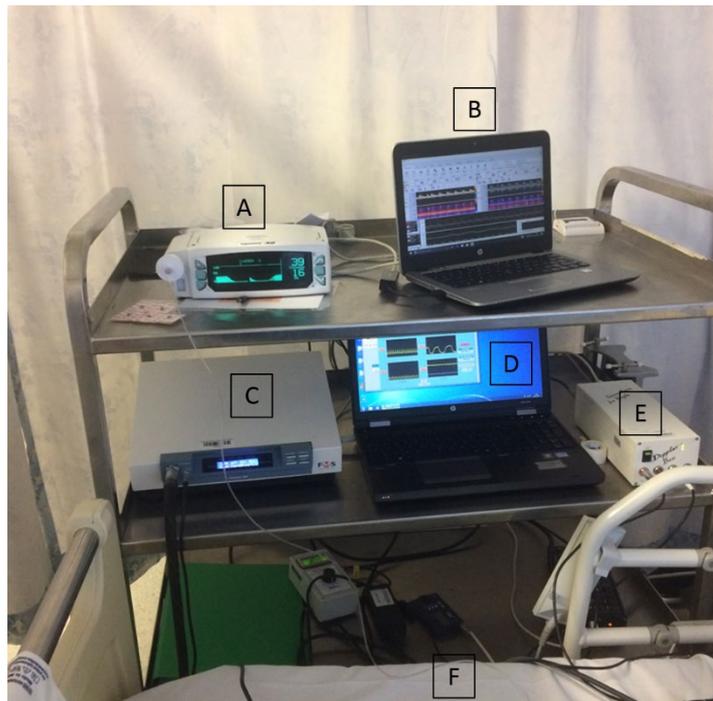
7.2.7 Assessment of Haemorrhage Volume, Intraventricular and Subarachnoid Extension

A CT scan within 48 hours was analysed for haematoma size, perihematoma oedema, intraventricular and subarachnoid extension. The assessor of these imaging parameters was blinded to any autoregulation data from initial CT or magnetic resonance imaging according to the ABC criteria (assess the length, width and depth of haemorrhage in centimetres) associated with the shape of the haemorrhagic lesion.¹⁵⁹ The ICH volume was calculated from the first CT scan using the $a \times b \times c \times 0.5$ (ABC/2) method as described above. The CT imaging underwent adjudication by a radiologist (Dr David Swinton, Consultant Radiologist, LRI).

7.2.8 Assessment of Clinical Parameters

At admission, patients were scored according to the National Institutes of Health Stroke Scale (NIHSS) (Appendix 10.11). Demographic, baseline clinical and laboratory parameters including age, sex and history of hypertension, previous antihypertensive therapy, admission NIHSS score, and admission BP were recorded.

Figure 7.1 Bedside physiological measurement set-up for BREATHE-ICH study.



A Capnograph; B Cerebral blood velocity waveforms from Dopplerbox (10.5.1 Software) dedicated laptop; C Finometer; D PHYSIDAS data acquisition system; E DWL Dopplerbox; F Korg Metronome.

7.2.9 Data Analysis

Following transfer function analysis (TFA), a measure of dCA, ARI, was calculated. TFA is widely used and validated technique of examining the relationship between CBF and BP. TFA allows quantification of the close transfer of spontaneous BP fluctuations to CBF, providing an objective measure of dCA. Furthermore, TFA and coherence (linearity between input and output signals) is calculated using Welch's method. Coherence was used to determine acceptability of good quality data taking into consideration appropriate number of degrees of freedom.⁸⁵ Beyond these parameters, phase and gain were calculated giving an explanation as to the timing of input (BP) and output (CBFV) signals. Lastly, to derive ARI, a time-domain approach was used as described by Tiecks *et al.*⁴⁰ from a standardised set of curves based on CBFV response to sudden step changes in BP following thigh cuff deflation (as outlined in Chapter 1).⁴⁰

7.2.10 Statistical Analysis

All normally distributed continuous variables are described as mean (SD) and non-Gaussian with skewness as median (IQR) where appropriate. Comparison of baseline data in acute ICH patients was made using the Student t-test for normally distributed data, or by appropriate non-parametric tests. Values of $p < 0.05$ were considered statistically significant. All statistics were performed using statistical software GraphPad Prism 7 for Windows.

7.3 Ethics

7.3.1 Ethics and Safety Considerations

The East Midlands – Nottingham 1 Research Ethics Committee (REC), reviewed the BREATHE-ICH study protocol. The committee appraised the ethical implications of the intervention that would be carried out during the study and potential impact on patient rights and wellbeing. Favourable opinion from the REC meant that due care and consideration had been made to preserve patient rights and the methodology was ethically justified; favourable opinion was provided on the 31st August 2017 (17/EM/0283). Further to this, University Hospitals of Leicester NHS Trust provided local research and innovation approval on 25th September 2017.

Informed consent was obtained from participants in line with recommendations set out in the E6(R1) of the International Council for Harmonisation of Technical Requirements or Pharmaceuticals for Human Use (ICH) Good Clinical Practice Guidelines. All patients provided written informed consent and all study procedures were completed in accordance with the most recent revision of the Declaration of Helsinki.⁹⁸

7.4 Results

Altogether 18 patients with acute ICH met the inclusion criteria, though only 12 entered the analysis. Five patients were excluded due to lack of acceptable TCD windows (see Appendix 10.1). A further patient was excluded due to CT confirmation at a later date of haemorrhagic transformation of cerebral infarction, as opposed to primary acute ICH. Furthermore, follow-up assessments were performed on 9 patients, as 3 patients were resident in local community hospitals and not able to return for the repeat assessments due to ongoing stroke specific rehabilitation needs. The mean age was 68 (range 30 to 91 years), mean haemorrhage volume was 5.78 mL (range 0.21 to 14.76 mL) and none had intraventricular extension, though 17% had subarachnoid extension. For detailed demographic, clinical and radiological characteristics of the study population see Table 7.1.

Table 7.1 Demographic and baseline characteristics.

	Subjects (n=12)
Age, y	68 (16)
Sex, male/female	8/4
Time to assessment, hours	24 (11)
Systolic BP on admission, mmHg	160 (30)
Diastolic BP on admission, mmHg	87 (17)
NIHSS on admission, points	4 (3)
NIHSS on assessment, points	4 (3)
GCS	15 (0)
Haematoma side, left/right	7/5
Haematoma location, n (%)	
Basal ganglia	2 (17)
Thalamus	3 (25)
Lobar	7 (58)
ICH volume, mL	5.78 (4.96)
Intraventricular haemorrhage, n (%)	0 (0)
Subarachnoid haemorrhage, n (%)	2 (17)
Hypertension, n (%)	5 (42)
Diabetes mellitus, n (%)	1 (8)
Hyperlipidaemia, n (%)	1 (8)
Smoking, n (%)	1 (8)
Excessive drinking, n (%)	0 (0)

Values are mean (SD) unless otherwise stated

The intervention was tolerated without any adverse clinical consequences including headache, syncope, ischaemic stroke or any hypocapnia related paraesthesia.

7.4.1 Results BREATHE-ICH Intervention and Haemodynamics at <48 hours

The trend in response pre- and post-hypocapnic intervention is demonstrated in Table 7.2 and in Figures 7.2 and 7.3. There was an overall trend for CBFV to decrease, ARI to increase, MAP to decrease, HR to increase, and RAP to decrease, with CrCP remaining static. Figure 7.4 demonstrates individual responses with 'responders' defined as those demonstrating the lower CBFV and improved ARI (as seen in Chapter 5) in response to lower EtCO₂. Both *Patient 6* and *Patient 11* were 'non-responders' for CBFV and *Patient 7* for ARI, with *Patient 2* exhibiting no ARI change in response to a -3mmHg EtCO₂ decrease.

There was no significant difference between baseline EtCO₂ (35.4 mmHg) and EtCO₂ for hypocapnic intervention (32.4 mmHg) ($p=0.08$, Table 7.2). However, a significant difference was noted, despite the lack of significant reduction in EtCO₂, between ipsilateral ARI at baseline 4.8 (1.7) and ARI during hypocapnic intervention 7.0 (0.8) ($p=0.0004$, Table 7.2).

Table 7.2 Haemodynamic characteristics at baseline assessment and during intervention (<48 hours).

	Baseline	Hypocapnic Intervention	p-value
EtCO₂ (mmHg)	35.4 (4.0)	32.4 (4.2)	0.08
CBFV Ipsilateral (cm.s⁻¹)	49.2 (18.7)	44.0 (12.6)	0.44
CBFV Contralateral (cm.s⁻¹)	50.1 (15.8)	45.7 (14.1)	0.54
ARI Ipsilateral	4.8 (1.7)	7.0 (0.8)	0.0004
ARI Contralateral	4.7 (2.1)	5.9 (1.7)	0.21
Mean ABP (mmHg)	102.9 (15.5)	95.2 (13.0)	0.20
Heart Rate (bpm)	71.9 (13.1)	74.6 (11.2)	0.59
CrCP Ipsilateral (mmHg)	45.8 (21.2)	48.1 (18.6)	0.78
CrCP Contralateral (mmHg)	44.8 (13.1)	45.2 (14.3)	0.95
RAP Ipsilateral (mmHg.s/cm)	1.3 (0.7)	1.1 (0.4)	0.56
RAP Contralateral (mmHg.s/cm)	1.2 (0.6)	1.1 (0.5)	0.92

Values are mean (SD). CBFV, cerebral blood velocity; ABP, arterial blood pressure; EtCO₂, end-tidal arterial pressure of carbon dioxide; CrCP, critical closing pressure; RAP, resistance area product; ARI, Autoregulation Index.

Individualised plots demonstrating variability in response for the 12 patients undergoing initial assessment (<48 hours) post-acute ICH for ipsilateral and contralateral CBFV (Figure 7.2.A-B), ARI (Figure 7.2.C-D), CrCP (Figure 7.3.E-F), ABP (Figure 7.3.G), RAP (Figure 7.3.H-I) and HR (Figure 7.3.J) are shown. In addition, individualised delta (Δ) EtCO₂ change, and corresponding delta (Δ) CBFV and ARI changes are demonstrated in Figure 7.4. These provide an overview of the response obtained by each individual in response to the intervention of EtCO₂ lowering.

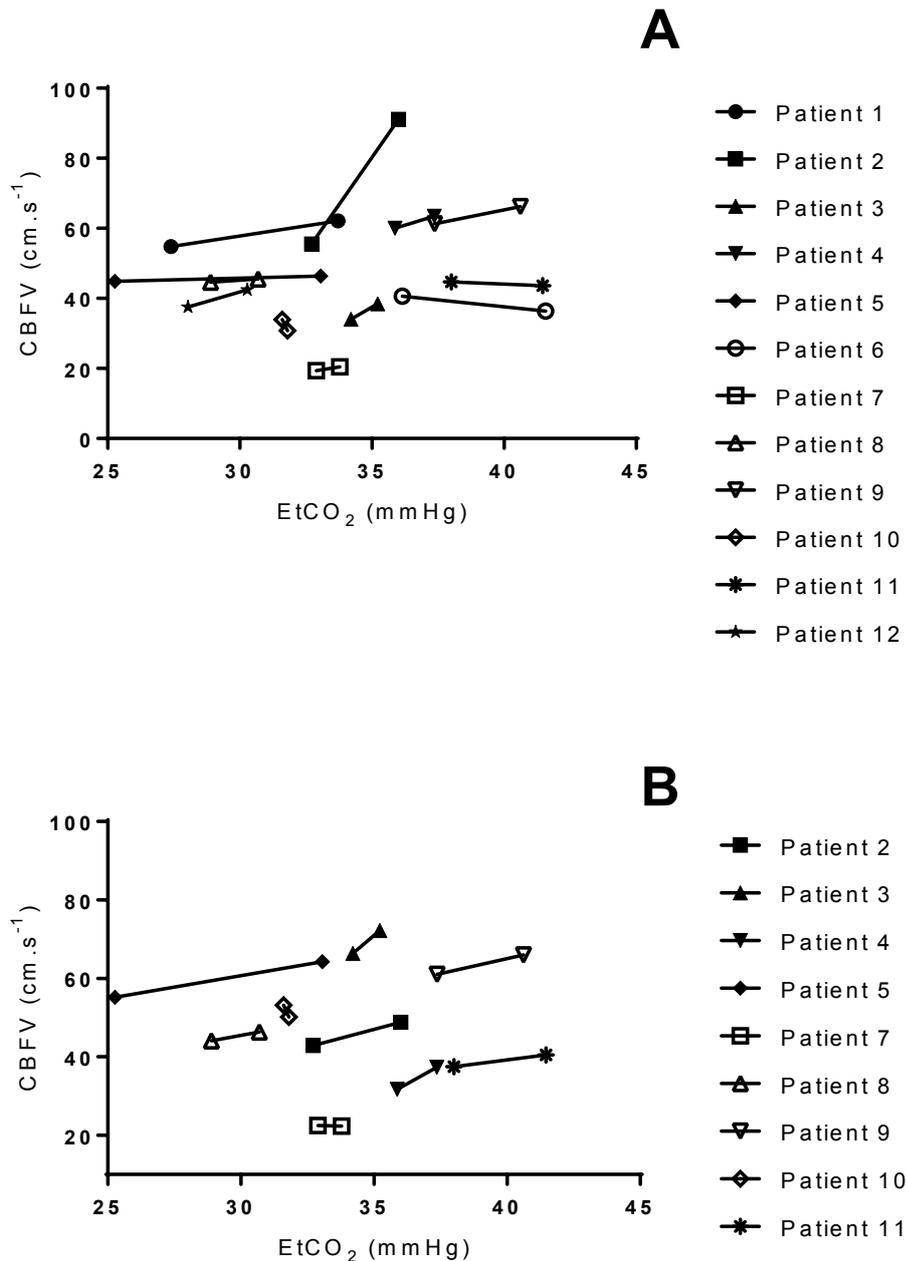
7.4.2 Results BREATHE-ICH Intervention and Haemodynamics at 10-14 days

The trend in response pre- and post-hypocapnic intervention is demonstrated in Table 7.3 and in Figures 7.5 and 7.6. There was an overall trend for CBFV to decrease, ARI to increase, MAP to decrease, HR to increase, CrCP to increase and RAP to decrease.

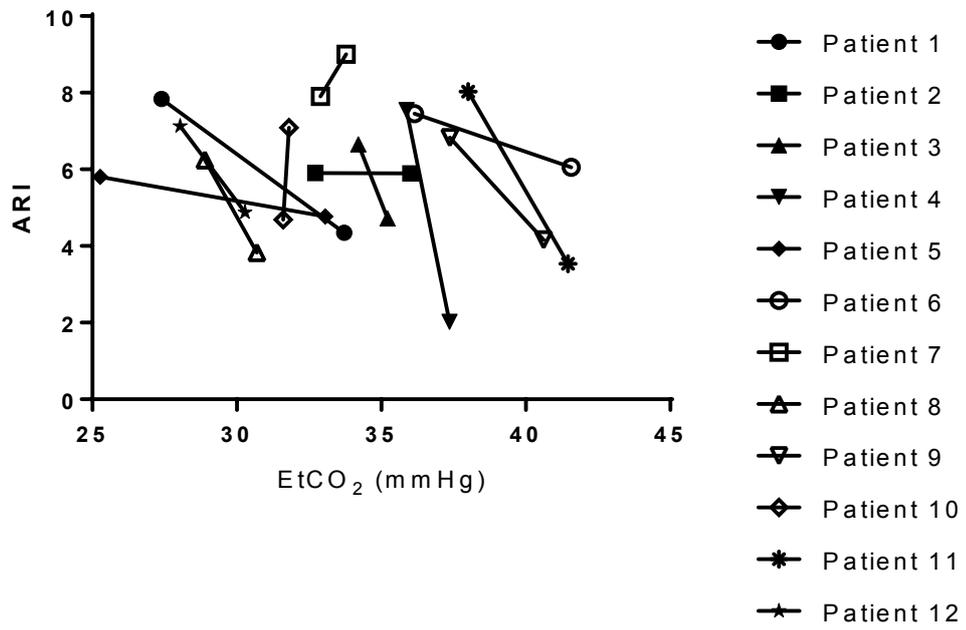
Follow-up haemodynamic data for the 10-14 days post-acute ICH are provided in Table 7.3. Furthermore, individualised plots demonstrating variability in response for the 9 patients followed-up (10-14 days) post-acute ICH for ipsilateral and contralateral CBFV (Figure 7.5.A-B), ARI (Figure 7.5.C-D), CrCP (Figure 7.6.E-F), ABP (Figure 7.6.G), RAP (Figure 7.6.H-I) and HR (Figure 7.6.J) are shown. Lastly, baseline ARI was examined relative to haematoma volume,

demonstrating a focus of values around an ARI of 4-5, with higher values of ARI appearing to be associated with larger haematoma volumes.

Figure 7.2 Individualised plots demonstrating variability in response for the dependence of A) CBFV ipsilateral, B) CBFV contralateral, C) ARI ipsilateral and D) ARI contralateral on end-tidal CO₂ change from baseline assessment to hypocapnia intervention.



C



D

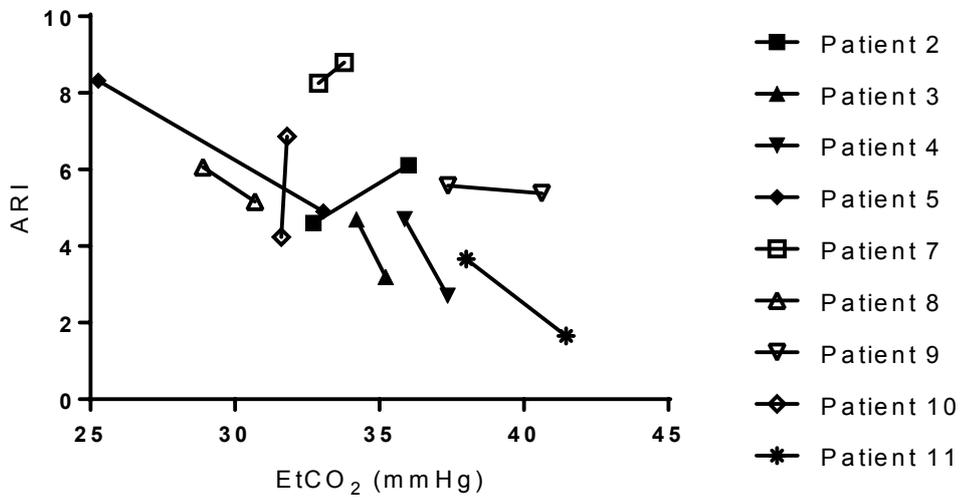
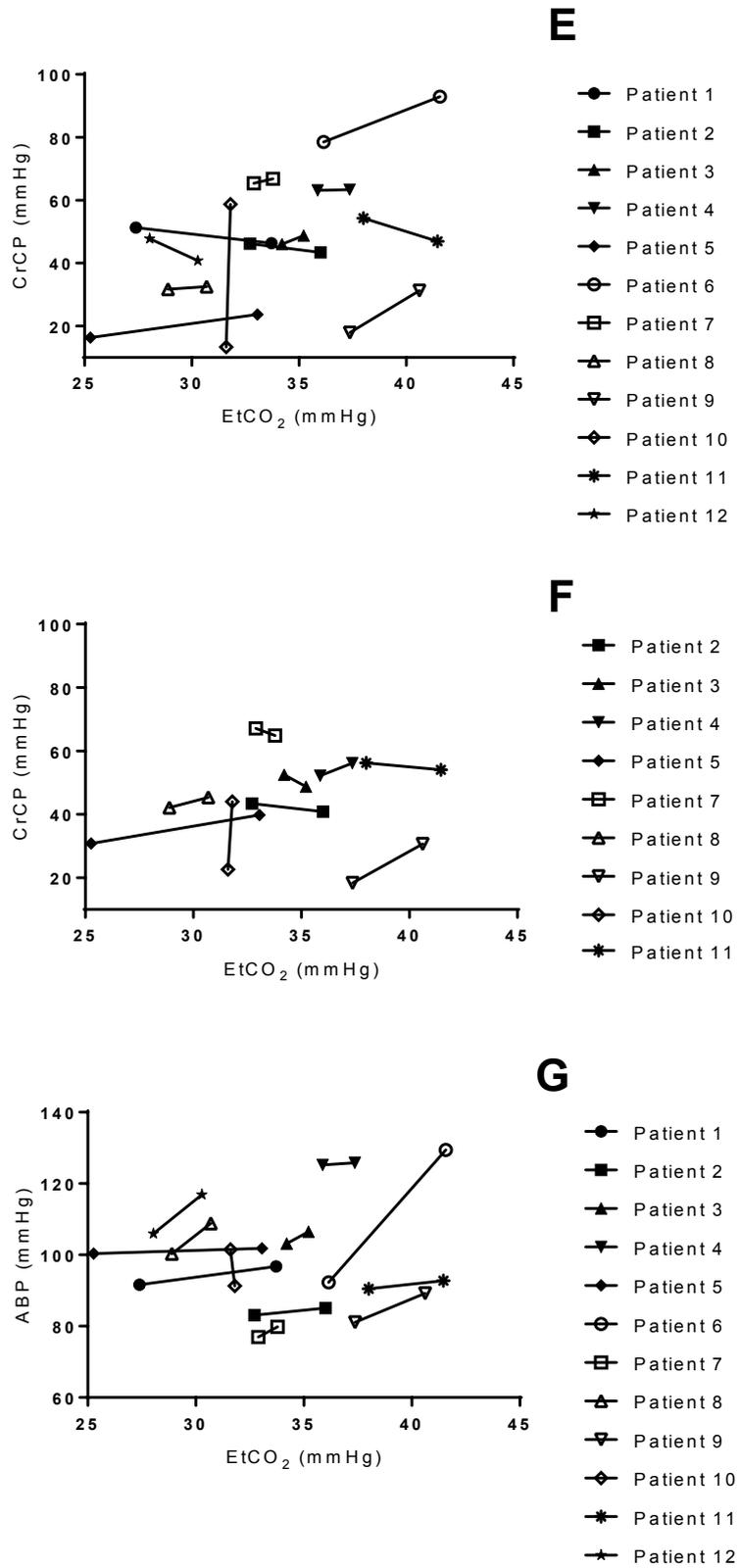


Figure 7.3 Individualised plots for the dependence of CrCP ipsilateral (E), F) CrCP contralateral, G) ABP, H) RAP ipsilateral, I) RAP contralateral and J) Heart Rate on end-tidal CO₂ change from baseline assessment to hypocapnia intervention.



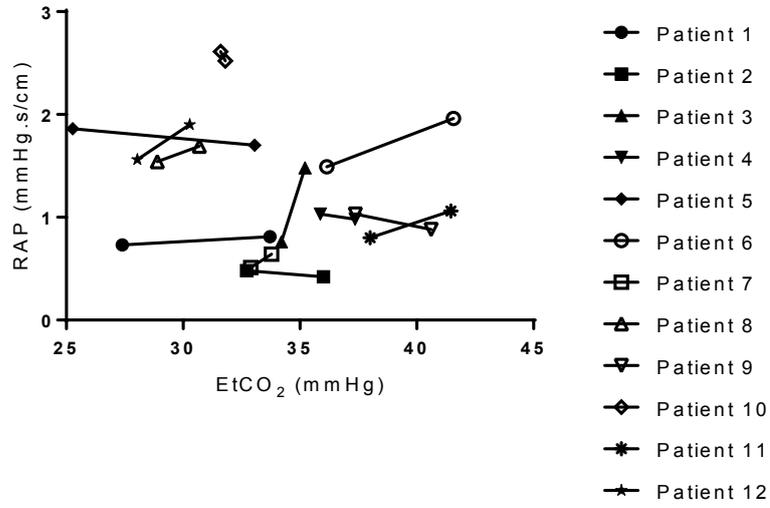
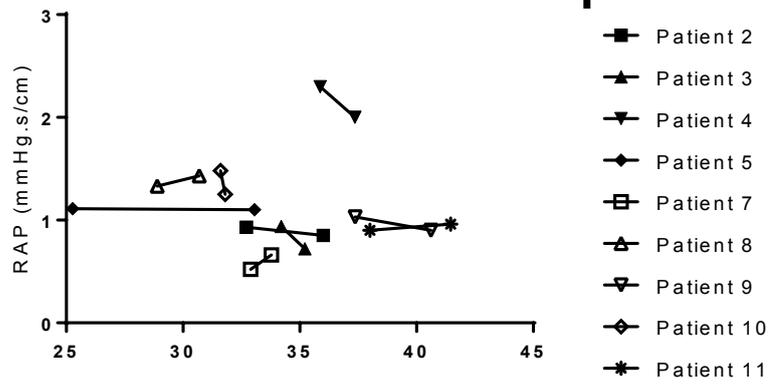
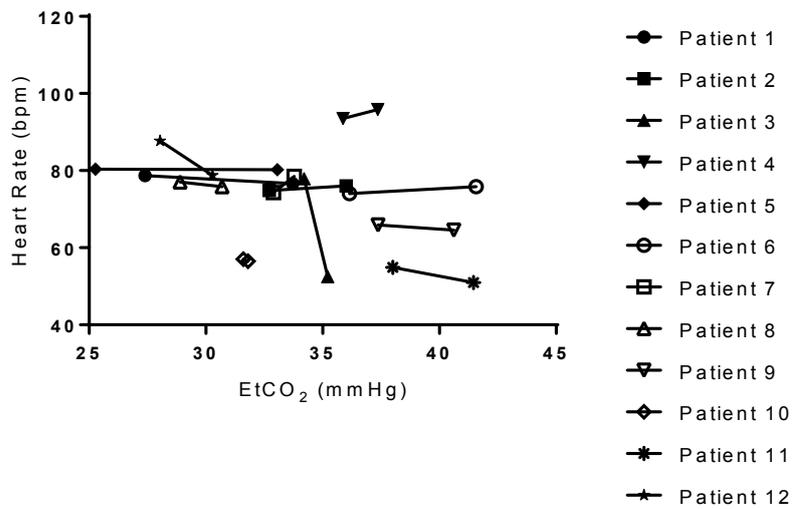
H**I****J**

Figure 7.4 Individualised delta (Δ) EtCO₂ change and corresponding delta (Δ) CBFV and ARI changes.

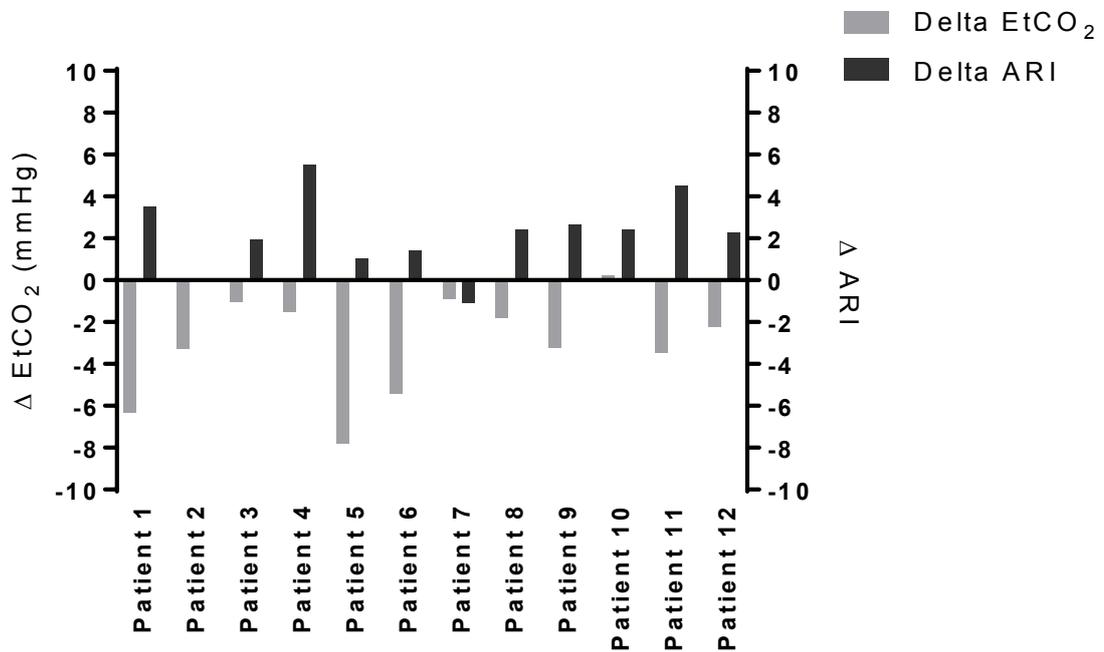
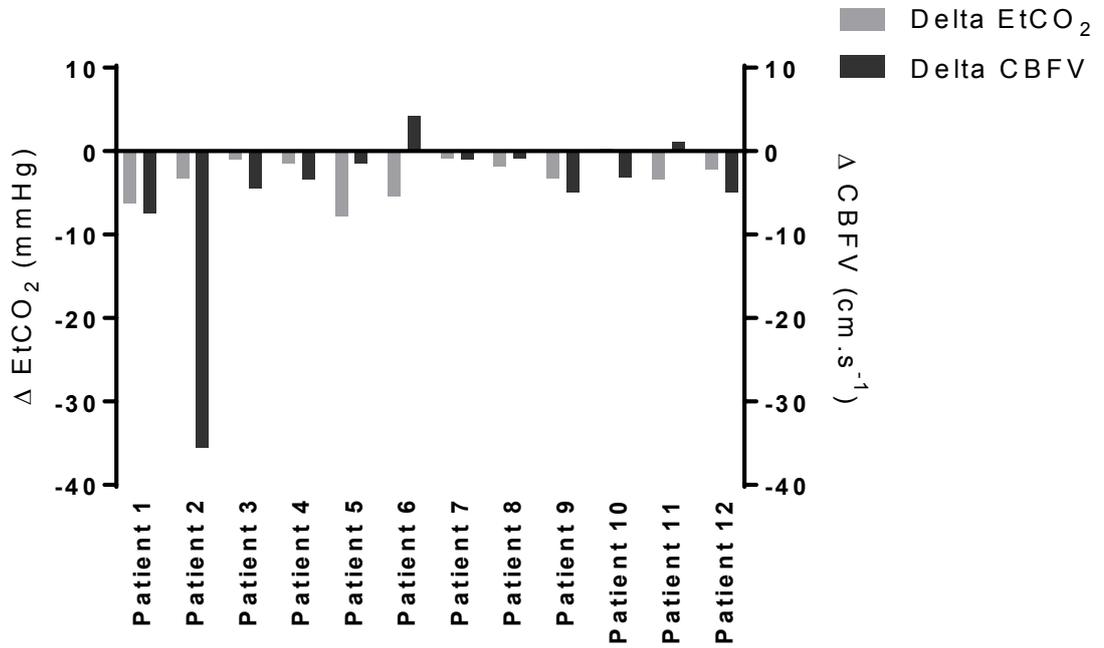
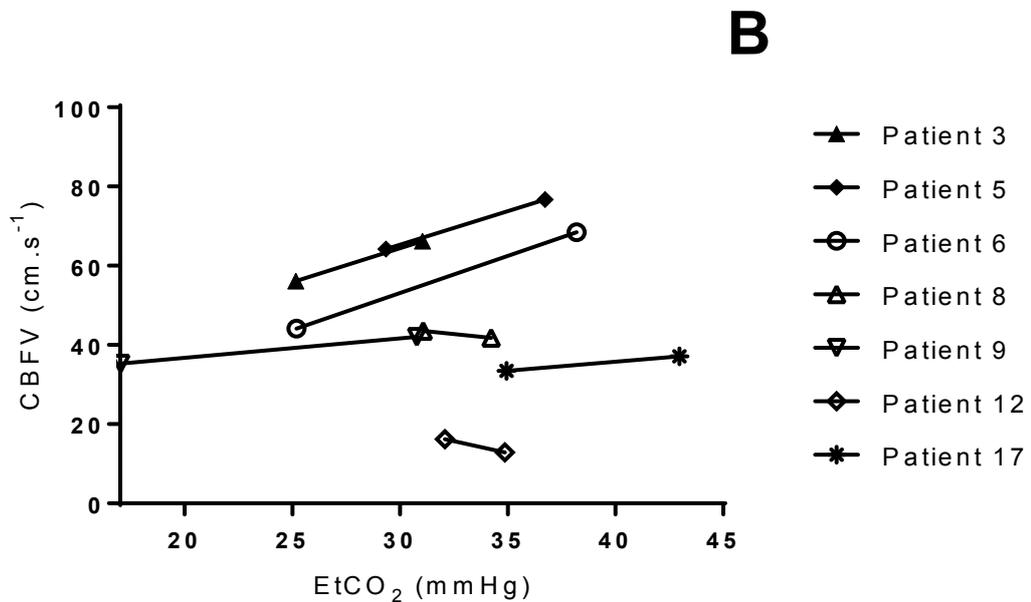
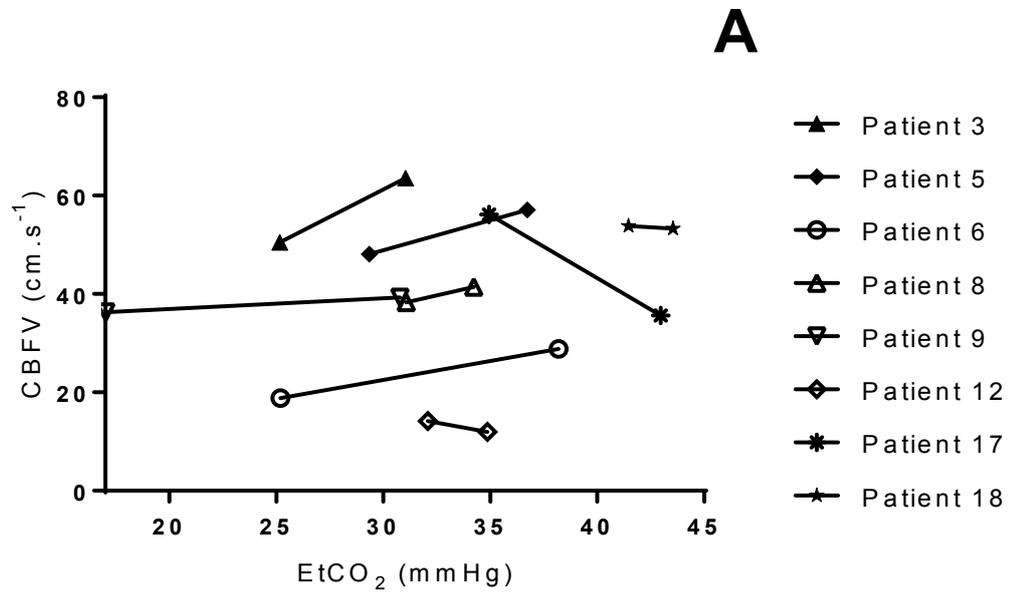


Table 7.3 Haemodynamic characteristics at 10-14 day follow-up.

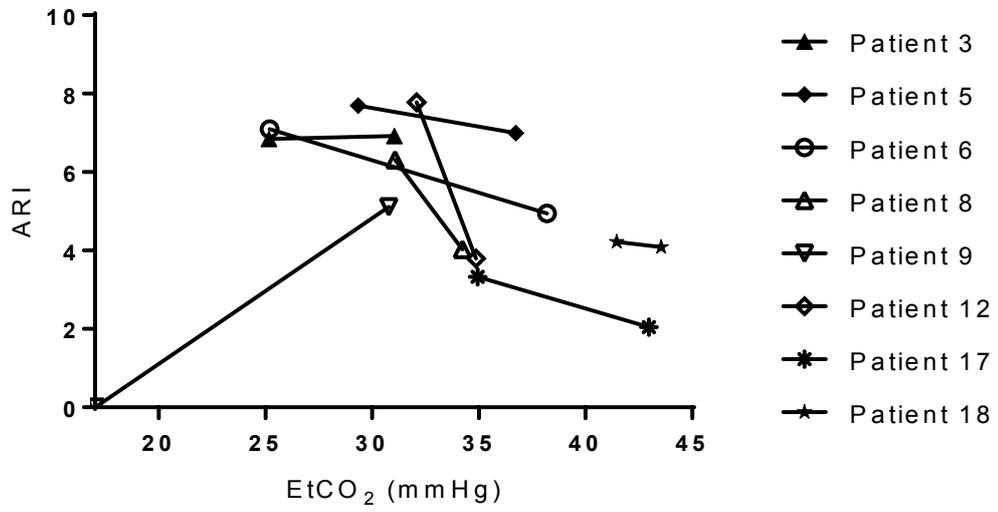
	Baseline	Hypocapnic Intervention	p-value
EtCO₂ (mmHg)	36.5 (4.9)	29.5 (7.3)	0.04
CBFV Ipsilateral (cm.s⁻¹)	41.4 (16.7)	39.5 (15.9)	0.82
CBFV Contralateral (cm.s⁻¹)	49.3 (22.3)	41.8 (15.7)	0.48
ARI Ipsilateral	4.7 (1.7)	5.4 (2.7)	0.56
ARI Contralateral	4.5 (1.9)	6.1 (2.0)	0.15
Mean ABP (mmHg)	115.6 (19.5)	113.7 (12.3)	0.82
Heart Rate (bpm)	64.7 (10.3)	71.4 (8.0)	0.17
CrCP Ipsilateral (mmHg)	50.3 (27.5)	55.5 (28.2)	0.71
CrCP Contralateral (mmHg)	52.6 (13.1)	54.0 (23.4)	0.92
RAP Ipsilateral (mmHg.s/cm)	1.6 (0.5)	1.5 (0.3)	0.72
RAP Contralateral (mmHg.s/cm)	1.3 (0.5)	1.4 (0.4)	0.74

Values are mean (SD). CBFV, cerebral blood velocity; ABP, arterial blood pressure; EtCO₂, end-tidal arterial pressure of carbon dioxide; CrCP, critical closing pressure; RAP, resistance area product; ARI, Autoregulation Index.

Figure 7.5 Individualised plots for the dependence of A) CBFV ipsilateral, B) CBFV contralateral, C) ARI ipsilateral and D) ARI contralateral on end-tidal CO₂ change from baseline assessment to hypocapnia intervention.



C



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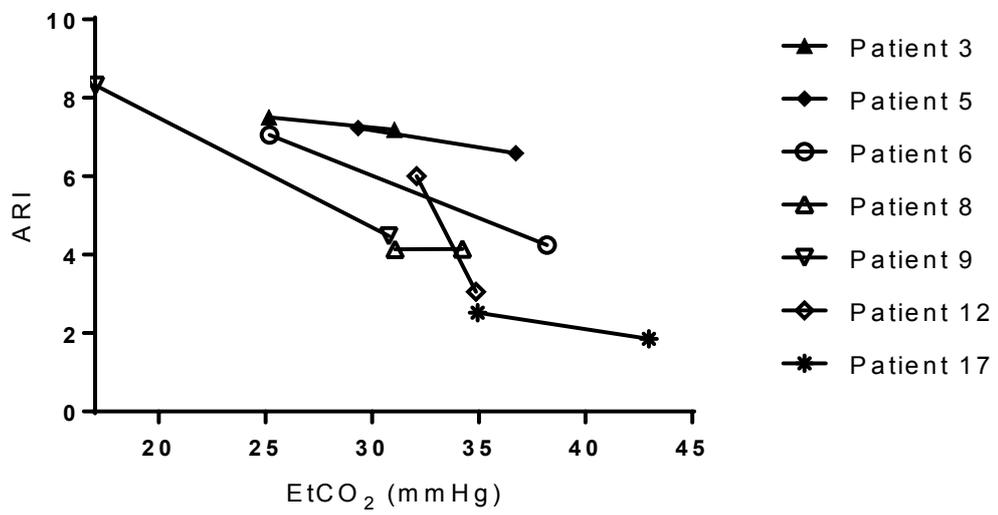
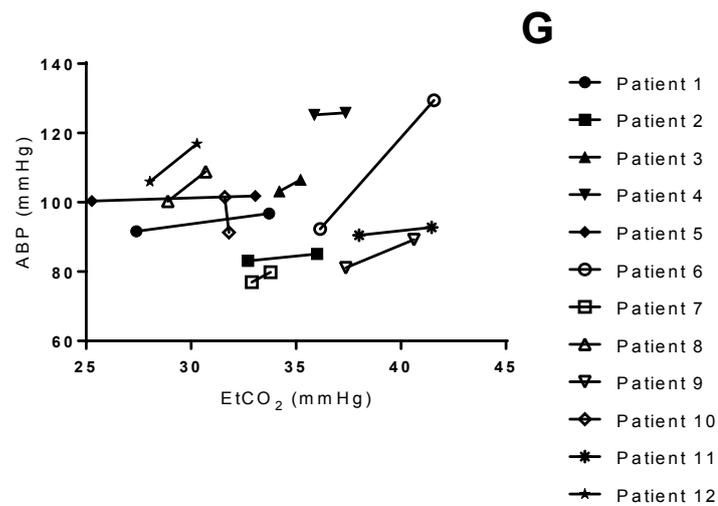
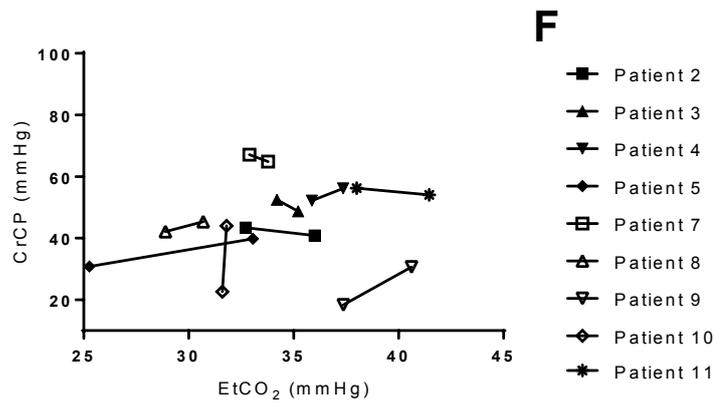
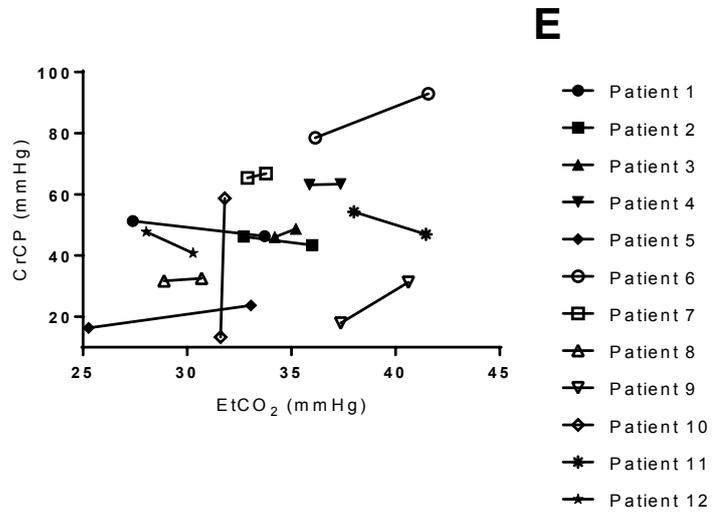
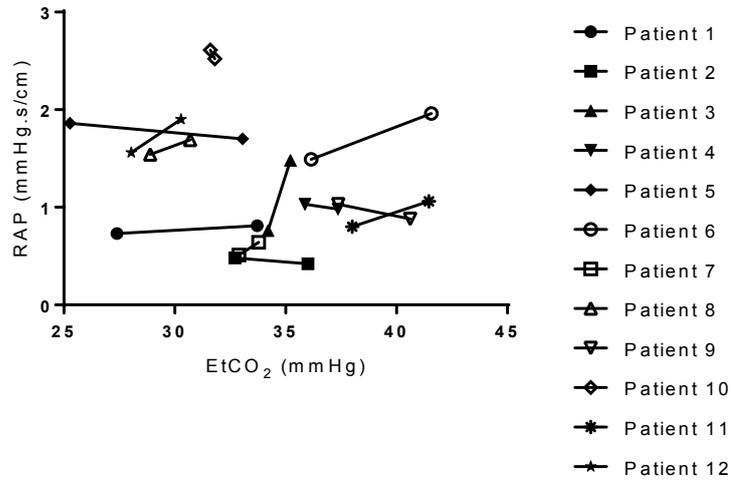


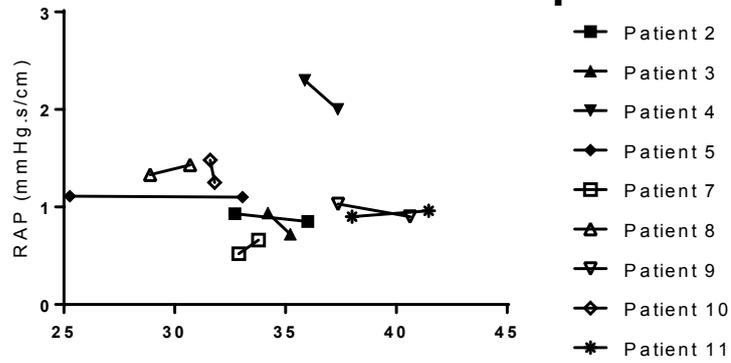
Figure 7.6 Individualised plots for the dependence of CrCP ipsilateral (E), F) CrCP contralateral, G) ABP, H) RAP ipsilateral, I) RAP contralateral and J) Heart Rate on end-tidal CO₂ change from baseline assessment to hypocapnia intervention.



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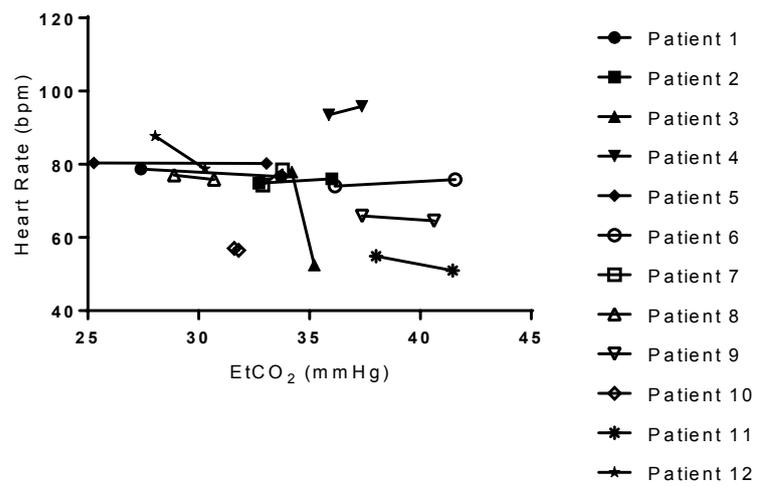
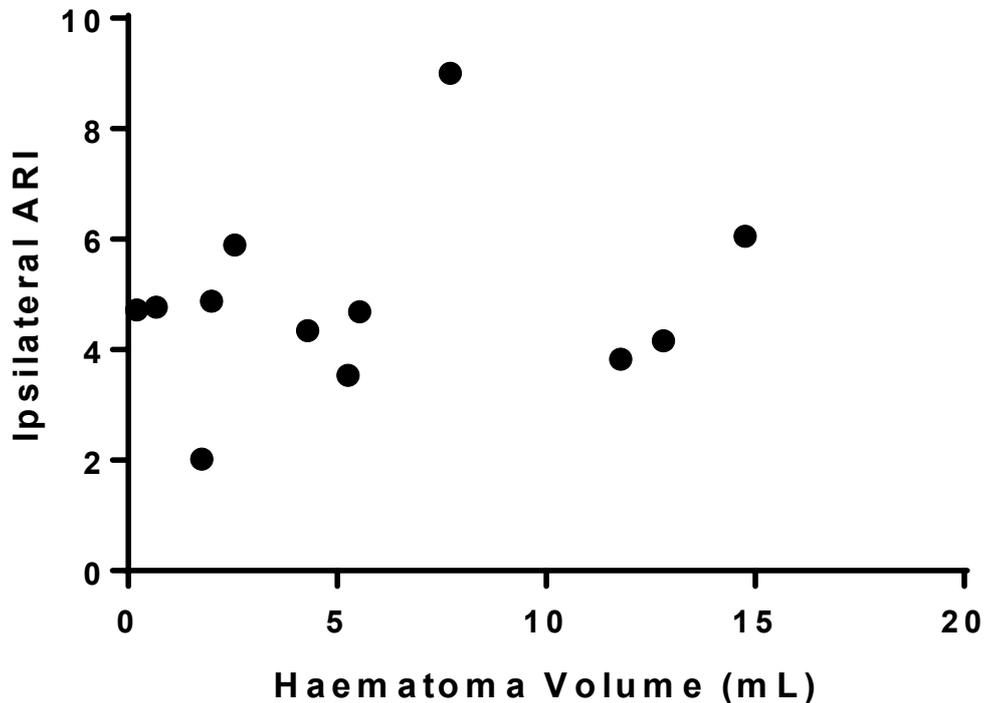


Figure 7.7 Correlation between baseline ipsilateral ARI and haematoma volume (mL).



7.5 Discussion

7.5.1 Main Findings

The authors report the feasibility of the first interventional study designed to manipulate EtCO₂ to improve CA in acute stroke. In a cohort of mild (NIHSS 4) supratentorial ICH patients with small volume haematomas without intraventricular extension, we demonstrated dCA could be improved from an acceptable mean ARI value (around 4) to a significantly improved one (>5). The data supports an individualised response by patients suffering an ICH, with ARI at or below the impaired cut-off of <4, improving towards ARI values in excess of 4.¹⁶³ Furthermore, this study affirms prior observational physiological measurement stroke studies that demonstrate the presence of hypocapnia post-stroke. The lack of a clear stepwise drop between averaged EtCO₂ values is likely associated with statistical power, though assessment of individualised responses to the intervention demonstrates the feasibility of lowering EtCO₂ in acute ICH.

Importantly, despite a small patient cohort, there was a significant difference in ARI despite a lack of significant reduction in EtCO₂ on a whole study population level.

7.5.2 CA and Hypocapnia at <48 hours in the BREATHE-ICH study

The patients recruited to the study were comparable to prior dCA in acute ICH studies with similar age, systolic BP, diastolic BP, and haematoma locations (as described in Chapter 2). Arguably, as demonstrated in Chapter 2 Table 2.2, the severity was greater (NIHSS >10), however, these studies were not designed to deliver an intervention to this patient population. Furthermore, haematoma volumes were smaller than the other dCA studies in ICH for the same reason, though almost a fifth of the cohort had evidence of subarachnoid extension, a poor prognostic feature in ICH.¹⁶⁴

The demonstration of a 'response' on individualised assessment (Figure 7.4) of all participants except two (83.3%) is encouraging. This supports the translation of the physiologically expected response from healthy volunteers to those with acute ICH. Furthermore, the lack of any significant adverse physiological change, aside from a dramatic drop in CBFV for *Patient 2* of 36 cm.s⁻¹ with a lack of ARI improvement. This could be explained by the presence of chronic hypertension (with three prior anti-hypertensives being taken) and ongoing acute BP lowering therapy being delivered (i.e. labetalol or glyceryl trinitrate therapy) as anti-hypertensives that have been demonstrated to affect both BP variability and autoregulatory response in both a positive and negative manner.¹⁶⁵⁻¹⁶⁷ Interestingly, calcium channel blockers have a selective action on vasoconstricted vessels and have differential effects in different regional vascular beds, this is particularly relevant in hypocapnic conditions as the exerted effect on the MCA may lead to unexpected variation in CBFV and autoregulatory function (either significantly accentuated or indeed minimal change).^{168,169}

Central and peripheral haemodynamic parameters behaved as expected though CrCP exhibited reduced sensitivity to EtCO₂ change (Figure 7.3). Prior work has demonstrated CrCP tends to increase with hypocapnia, though this is usually associated as reported previously in patients with syncope with a large fall in CBFV.^{118,170} Interestingly, the behaviour of CrCP and indeed ARI at the 10-14 day follow-up was more comparable to trends described in Chapter 5.

7.5.3 CA and Hypocapnia at 10-14 days in the BREATHE-ICH study

During the follow-up period, the significant difference in EtCO₂ between the baseline measurement and the intervention suggests compliance with protocol was better in less acutely unwell patients after 10-14 days post stroke. Importantly, though no significant benefit (but also no harm) was seen in this assessment period (as compared to the acute period), this is important to explore in a larger group and to consider alternative options to reduce PaCO₂ in acutely unwell patients, particularly those with larger haematoma volumes or a more severe NIHSS.^{90,91} CrCP demonstrated a less static trend during this delayed follow-up period post ICH with a more noticeable improvement seen during hypocapnia. Furthermore, delta EtCO₂ was larger and hence ARI improved more in the contralateral hemisphere during hypocapnia during the 10-14 day follow-up as opposed to the <48 hour assessment (Tables 7.2 and 7.3). However, the heightened ARI response ipsilateral to the haematoma was dampened at the follow-up period, suggesting the neurovascular response to EtCO₂ within the affected hemisphere appeared to be heightened when a haematoma was present. Studies in animal models of SAH have concluded that post haemorrhagic microspasm leads to complete loss of CO₂ reactivity for up to 24 hours and therefore CBF cannot be increased during hypercapnia.¹⁷¹ However, in other experimental models using hyperventilation to induce hypocapnia, as opposed to hypercapnic conditions, graded hyperventilation has been sufficient for re-establishing impaired autoregulation after SAH. This is mechanistically explained in SAH by the association between defective autoregulation increasing cerebrospinal fluid lactate, and therefore arteriolar wall and

perivascular space pH governing autoregulatory function.¹⁵⁶ During hyperventilation, pH increases and as such acidotic conditions can be balanced. There is a paucity of comparable research in acute ICH and clearly with vasospasm predominating in subarachnoid haemorrhage, the mechanisms are distinct. However, the suggestion is that despite ipsilateral haemorrhage being present, CA can be improved as this has study demonstrated.¹⁵⁶

7.5.4 CA and the 'Autoregulatory Reserve' Hypothesis

The demonstration that ipsilateral CA can be improved raises an important question about the presence of 'reserve' CA that could be recruited in a potentially neurologically vulnerable circumstance. The main secondary brain injury post ICH involves three cascades: inflammation, red cell lysis and iron deposition, and thrombin production.¹⁷² Combined with other pathophysiological mechanisms including oxidative stress, apoptosis, and factors precipitating blood brain barrier disruption, brain oedema and hydrocephalus, these all contribute to raised intracranial pressure and a consequent vicious cycle henceforth.¹⁷² These factors all have the potential to influence cerebral autoregulatory function and reserve. The demonstration of improvement in ARI to significantly better levels in the acute period suggests the presence of 'reserve' autoregulatory function. The presence of 'reserve' cerebrovascular function precipitated by hypocapnia has been demonstrated in animal, healthy volunteer and disease states. In studies of dogs, profound hypotension induced with adenosine did not eliminate CO₂ reactivity or lower CBF to ischaemic levels in the presence of severe hypocapnia.¹⁷³ In healthy humans, the vasoconstrictive response precipitated by hypoxic hypocapnia differed between high altitude and sea level but the impairment was rapidly reversible.¹⁷⁴ Lastly, in disease states, this phenomenon has been seen in carotid stenosis,¹⁷⁵ cerebral amyloid angiopathy,¹⁷⁶ and type II diabetes mellitus.¹⁷⁷ Of particular relevance is the severely impaired cerebrovascular reserve in perilesional brain tissue and surrounding abnormal vessels as assessed by TCD.¹⁷⁶

7.5.5 CA and Clinical Parameters within the BREATHE-ICH study

The demonstration of ipsilateral improvement in ARI and the lack of significant adverse physiological change during individualised assessment of responses to this interventional protocol is encouraging. The careful process of translation from healthy volunteers to ICH patients has involved several steps in both logistical and scientific understanding as to how PaCO₂ change influences CBF and autoregulatory function. Further exploration beyond haematoma volume in larger clinical cohorts would be useful to provide further perspectives on associations between CA and poor prognostic markers demonstrated in prior work discussed in Chapter 2. The results form a strong basis for larger trials which can focus on outcomes for ‘responders’ and ‘non-responders’ to the intervention. If this distinction is made during initial clinical assessment post-acute ICH, it may provide a basis for offering personalised interventions to specific ICH sufferers in an effort to expand current treatment options and provide insight during discussions about prognosis.

7.5.6 Limitations

These results alongside those of prior studies in ICH have indicated that the pathophysiology of dCA in ICH patients is complex (as described in Chapter 2). Despite prior work demonstrating ipsilateral autoregulation is closely correlated with haematoma volume, contralateral autoregulation of ICH can also provide information about the presence of small vessel disease and baseline intracranial leukoarioriosis and therefore may not be a reliable marker of preceding autoregulation status.⁹⁰ Furthermore, if the sample size had been larger and nearer the second power threshold (n=40), a more accurate assessment of other parameters aside from ARI, as seen in studies discussed in Chapter 2, may have been obtained.

There was evidence of selection bias, towards mild to moderate ICH as dCA monitoring was not always possible in severe ICH, particularly with the involvement of a ventilatory manoeuvre.

The depth of understanding of impaired dCA in severe haemorrhage is unclear and has not been clarified by this study. This limitation prevents generalisability of findings to those with larger haematomas, higher NIHSS scores and lower level of consciousness.

The methodological setup was optimised through pre-pilot and pilot studies as demonstrated in Chapter 5. However, based on sample size, we were unable to draw any conclusions on the influence of sex differences as discussed in Chapter 6. The study limitations were carefully considered in the context of current work. First, patients were likely to be mild-to-moderate stroke severity, as evidenced by neurological impairment (NIHSS 5-12) and GCS >8 in similar experimental studies. Furthermore, there was the possibility that in order to comply with the respiratory manoeuvre; patients had milder stroke severity (NIHSS 1-4). Secondly, this study used BP instead of cerebral perfusion pressure to calculate dCA parameters, rather than the more invasive ICP measurement. This is an acceptable approach as we were unlikely to find significantly elevated ICP in this patient population, as their neurological status would not permit involvement in the study. Thirdly, we did not directly assess the effects of BP-lowering therapy on dCA, so there will remain limited data on the impact of intensive BP lowering on cerebral haemodynamics. Therefore, a mechanistic understanding from a cerebral haemodynamic perspective of different targets for intensive BP lowering is lacking. Fourthly, use of nasal cannulae for PaCO₂ measurements had benefits associated with rapid response time to changes, though concerns exist over accuracy compared to face mask measurements.¹⁶⁰ Lastly, with reference to TCD studies, changes in CBF can be accurately expressed by CBFV, as long as the diameter of the MCA remains constant. This assumption is usually acceptable at normocapnia or mild hypercapnia, but at moderate levels of hypocapnia, as we achieved in our subjects, it is likely that CBFV underestimated CBF. There are no studies to date showing MCA diameter change with hypocapnia despite evidence being shown in hypercapnia.¹²⁵

TCD studies have non-modifiable limitations. The study could be improved using isocapnic clamping during baseline periods to ensure the exact deviation of EtCO₂ during hypocapnia is deduced. However, as demonstrated in prior studies, there is an acceptable level of accuracy using non-invasive EtCO₂ assessment using capnography at the bedside.¹⁶⁰ Despite the benefits of repeating the hypocapnic intervention to ensure compliance and therefore strength of the response was optimised, there remains the possibility of inadequate washout and hence ongoing vasoconstrictive processes on a microvascular level, particularly if biochemical processes govern the process of reversibility post hypocapnia. Prior studies have shown that hypocapnic cerebral vasoconstriction is maintained even after ischaemia and/or reperfusion is reversed. This mechanism is thought to be driven by increased pH acting directly on vascular smooth muscle via second messengers such as inositol 1,4,5-triphosphate.¹⁷⁸ The suggestion is that these second messengers increase cytosolic calcium concentrations and hence induce vasoconstriction. However, as discussed previously, the benefits of prolonged vasoconstriction are unclear in the presence of a haematoma, as though the increased pH balances any acidosis, there is a risk of 'vasoparalysis' and hence vulnerability to secondary insults as seen in hypocapnic studies of mild TBI and moderate to severe hypoxic ischaemic encephalopathy.^{179,180}

7.6 Conclusion

dCA can be improved in acute ICH using an interventional manoeuvre, namely generating hypocapnia via hyperventilation. This was demonstrated to be safe and feasible with no adverse clinical outcomes. Further studies are required to examine the possibility of improving clinical outcomes at 30 days, as well as the assessment of alternative methods in a more severe ICH population that would be less able to comply with a hyperventilatory technique.

8 Conclusions

8.1 Main Thesis Findings

Prior to completion of this thesis, several studies had examined cerebral and peripheral haemodynamic responses to PaCO₂ change, but, with rare exceptions, these studies described the effects of PaCO₂ changes over a limited range. As a consequence there remained a lack of clarity as to the responses over the entire physiological range of PaCO₂ of key peripheral and cerebral haemodynamic variables. In addition, our understanding as to the state of impaired CA in acute ICH was unclear with several studies presenting contradictory findings. Furthermore, the knowledge that hypocapnia can improve CA in a healthy state had yet to be translated into an acute ICH setting. This thesis represents a novel study design, which in addition to being the first study to assess ARI in acute ICH, endeavoured to target CA improvement via an interventional approach in acute ICH patients. This chapter will briefly summarise the key findings in the thesis and discuss their potential implications for future clinical practice.

Chapter 2 provided systematic review and meta-analysis qualitative and quantitative data, respectively, to support the presence of lower CBFV bilaterally in acute ICH. Furthermore, evidence was demonstrated for impaired CA metrics and their association with worsening clinical status. These findings provide a mechanistic understanding of the potential impact of intensive BP lowering in ICH as lower CBFV in the presence of such interventions could have implications. This review permitted the development of a novel hypothetical relationship between CBFV, CA metrics and clinical status.

Chapter 3 provided an assessment of current methodological practice with regards to EtCO₂ in physiological measurement studies. This was primarily designed to ensure optimisation of the current setup and to understand how best we can assess EtCO₂ change at the bed-side without

the ability to derive isocapnic conditions. This study showed for the first time that use of FM and NC for measurement of EtCO₂ is associated with differing physiological changes and differences in parameter values.

Chapter 4 is a more detailed assessment of the methods of inducing and maintaining hypocapnia. Hypocapnia via hyperventilation is usually generated using a metronome. This study provides an important resolution to dysautoregulation previously shown by Dineen *et al.*⁴⁷ during the induction of hypocapnia. The use of a continuous metronome to induce hypocapnia rather than a sudden inception of an auditory stimulus appeared to reduce the initial decrease in autoregulatory capacity seen in prior studies. This has important implications for clinical translation of hypocapnia as dysautoregulation can be minimised by continuous metronome use and also supports the need for looking into the continuous time series of ARI values (ARI(t)) as a more robust and sensitive indicator of dCA status.

Chapter 5 detailed a novel logistic model, which assessed CBFV, ARI, HR, ABP, CrCP and RAP dependency to PaCO₂ over its entire physiological range. This model is a powerful tool for both physiological and clinical studies, providing a mechanism to adjust for variation in disease populations with differing values of baseline PaCO₂. This model instantly solves issues raised by Salinet *et al.*^{60,65} during prior clinical stroke studies. This model has the potential to be applied to other dCA metrics and be considered for application in licensed programs currently measuring physiological variables in a neurointensive setting where PaCO₂ variation is expected and closely monitored.

Chapter 6 describes the first study to examine sex within the context of a multi-level CO₂ protocol. The demonstration that the logistic curve parameters described in Chapter 5 are influenced by sex, highlights the need to take into account sex differences, which may be relevant in the context of the often older, predominantly female stroke population. Crucially,

with significant differences in CrCP, concern over vasoconstriction differences are relevant when considering interventional manoeuvres in clinical populations.

Chapter 7 describes the detailed protocol and results for the first CA targeted interventional study (BREATHE-ICH) designed to determine whether a simple breathing manoeuvre in acute ICH is safe and feasible and improves CA. This protocol was devised following improved understanding of the extent of impaired CA and CBFV changes in acute ICH (Chapter 2), pre-pilot studies in health volunteers (Chapter 5) and application of several decades experience of conducting TCD studies. This study recruited above its planned lower power threshold and demonstrated positive findings including an improvement in ARI ipsilateral to the haematoma during hypocapnia. Furthermore, early exploratory analyses have begun on clinical associations with CA parameters.

8.2 Further Work

This work will be continued beyond this MD in the form of further manuscripts designed to draw comparisons between healthy volunteers and ICH patients under hypocapnic conditions, and the relationship between dCA metrics and clinical stroke parameters. Given the finding that the ARI is close to the ARI <4 threshold for impairment and ipsilateral to the haematoma the ARI can be significantly improved, there are exciting avenues for further investigation. The author hopes to transition this clearly defined work stream into an Academic Clinical Lecturer post with a view to gathering evidence to advocate the use of capnography in an acute stroke setting. In particular, assessing manipulation of PaCO₂ in the acute stroke setting including the development of methodologies that can include all ICH stroke severity. The intention is to understand the variation that exists post-stroke, understand correlations with poor prognostic markers and further understand the relationship with CA. The author has recently contributed to a large meta-analysis demonstrating the presence of hypocapnia post-stroke.

Despite the significant advancement in the understanding of the relationship between PaCO₂ and cerebral haemodynamics presented, there remain several interesting avenues for further research. These include the hypocapnic 'dose' conundrum. The author observed variation in the post hypocapnic return of EtCO₂ to baseline. This variation in vasoconstrictive responses and clarification as to whether the benefits of improved ARI can be sustained for an optimal period of hypocapnia are yet to be determined. This research is supported by robust data supporting a lack of cerebral ischaemic risk during sustained hypocapnia.¹⁸¹

Furthermore, following positive feedback at a recent conference presentation on the logistic modelling of peripheral and cerebral haemodynamic parameters, other groups have enquired about the possibility of modelling their preferred 'metrics' in a similar manner. As stated previously, understanding the scatter of the four parameter logistic model in alternative dCA metrics would provide very useful information on metric specific CO₂ vasomotor reactivity and permit comparisons to be drawn.

Lastly, the influence of sex on effects of PaCO₂ on CBFV and dCA is a very important finding that warrants further assessment in older and diseased individuals. This has implications for physiological measurement work within the area of CO₂ vasomotor reactivity particularly at the modest sample sizes that drew significant results within this project.

In conclusion, this thesis represents a considerable step forward in our understanding of the physiological responses to PaCO₂ change in both healthy and clinical stroke populations. Crucially, it has successfully translated a significant wealth of understanding from biochemical, physiological and radiological CO₂ work from the bench to the bed-side in a carefully designed and delivered first of its kind interventional study. The author hopes this forward thinking approach will be applied robustly to other autoregulatory models currently solely examined in healthy populations without consideration of clinical applicability.

9 References

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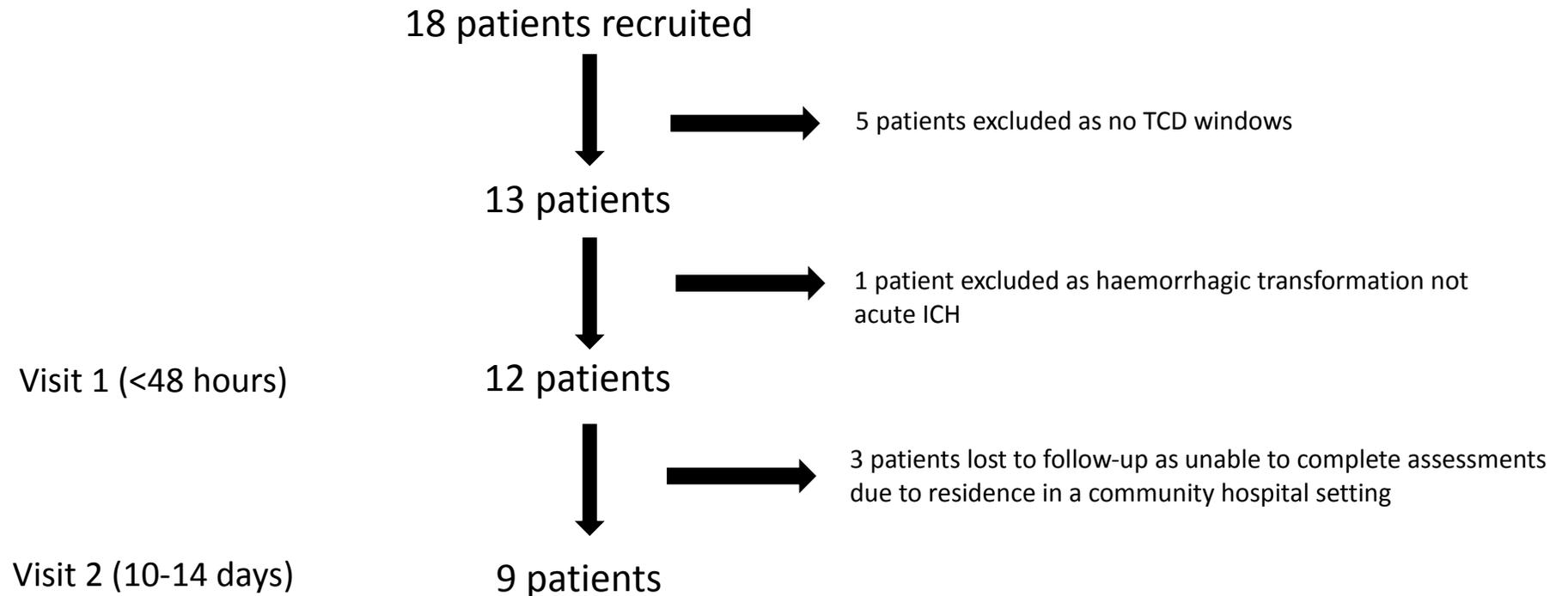
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10 Appendices

10.1 Summary of patient recruitment and inclusion in data analyses

SUMMARY OF PATIENT RECRUITMENT AND SUBSEQUENT INCLUSION IN DATA ANALYSES



10.2 Ethical Approval for the Patient Study



Health Research Authority

Professor Thompson G Robinson
University of Leicester
Cardiovascular Research Centre
Groby Road
LE3 9QP

Email: hra.approval@nhs.net

24 July 2017

Dear Professor Robinson,

Letter of HRA Approval

Study title:	Feasibility of Improving Cerebral Autoregulation in Acute Intracerebral Haemorrhage (BREATHE-ICH)
IRAS project ID:	230925
Protocol number:	0624
REC reference:	17/EM/0283
Sponsor	University of Leicester

I am pleased to confirm that **HRA Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications noted in this letter.

Participation of NHS Organisations in England

The sponsor should now provide a copy of this letter to all participating NHS organisations in England.

Appendix B provides important information for sponsors and participating NHS organisations in England for arranging and confirming capacity and capability. Please read *Appendix B* carefully, in particular the following sections:

- *Participating NHS organisations in England* – this clarifies the types of participating organisations in the study and whether or not all organisations will be undertaking the same activities
- *Confirmation of capacity and capability* - this confirms whether or not each type of participating NHS organisation in England is expected to give formal confirmation of capacity and capability. Where formal confirmation is not expected, the section also provides details on the time limit given to participating organisations to opt out of the study, or request additional time, before their participation is assumed.
- *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* - this provides detail on the form of agreement to be used in the study to confirm capacity and capability, where applicable.

Further information on funding, HR processes, and compliance with HRA criteria and standards is also provided.

10.3 R & D Approval for the Study



UNIVERSITY OF
LEICESTER

25 September 2017

Professor Thompson Robinson
Head of Department and Professor of Stroke Medicine
University of Leicester
Robert Kilpatrick Clinical Sciences Building
LE2 7LX

Research & Enterprise Division
University of Leicester
Research Governance Office
Fielding Johnson Building
University Road
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Admin Tel 0116 373 6410 / 223 1660

Dear Professor Robinson

Ref: UOL 0624 / IRAS project ID: 230925
Title: Feasibility of Improving Cerebral Autoregulation in Acute Intracerebral Haemorrhage (BREATHE-ICH) Study
Status: Approved
End Date: 02/08/2019
Site: University Hospitals of Leicester NHS Trust

I am pleased to advise you that following confirmation of a Favourable Opinion from an Ethics Committee, HRA, NHS Trust R&D Approval, and where relevant regulatory authority agreements have been received, the University are able to confirm sponsorship for the above research at the above site.

I would be grateful if you can forward a copy of this letter to the Principal Investigator for their Site File.

Please note you are required to notify the Sponsor and provide copies of:

- Changes in personnel to the Study
- Changes to the end date
- All substantial amendments and provisional and favourable opinions
- All minor amendments
- All serious adverse events (SAEs) and SUSARS
- Annual progress reports
- Annual MHRA (DSUR) safety reports (if applicable)
- End of study declaration form
- Notifications of significant breaches of Good Clinical Practices (GCP) or Protocol

Please copy the Sponsor into all correspondence and emails by using uolsponsor@le.ac.uk.

Please note it is essential that you notify us as soon as you have recruited your first patient to the study.

I would like to wish you well with your study and if you require further information or guidance please do not hesitate to contact me.

Yours sincerely

Dr Michelle Muessel
Research Governance Manager

10.4 Patient Information Sheet



University Hospitals of Leicester **NHS**
NHS Trust

Leicester Royal Infirmary
Infirmary Square
Leicester
LE1 5WW
Tel: 0300 303 1573 (Ext 7257)
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PARTICIPANT INFORMATION SHEET

Feasibility of Improving Cerebral Autoregulation in Acute Intracerebral Haemorrhage (BREATHE-ICH) Study

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

1. What is the purpose of the study?

Blood flow to the brain has to be carefully controlled, otherwise there is a risk of too much or too little blood reaching the brain, both of which may be associated with risk and damage. The ability of the brain to control its blood supply is called autoregulation and it may range from no control to perfect control. Certain things affect brain blood flow (autoregulation) including changes in breathing rates and movement. Cerebral Autoregulation (CA) is assessed using the Autoregulation Index (ARI) which assigns values between 0 and 9 (0 being poor and 9 being the most efficient CA observed) to gauge how good the control over blood flow is at a given time. Following acute intracerebral haemorrhage (or brain bleeding) autoregulation has been shown to be poor, but research has not shown whether the severity of the bleed is related to the poorest autoregulation values. However, there is some evidence to suggest that poor autoregulation in some stroke types (clot type and bleeding type) is associated with complications (worsening bleeding and swelling), which could lead to an increased risk of disability or even death.

It is important to understand better the relationship between poor autoregulation and outcome, since our preliminary work has recently shown that changes in carbon dioxide using simple breathing exercises can improve autoregulation. This may provide an entirely new and simple treatment, using a breathing exercise, as a potential method of improving the way we control blood flow and importantly prevent complications that cause the poor outcome associated with brain bleeding.

2. Why have I been chosen?

You are being invited to participate in this study as you have had an acute stroke related to bleeding in the brain.

Trust Headquarters, Level 3, Balmoral Building, Leicester Royal Infirmary, Infirmary Square, Leicester, Leicestershire, LE1 5WW
Tel: 03003031573 Fax: 0116 252 5847 Website: www.uhl-tr.nhs.uk
Chairman Mr. Karamjit Singh CBE Chief Executive Mr. John Adler

Participant Information Sheet V2.0 Dated 22 August 2017. IRAS Number: 230925.
Page 1 of 5

3. Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information leaflet to keep and be asked to sign a consent form. You are very welcome to ask questions at any stage of the study. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

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

If you agree to join this study, you will have two study tests on two separate days. The first measurement will happen during the period of time immediately after your stroke for approximately 1 hour. The second will occur at 10-14 days after your stroke and will be either when you are still in hospital or will involve your return should you be at home at the time. This will also last an hour. In order to participate in the study, you will first be required to discuss this information leaflet and sign a consent form. You will then be asked to lie quietly on a bed whilst a small cuff is attached to the fingers of one hand to measure your blood pressure, 3 stickers to your chest to monitor your heart rate, and a small mask over your nose to measure the waste gas from your breathing. You will be asked to wear a head-frame, which will hold the small ultrasound probes against both sides of your head that are used to measure the blood flow to the brain. The head-frame is made of plastic material and able to adjust according to head size (see figure). Both the head-frame and ultrasound probe will exert a slight pressure on the head. However, this is not painful and is used routinely in many medical units to monitor blood flow to the brain.

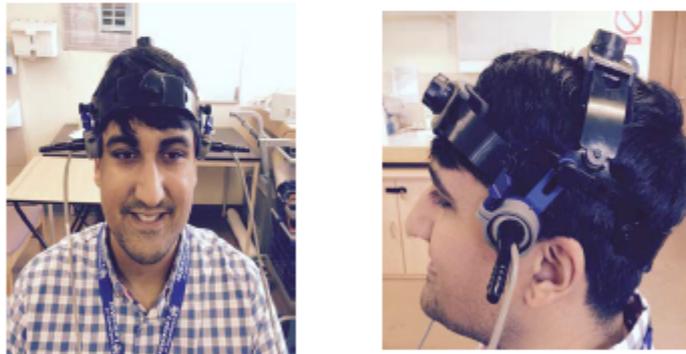


Figure: Head-frame and ultrasound probes

After the readings have stabilised, a recording will be during a period of hyperventilation (breathing faster) that lasts for 2 minutes. This will be followed by another recording when you are breathing normally. We will then repeat this process again.

Overall, the whole assessment will take approximately 1 hour.

For the second assessment (10-14 days later), you will be asked to discuss the progress of your stroke, new problems with your health, medication changes or intervention carried out during this period of time. We will then carry out the same set of measurements as before.

Trust Headquarters, Level 3, Balmoral Building, Leicester Royal Infirmary, Infirmary Square, Leicester, Leicestershire, LE1 5WW
Tel: 03003031573 Fax: 0116 252 5847 Website: www.uhl-tr.nhs.uk
Chairman Mr. Karamjit Singh CBE Chief Executive Mr. John Adler

Participant Information Sheet V2.0 Dated 22 August 2017. IRAS Number: 230925.
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There is a possibility abnormalities may be picked up on routine measurements including blood pressure and heart tracings. In such an instance, your GP will be notified you have participated in the above study and that health problems have been identified that need further tests or treatment (e.g. abnormalities on the heart tracing (ECG)).

5. What treatments will be used?

No specific treatments are given as part of this small study. The measurement of blood flow is designed to assess whether breathing faster is a potential treatment strategy to use in the future in patients with a similar stroke to yours.

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

The blood pressure cuff applies only a gentle pressure to your fingers to enable a blood pressure recording to be made every heartbeat. This may cause a slight tingling in your fingers, but this should not be painful or cause any harm. Indeed, this type of blood pressure monitoring is often used routinely, e.g. in patients under general anaesthetic or in intensive care. The head-frame and ultrasound probes will exert a slight pressure against your head. However, this is not painful, and again is routinely used in many units to monitor blood flow to the brain. Lastly, the mask may make some participants feel claustrophobic, however, as no gas is breathed in (just breathed out), this is usually well tolerated. Furthermore, the hyperventilation may make you feel a little dizzy or cause tingling if prolonged, and hence the short length of time chosen and careful limits on amount of gas expired. Importantly, this protocol was well tolerated amongst many healthy volunteers.

7. What are the possible benefits of taking part?

You should not expect to receive any personal benefit from taking part in this study. The study procedures are not diagnostic, and you will not routinely receive the test results. However, it is hoped that this study will help us all to learn more about the stroke disease and design a new treatment strategy for future patients with a similar stroke as the one you had.

8. What if I withdraw from this research study?

As mentioned previously, if you decide to take part in the study you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive. Furthermore, the research team can also decide to withdraw you from the study if they believe it is in your best interest, e.g. progression of the stroke disease.

If you decide to leave the study, the researchers would like to keep the health information and the data about you that has been collected. This is to help them make sure that the results of the research can be assessed properly. If you do not want them to do this, you must tell them before you join the research study.

In the extremely unfortunate and unusual circumstance that you become more unwell and lose the ability to decide whether you should continue to participate in this research study, the research team would not invite you to continue participate in this research study. However, the research team would still like to keep the health information and the data about you that has been collected so far for the final analysis. If you do not want them to do this, you must also tell them before you join the research study.

Trust Headquarters, Level 3, Balmoral Building, Leicester Royal Infirmary, Infirmary Square, Leicester, Leicestershire, LE1 5WW
Tel: 03003031573 Fax: 0116 252 5847 Website: www.uhl-tr.nhs.uk
Chairman Mr. Karamjit Singh CBE Chief Executive Mr. John Adler



9. Will travel expenses be paid?

Yes, you will not be out of pocket if you decide to take part in this study. Travel costs to and from the hospital for the study will be reimbursed to a maximum of £40 (return journey) and will be payable upon production of a receipt.

10. What if something goes wrong?

It is very unlikely that you will be harmed by taking part in this type of research study. However, if you wish to complain or have any concerns about the way you have been approached or treated in connection with the study, you should contact the Patient Information & Liaison Service, an independent service, by email (pils.complaints.compliments@uhl-tr.nhs.uk) or post (Patient Information Liaison Service, The Firs, c/o Glenfield Hospital, Groby Road, Leicester, LE3 9QP). Freephone: 0808 1788337.

In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence, then you may have grounds for a legal action for compensation against the University of Leicester but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you (if appropriate).

11. Will my taking part be kept confidential?

The blood pressure and blood flow data recorded during the study will be stored on a computer for subsequent analysis. However, you will not be identified by name, and only the researcher will know that the information is related to you. Any information collected during the study will be treated with the usual degree of confidentiality under the Data Protection Act and will not be passed to anyone else without your express permission. Data may be accessed by authorised individuals from the Sponsor, regulatory authorities or host NHS organisation (e.g. UHL) for monitoring and audit purposes. Furthermore, data will be stored on University Computers. Your identity will not be revealed in any publication or presentation of the results from this study. However, with your permission, your own doctor (your GP) will be notified you have participated in the above study and if any health problems are identified that need further tests or treatment (e.g. abnormalities on the heart tracing (ECG)). Anonymised data will be stored in the Leicester Research Archive (LRA) at the end of study, as detailed in clause 6 of the consent form.

12. Who is organising and funding the research?

This research is coordinated by Professor Robinson and Professor Panerai from the University of Leicester. The funding for the study is provided by a Dunhill Medical Trust Research Training Fellowship Grant (RTF97/0117).

13. How will I find out the results of the research?

At the end of the study, you will be sent a written letter, in plain English, with a summary of our study findings and conclusions.

14. Whom can I contact?

For further information or appointments:

Trust Headquarters, Level 3, Balmoral Building, Leicester Royal Infirmary, Infirmary Square, Leicester, Leicestershire, LE1 5WW
Tel: 03003031573 Fax: 0116 252 5847 Website: www.uhl-tr.nhs.uk
Chairman Mr. Karamjit Singh CBE Chief Executive Mr. John Adler



If you have any concerns or other questions about the study, or the way it has been carried out, you should contact the principal research investigator on 0116 252 3182 or any of the following people:

Name: Dr Jatinder S. Minhas

Role: Clinical Research Fellow of Stroke Medicine

Telephone: 0116 252 5841

Email: jm591@le.ac.uk

For complaints:

If you have any complaints about any aspect of the project, the way it is being conducted or any questions about being a research participant in general, then you may contact an independent complaints body:

Patient Information and Liaison Service (PILS)

Address: The Firs, C/O Glenfield Hospital, Groby Road, Leicester, LE3 9QP, UK

Free phone line: 08081 788 337

Facsimile: 0116 258 8661

Email: pils@uhl-tr.nhs.uk

Once again, thank you for taking the time to read this information sheet and for considering taking part in this study.

10.5 Ethical Approval for Healthy Volunteer Study



University of Leicester Ethics Review Sign Off Document

To: **Dr Jatinder Minhas**

Subject: Ethical Application Ref: **jm591-c033**

(Please quote this ref on all correspondence)

07/05/2015 16:16:10

Cardiovascular Sciences

Project Title: **Defining a 'Dose-Response'™ Curve for the influence of PaCO₂ on Dynamic Cerebral Autoregulation**

Thank you for submitting your application which has been considered.

This study has been given ethical approval, subject to any conditions quoted in the attached notes.

Any significant departure from the programme of research as outlined in the application for research ethics approval (such as changes in methodological approach, large delays in commencement of research, additional forms of data collection or major expansions in sample size) must be reported to your Departmental Research Ethics Officer.

Approval is given on the understanding that the University Research Ethics Code of Practice and other research ethics guidelines and protocols will be compiled with

- <http://www2.le.ac.uk/institution/committees/research-ethics/code-of-practice>
- <http://www.le.ac.uk/safety/>

The following is a record of correspondence notes from your application jm591-c033. Please ensure that any proviso notes have been adhered to:-

May 7 2015 4:16PM Happy to approve. The picture in the patient information sheet is particularly useful

Prof DG Lambert / Dr J McDonald

--- END OF NOTES ---

PARTICIPANT INFORMATION LEAFLET

Regulation of brain blood flow in response to carbon dioxide changes in people of different ages

Thank you for taking time to consider participation in this research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

This is a small research study, which will involve a 60-70 minute measurement period of your blood pressure and blood vessels following some breathing exercises.

1. What is the purpose of the study?

The brain requires a constant supply of blood in order to maintain its function. Blood is under pressure in the arteries (tubes that take blood from the heart to the body) so that it can reach all parts of the body. When blood pressure drops, small arteries widen to bring back flow levels, and when pressure rises, they tighten to protect the most delicate blood vessels and avoid bleeding and swelling in the brain. Failure of this control system (called cerebral autoregulation) following injury to the brain can worsen health, and also influence how to control the changes happening in blood pressure. This project aims to assess the impact of changes in carbon dioxide on cerebral autoregulation. This will enable a deeper understanding of the complex relationship between blood pressure, carbon dioxide levels and blood flow in healthy individuals as well as in patients following stroke (a "brain attack" that occurs when a blood clot blocks an artery). Using advanced data analysis methods we aim to estimate the damages that happen to this "control system" which keeps the blood flow and blood pressure controlled, with the hope to better understand how carbon dioxide changes could influence the management of an individual patient's blood pressure in the acute (critical) and chronic (long term) phases following stroke. Furthermore, by conducting this study on participants of different ages, we will gain information as to whether a relationship exists with the ageing process.

2. Why am I eligible?

You are a healthy volunteer between the age of 20 and 69 years of age.

3. Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

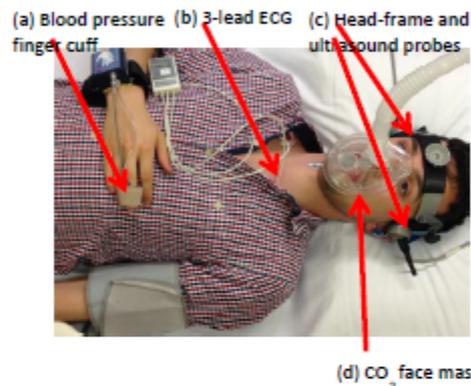
4. What if I withdraw from the study?

If you withdraw, no data will be retained.

5. What will happen to me if I take part?

If you agree to join this study, you will undertake one assessment lasting up to 70 minutes.

The assessment methodology described below on the 'representative subject', has been performed by our group in more than 150 individuals without any complications. For the assessment, you will be asked to lie



quietly on the bed whilst a small cuff is attached to the fingers of one hand to measure your blood pressure (photograph [a]), 3 stickers to your chest to monitor your heart rate (photograph [b]), and small tubes placed at the base of your nose to monitor your breathing. You will be asked to wear a head-frame, which will hold the small ultrasound probes that are used to measure blood flow to the brain against both sides of your head (photograph [c]). After about 15 minutes the readings will have stabilised, and we will make recordings for 15 minutes: at rest (5 minutes), when breathing a slight higher but safe concentration of waste gas (5% carbon dioxide) in addition to oxygen via a mask placed over your mouth and nose (5 minutes) (photograph [d]), and during a further period with a further safe level of waste gas (8% carbon dioxide) via the same method (5 minutes). A five minute rest will then occur prior to measurements involving breathing exercises (hyperventilation): will make recordings for 15 minutes: at rest (5 minutes), when increasing your breathing rate higher to alter your carbon dioxide (5 minutes), and then further increasing your breathing rate to lower the level of carbon dioxide (5 minutes). We will repeat each of the breathing related recordings twice and will require you to breathe faster (hyperventilate) for 90 seconds ideally during each episode.

6. Is there a follow up part to this study?

No.

7. What treatments will be used?

None.

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

The blood pressure cuff applies only a gentle pressure to your fingers to enable a blood pressure recording to be made every heart beat. This may cause a slight tingling in your fingers, but this should not be painful or cause any harm. Indeed, this type of blood pressure monitoring is often used routinely, e.g. in patients under general anaesthetic or in intensive care.

The head-frame (which is used to hold the ultrasound probes in place), and ultrasound probes will exert a slight pressure against your head. However, this is not painful, and again is routinely used in many units to monitor blood flow to the brain.

For the manoeuvre to breathe in 5% and 8% carbon dioxide, a face mask will be placed covering your nose and mouth, some people find this uncomfortable, but there are no side effects of the carbon dioxide as the concentration being used for the purpose of this study is low.

Breathing exercises using hyperventilation may make you feel a little light headed, if this occurs please notify the researcher present.

9. What are the possible benefits of taking part?

You should not expect to receive any personal benefit from taking part in this study.

10. What if something goes wrong?

We do not expect anything to go wrong, should anything occur we have protocols by which you can raise this with the department.

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

The blood pressure and blood flow data recorded during the study will be stored on computer for subsequent analysis. However, you will not be identified by name, and only the investigator will know that the information is related to you. Any information collected during the study will be treated with the usual degree of confidentiality under the data protection act. Your identity will not be revealed in any publication or presentation of the results from this study.

12. How will I be informed of the results of this research study?

It is anticipated that the results from this study will be provided in a final study report. Please ask if you are interested in any aspect of this report.

The results will also be published in scientific journals. Because the results need to be analysed it may take some time for publication of results to occur.

13. Who is organizing and funding the research?

This research is coordinated by Professors Robinson and Panerai from the University of Leicester.

14. What if I have any concerns?

If you have any concerns or other questions about this study or the way it has been carried out, you should contact the investigator (Prof Tom Robinson, Telephone Number 0116 252 3182).

15. What if I want further information from an independent clinician?.

You may contact Dr Amit Mistri (a Stroke Consultant unrelated to this research study). Email: jennifer.kerry@uhl-tr.nhs.uk Telephone: 0116 252 3182.

Once again, thank you for taking the time to read this information sheet and for considering taking part in this study.

10.7 Patient Consent Form



University Hospitals of Leicester **NHS**
NHS Trust

Leicester Royal Infirmary
Infirmary Square
Leicester, LE1 5WW
Tel: 0300 303 1573 (Ext 7257)
Fax: 0116 252 5847

PARTICIPANT CONSENT FORM

Feasibility of Improving Cerebral Autoregulation in Acute Intracerebral Haemorrhage (BREATHE-ICH) Study

Researcher Name: Dr _____

Please initial

Patient Study ID Number: _____

1. I confirm that I have read and understood the Participant Information Sheet (V2.0, Dated 22nd August, 2017) for the above study, and have had the opportunity to ask questions and have had these answered satisfactorily.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving reason, without my medical care or legal rights being affected.

3. I understand that if I decide to withdraw prior to the completion of the study, the research team would like to keep all the health information and data that has been collected so far for the final analysis and I am granting permission for this. This includes active withdrawal or no longer being able to give consent for study.

4. I understand that my GP will be informed about my participation in this study, and by signing this consent form I am granting permission for this. Furthermore, I understand my GP will be informed about any abnormalities that arise (e.g ECG).

5. I understand that in the unusual circumstance that I became unwell and lose the ability to decide whether I should continue to participate in the research study, the research team would withdraw me from the study. However, the research team would like to keep all the health information and data that has been collected so far for the final analysis and I am granting permission for this.

6. I understand that relevant sections of my medical notes and/or data collected during the study, may be looked at by responsible individuals from the study team, the sponsor, NHS Trust or from regulatory authorities, where it is relevant to taking part in this research. I give permission for these individuals to have access to my records. Furthermore, I give permission for anonymised data to be stored in the Leicester Research Archive (LRA) at the end of study.

7. I agree to take part in the above study.

Participant Name: _____ Date: _____ Signature: _____

Researcher: _____ Date: _____ Signature: _____

(File: 1 for patient, 1 for researcher, 1 for hospital notes)
Version 2.0, 22nd August 2017

Trust Headquarters, Level 3, Balmoral Building, Leicester Royal Infirmary, Infirmary Square, Leicester, Leicestershire, LE1 5WW

Tel: 03003031573 Fax: 0116 252 5847 Website: www.uhl-tr.nhs.uk

Chairman Mr. Karamjit Singh CBE Chief Executive Mr. John Adler

Participant Consent Form V2.0 Dated 22nd August 2017. IRAS Number: 230925.

Page 1 of 1

10.8 Healthy Volunteer Consent Form



VOLUNTEER CONSENT FORM

Regulation of brain blood flow in response to carbon dioxide changes in people of different ages

Researcher Name: Dr

I confirm that I have read and understand the volunteer information sheet version 1.0 dated 22 April 2015 for the above study, and had the study fully explained to me, by the above named, that the risks, and benefits and treatments have been discussed, explained in detail, and all my questions have been satisfactorily answered.

Please Initial

I understand that my participation is voluntary and that I am free to withdraw at any time, without giving reason, without my medical care or legal rights being affected.

I am aware that records may be examined by sponsor, regulatory and ethical authorities. I am also aware that should I wish, my GP can be informed of involvement in the study.

I agree to take part in the above study.

Volunteer Name

Date

Signature

Researcher

Date

Signature

Version 1.0, 22/04/15

10.9 GP Information Sheet



University Hospitals of Leicester **NHS**
NHS Trust

Leicester Royal Infirmary
Infirmary Square
Leicester
LE1 5WW
Tel: 0300 303 1573 (Ext 7257)
Fax: 0116 252 5847

GP INFORMATION LETTER

Feasibility of Improving Cerebral Autoregulation in Acute Intracerebral Haemorrhage (BREATHE-ICH) Study

Dear Dr GP

Re:
Name
DOB:
Address:

Your patient named above has been recruited to the above study, which is being carried out by researchers at the University of Leicester. The study involves repeated hyperventilation manoeuvres (90 seconds) designed to improve impaired cerebral autoregulation seen post-acute intracerebral haemorrhage. Careful physiological measurement including beat to beat blood pressure and heart rate monitoring alongside measurement of cerebral blood flow velocity using Transcranial Doppler will be conducted.

As part of this study, your patient will be required to attend 1 (one) follow-up assessment at 10-14 days post initial acute intracerebral haemorrhage. They have been counselled regarding this. Any abnormalities detected (e.g. ECG or Blood Pressure), will be communicated to you.

Participation in the above study is entirely voluntary.

Please find enclosed a copy of the Participant Information Sheet which provides further information on the study. If you have any further questions regarding this trial, please contact us on the details below.

Yours Faithfully,

Dr Jatinder S. Minhas
Honorary Clinical Research Fellow/Honorary Specialist Registrar in Stroke Medicine
Telephone +44 116 252 3134 / Email jm591@le.ac.uk

On behalf of Professor T G Robinson BMedSci, MBBS, MD, FRCP, FESO
Professor of Stroke Medicine/Honorary Consultant Physician

Trust Headquarters, Level 3, Balmoral Building, Leicester Royal Infirmary, Infirmary Square, Leicester, Leicestershire, LE1 5WW
Tel: 03003031573 Fax: 0116 252 5847 Website: www.uhl-tr.nhs.uk
Chairman Mr. Karamjit Singh CBE Chief Executive Mr. John Adler
GP Information Letter V1.0 Dated 29 June 2017. IRAS Number: 230925.
Page 1 of 1

BREATHE-ICH



Feasibility of Improving Cerebral Autoregulation
in Acute Intracerebral Haemorrhage
(BREATHE-ICH) Study

Please notify the study investigator if a patient
under your care has:

**Acute haemorrhagic stroke, within 48 hours of
onset, able to comply with a respiratory
manoeuvre (hyperventilation)**

Study Investigators: Dr Jatinder S. Minhas (Stroke Clinical Research Fellow),
Professor Thompson G. Robinson (Professor of Stroke Medicine)
Contact: 0116 252 5481 / Jatinder via Switchboard (24 hour contact)

**This study is designed to assess the feasibility of a simple bed-side respiratory manoeuvre
in acute haemorrhage patients assessed using non-invasive ultrasound of the brain**



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LEICESTER

University Hospitals of Leicester **NHS**
NHS Trust

Research Participant Poster V2.0 Dated 27 Sept 2017. IRAS Number: 230925.

10.11 National Institutes of Health Stroke Scale

Category	Score/Description	Date/Time	Date/Time	Date/Time	Date/Time	Date/Time
		Initials	Initials	Initials	Initials	Initials
1a. Level of Consciousness (Alert, drowsy, etc.)	0 = Alert 1 = Drowsy 2 = Stuporous 3 = Coma					
1b. LOC Questions (Month, age)	0 = Answers both correctly 1 = Answers one correctly 2 = Incorrect					
1c. LOC Commands (Open/close eyes, make fist/let go)	0 = Obeys both correctly 1 = Obeys one correctly 2 = Incorrect					
2. Best Gaze (Eyes open - patient follows examiner's finger or face)	0 = Normal 1 = Partial gaze palsy 2 = Forced deviation					
3. Visual Fields (Introduce visual stimulus/threat to pt's visual field quadrants)	0 = No visual loss 1 = Partial Hemianopia 2 = Complete Hemianopia 3 = Bilateral Hemianopia (Blind)					
4. Facial Paresis (Show teeth, raise eyebrows and squeeze eyes shut)	0 = Normal 1 = Minor 2 = Partial 3 = Complete					
5a. Motor Arm - Left 5b. Motor Arm - Right (Elevate arm to 90° if patient is sitting, 45° if supine)	0 = No drift 1 = Drift 2 = Can't resist gravity 3 = No effort against gravity 4 = No movement X = Untestable (Joint fusion or limb amp)	Left				
		Right				
6a. Motor Leg - Left 6b. Motor Leg - Right (Elevate leg 30° with patient supine)	0 = No drift 1 = Drift 2 = Can't resist gravity 3 = No effort against gravity 4 = No movement X = Untestable (Joint fusion or limb amp)	Left				
		Right				
7. Limb Ataxia (Finger-nose, heel down shin)	0 = No ataxia 1 = Present in one limb 2 = Present in two limbs					
8. Sensory (Pin prick to face, arm, trunk, and leg - compare side to side)	0 = Normal 1 = Partial loss 2 = Severe loss					
9. Best Language (Name item, describe a picture and read sentences)	0 = No aphasia 1 = Mild to moderate aphasia 2 = Severe aphasia 3 = Mute					
10. Dysarthria (Evaluate speech clarity by patient repeating listed words)	0 = Normal articulation 1 = Mild to moderate slurring of words 2 = Near to unintelligible or worse X = Intubated or other physical barrier					
11. Extinction and Inattention (Use information from prior testing to identify neglect or double simultaneous stimuli testing)	0 = No neglect 1 = Partial neglect 2 = Complete neglect					
TOTAL SCORE						