Physiological Measurement

Modelling the cerebral haemodynamic response in the physiological range of $PaCO_2$

To cite this article before publication: Jatinder Singh Minhas *et al* 2018 *Physiol. Meas.* in press https://doi.org/10.1088/1361-6579/aac76b

Modelling the cerebral haemodynamic response in the physiological range of $PaCO_2$

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Running Title: Cerebral haemodynamics in the physiological range of $PaCO_2$

Keywords: blood pressure, carbon dioxide, haemodynamics

Abbreviations list

ARI	Autoregulation index
BP	Blood pressure
CA	Cerebral autoregulation
CBFV	Cerebral blood flow velocity
CO_2	Carbon dioxide
CrCP	Critical closing pressure
CVMR	Cerebral vasomotor reactivity
dCA	Dynamic cerebral autoregulation
ECG	Electrocardiogram
$EtCO_2$	End-tidal CO_2
HR	Heart rate
MABP	Mean arterial blood pressure
MCA	Middle cerebral artery
$PaCO_2$	Partial pressure carbon dioxide
RAP	Resistance-area product
SD	Standard deviation
TCD	Transcranial Doppler
TFA	Transfer function analysis

Abstract

Objective

Arterial CO_2 ($PaCO_2$) has a strong effect on cerebral blood flow (CBF), but its influence on CBF regulatory mechanisms and circulatory systemic variables has not been fully described over the entire physiological range of $PaCO_2$.

Approach

CBF velocity (CBFV, transcranial Doppler), blood pressure (BP, Finometer) and end-tidal CO_2 ($EtCO_2$, capnography) were measured in 45 healthy volunteers (19 male, mean age 37.5 years, range 21-71) at baseline, and in response to hypo- (-5mm Hg and -10mm Hg below baseline) and hypercapnia (5% and 8% CO_2), applied in random order.

Main Results

CBFV, cerebral dynamic autoregulation index (ARI), heart rate (HR), arterial blood pressure (ABP), critical closing pressure (CrCP) and resistance-area product (RAP) changed significantly (all $p<0.0001$) for hypo- and hyper-capnia. These parameters were shown to follow a logistic curve relationship representing a 'dose-response' curve for the effects of $PaCO_2$ on the cerebral and systemic circulations. The four logistic model parameters describing each 'dose-response' curve were specific to each of the modelled variables (ANOVA $p<0.0001$).

Significance

The ability to model the CBFV, ARI, HR, ABP, CrCP and RAP dependency of $PaCO_2$ over its entire physiological range is a powerful tool for physiological and clinical studies, including the need to perform adjustments in disease populations with differing values of baseline $PaCO_2$.

Introduction

Cerebral autoregulation (CA) is usually defined as the tendency of cerebral blood flow (CBF) to remain approximately constant despite changes in blood pressure (BP) within the range 50 to 170 mmHg (Lassen 1959, Paulson *et alet al* 1990). However, outside these limits, CA becomes passive and CBF follows changes in response to BP. Importantly, this classical relationship, usually referred to as 'static' CA, has been challenged and ultimately the physiological properties of CA remain largely inconclusive (Willie *et alet al* 2014, Tzeng, Ainslie 2014, Tymko, Ainslie 2017). Dynamic CA (dCA) can be estimated from the transient response of CBF to rapid changes in BP (Aaslid *et alet al* 1989) and this has been the preferred approach for the assessment of CA in human physiological and clinical studies (Reivick 1964, Ogoh *et alet al* 2010, Battisti-Charbonney *et alet al* 2011). Understanding the dCA response to physiological manoeuvres, such as exercise and changes in respiratory patterns, has often been confounded by simultaneous changes in the arterial partial pressure of carbon dioxide ($PaCO_2$) (Ogoh *et al* 2008, Dineen *et al* 2010). Hypercapnia leads to vasodilation of cerebral vessels (Markwalder *et al* 1984) and overall causes deterioration in CA (Aaslid *et al* 1989). Conversely, hypocapnia has a vasoconstrictive effect, improving CA (Aaslid *et al* 1989, Ogoh *et al* 2008, Ainslie *et al* 2008, Dineen *et al* 2010). Indeed, experimental work has suggested hypercapnia can be used to emulate a state of impaired dCA (Maggio *et al* 2013). Although these effects of $PaCO_2$ changes on CBF and dCA are widely accepted qualitatively, there is a need for a comprehensive quantitative model covering the entire physiological range of $PaCO_2$, 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 $PaCO_2$ induced by transient breath-by-breath adjustment demonstrates non-linear effects on CBF (Poulin *et al* 1996, Ide *et al* 2003, Claassen *et al* 2007, Ainslie *et al* 2008, Duffin *et al* 2017). Amongst different potential non-linear models, the logistic function has been shown to provide a realistic description of the cerebral blood flow velocity (CBFV), responding to changes in $PaCO_2$ (Claassen *et al* 2007). Logistic models have also proved successful to describe the effects of $PaCO_2$ on cerebrovascular conductance index (CVCi) (Claassen *et al* 2007) and cerebrovascular resistance (Duffin *et al* 2017).

Current understanding of vascular physiology principles has led to models adopting the principle that vascular bed resistance-CO_2 response relationships are sigmoidal (Duffin *et al* 2017). 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 CO_2 responsiveness.

We used a wide range of $PaCO_2$ within a multi-step protocol to test for the first time the hypotheses that i) a commonly used index of dCA, the autoregulation index (ARI) shows a dependence on $PaCO_2$ following a logistic non-linear model, similar to that described for CBFV; and ii) key cerebral haemodynamic parameters including ABP, heart rate (HR), critical closing pressure (CrCP) and resistance-area product (RAP) can also have their dependence on $PaCO_2$ described by a logistic non-linear model.

Methods

Subjects and measurements

The study was conducted in accordance with the Declaration of Helsinki (2000). 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). $EtCO_2$ 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 $EtCO_2$ was measured via nasal prongs (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.

Experimental protocol

All measurements were conducted at a single visit. Prior randomization of the order of hypo- and hypercapnia was conducted using a random number generator. An initial period of 15 minutes of stabilization preceded a 5-minute baseline recording supine at rest. This was followed by inspiring CO_2 in air, constantly ('fixed inspiration') for a minimum of 90 s (ideally 120 s) with either 5% CO_2 or 8% CO_2 in air (dependent on randomization). 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 stabilization, 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 $EtCO_2$ of 5mmHg and 10mmHg less than normocapnia for that individual. Hyperventilation was sustained for a minimum period of 90 s, or a 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 $EtCO_2$ 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.

Data Analysis

Data collected corresponded to six individual files for each participant: 2 at baseline, 2 hypercapnic and 2 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 used, but also visual inspection was undertaken with and manual correction whenever necessary. This allowed mean ABP, HR, $EtCO_2$ and mean CBFV to be calculated for each cardiac cycle. CrCP and RAP were estimated using the first harmonic method (Panerai 2003). Randomization was not disclosed until data collection was completed.

Given the non-stationary influence of $PaCO_2$ on dCA, ARI, proposed by Tiecks *et al* (Tiecks *et al* 1995) was calculated as a function of time (ARIt), 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* (1995) as described previously (Dineen *et al* 2010). In short, with V(t) representing beat-to-beat changes in CBFV and dP(t) corresponding changes in BP, normalised by CrCP (Tiecks *et al* 1995), the two quantities are linked by:

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

where *K* represents a gain parameter in the second order equation, and $x_2(t)$ is a state variable obtained from the following state equation system representing a second-order equation:

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

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

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 (Tiecks *et al* 1995).

From these equations, it is possible to demonstrate (Dineen *et al* 2010) that eq. 1 can be expressed as a discrete ARMA model, that is:

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

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* (Tiecks *et al* 1995). The complete time-series of ARIt values for each recording was obtained by moving the time-window at 0.6s intervals. Values of ARIt=0 represent absence of autoregulation, whilst ARIt=9 corresponds to the most efficient CA that can be observed. The ARIt time-series was computed for each subject separately for left and right hemispheres for each recording.

Logistic Model

Following the logistic model for the effects of CO_2 on CBFV (Claassen *et al* 2007), a similar model was adopted, and was also extended to test the feasibility of using the logistic relationship to express the influence of CO_2 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 either mean CBFV, ABP, HR, CrCP, RAP or ARIt. x is the $EtCO_2$ level and k is the exponential coefficient. Fitting the model allows estimation of its four parameters, namely y_{max}, y_{min}, k and x_0 (Figure 1).

Model parameters were obtained by combining least squares with a recursive bootstrap technique to remove outliers. After estimation of ARIt for each of the six different recordings (two each, for baseline, hyperventilation, and CO_2 breathing), each recording was divided into four segments and the mean CBFV, ABP, HR, CrCP, RAP, ARIt and $EtCO_2$ of each segment was used to fit equation 1 using 24 sample values (4 segments for 6 recordings) to express the effects of $PaCO_2$ on these six different parameters. After each stage of error minimization by least squares, the largest outlier was identified and the process repeated for a maximum of eight 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 eight or less. The advantages and limitations of this new approach will be discussed later.

Statistical Analysis

The study protocol was tested for differences in each level of CO_2 using one-way ANOVA. 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. Repeated measures ANOVA was used to assess for differences between model parameter values for each haemodynamic parameter group.

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 1.

Effect of hypo- and hypercapnia on cerebral haemodynamics

Highly significant differences in $EtCO_2$ resulted from breathing CO_2 in air and hyperventilation (ANOVA $p<0.0001$) leading to $EtCO_2$ values of 46.5 (3.7) mmHg (8% CO_2), 42.7 (3.5) mmHg (5% CO_2), 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 2, showing the temporal patterns of changes in ABP, CBFV and $EtCO_2$ observed during normo-, hypo- and hypercapnia. For the same subject, Figure 3 shows the corresponding fitting of the data to the logistic model for both CBFV and ARI. Similar models were obtained for ABP, HR, CrCP and RAP.

Population values of model parameters are given in Table 2, with corresponding population average logistic curves represented in Figure 4. In all cases the dependence on $EtCO_2$ reflects the expected physiological effects of $PaCO_2$ on each of the parameters modelled as will be discussed below.

The model parameters k and x_0 were different when assessed for between group differences for all haemodynamic parameters. The model parameter k was different between haemodynamic parameter groups ($p<0.0001$) with HR demonstrating the largest value (SD) of 1.00mmHg^{-1} (0.8). The model parameter x_0 was different between haemodynamic parameter groups ($p=0.004$) with CrCP demonstrating the largest value (SD) of 38.4mmHg (4.3). There were no outliers removed from CBFV, ARI, CrCP or RAP analyses. Eight outliers or less were removed for ABP (Median 6, IQR 4-7) and HR (Median 6, IQR 4-7).

Discussion

Main findings

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

Effects of carbon dioxide on cerebral autoregulation

For many pharmacological agents, regression of the stimulus on organ response is non-linear (Kenakin 1997). The dependence of CBF, usually estimated from non-invasive measurements of CBFV with TCD ultrasonography, on $PaCO_2$ has been previously quantified by exponential (Markwalder *et al* 1984) or logistic curves, using $EtCO_2$ as the independent variable (Claassen *et al* 2007). The demonstration that ARI, a widely used index of dCA, decreases as $EtCO_2$ 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 2 and 3) 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 $PaCO_2$ on CBF or CBFV, reporting non-linear relationships including sigmoidal curves as in our case (Claassen *et al* 2007, Battisti-Charbonney *et al* 2011). On the other hand, Ainslie *et al* (Ainslie *et al* 2008) reported on the effects of $PaCO_2$ on TFA measures of dynamic CO_2 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 $EtCO_2$ as we found for ARI. One possible explanation is the reduced sensitivity of using separate measures of gain and phase, and the fact that the ARI incorporates all the information obtained with TFA thus providing a more robust measure of dCA (Claassen *et al* 2016). Although other studies have described changes in dCA with different levels of $PaCO_2$, 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 $EtCO_2$ (Aaslid *et al* 1989, Panerai *et al* 1999, Dineen *et al* 2010, Ogoh *et al* 2010, Maggio *et al* 2013).

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 $PaCO_2$ (Willie *et al* 2012, Battisti-Charbonney, Fisher & Duffin 2011). For example in a healthy control population, Ogoh *et al* (Ogoh *et al* 2010) demonstrated that hypoxia disrupts dCA, but hypocapnia augments the dCA response. Furthermore, in our own previous work in an acute ischemic stroke population (Salinet *et al* 2015) 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 $PaCO_2$, including both hypo- and hyper-capnia that could be used to establish comparisons between individuals with different levels of $PaCO_2$.

Currently, there are no studies that have demonstrated $PaCO_2$ stimulus-response curves for an extended set of variables like CBFV, ARI, ABP, HR, CrCP, RAP, ARIt. Other associated work has demonstrated a sigmoidal relationship between $PaCO_2$ and vascular resistance using BOLD as a surrogate for CBF as well as speed of response to hypercapnic stimulus (Poublanc *et al* 2015, Duffin *et al* 2017). The 'model branch pressure' reported by Duffin *et al* (Duffin *et al* 2017) also has the potential to be represented by a logistic model as demonstrated within this study. With reference to RAP and CrCP, previous work (Panerai 2003, Ainslie *et al* 2008, Grune *et al* 2015) has shown RAP and CrCP decrease with $PaCO_2$ with this particular study providing no data on associated HR changes though highlighting a relatively static ABP (Grune *et al* 2015). Prior work has shown that RAP increases significantly with hypocapnia with similar findings as in our study population (McCulloch, Turner 2009).

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 3). On the other hand, the increase in BP with $EtCO_2$ has been explained by the increased sympathetic activity induced by hypercapnia

(Claassen *et al* 2007, Ainslie *et al* 2008, Grune *et al* 2015). The small reduction in HR across the range of $EtCO_2$ values represented in Figure 3 though, might be more controversial. Ainslie *et al* (Ainslie *et al* 2008) reported HR following a U-shaped curve when $EtCO_2$ 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 $PaCO_2$ on heart rate.

We provide a novel evolution from original logistic relationship studies (Markwalder *et al* 1984, Claassen *et al* 2007). This study provides a wider range of $PaCO_2$ and more participants than Markwalder *et al* (Markwalder *et al* 1984) originally used for corrective velocity experiments on $PaCO_2$ values in 31 individuals and the Claassen *et al* (Claassen *et al* 2007) study demonstrating modified logistic function of CBFV to transient changes in CO_2 in 10 subjects.

Clinical perspectives

An important outcome from this study is the potential to improve comparability of dCA estimates for different patients with different $PaCO_2$ readings. The clinical necessity of this previous limitation was demonstrated by Salinet *et al* (2015) who examined the effects of cerebral ischemia on neurovascular coupling. They found no difference between groups (patients vs. controls p=0.07). They noted $PaCO_2$ levels were lower in the stroke population, and concluded that if both groups were normalized to the same $PaCO_2$, 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 $EtCO_2$ and dCA. Our study also provides an example of how it would be possible to progress towards CO_2-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 CO_2 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 (Minhas *et al* 2016). However, previous studies in stroke patient populations (Salinet *et al* 2015) have shown less extreme variation in $EtCO_2$ values and hence

ARI (i.e. a trend towards hypocapnia). Instead of simply comparing values of ARI at single operating points, determined by stable $PaCO_2$ values, the approach we are proposing, of comparing the entire ARI curve as a function of $EtCO_2$ (Figure 3) might provide a much more robust and general approach.

Limitations of the study

Several potential limitations must be considered in this study. 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 (Coverdale *et al* 2014, Verbree *et al* 2014). 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 $PaCO_2$ in altering pressure-flow dynamics and further studies are needed to assess this (Smirl *et al* 2014). Secondly, based on previous studies (Ogoh *et al* 2010, Panerai *et al* 1999) we have used a maximum of 8% CO_2 in air. Higher levels of CO_2 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 2 & 3) can be reduced even further. Informed by previous work (Ainslie *et al* 2008), 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% CO_2 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 $PaCO_2$ 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 at both extremes of $PaCO_2$. Moreover, the fact that each curve is defined by four parameters (eq. 1) 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. 1). Finally, the relatively low values of MSE obtained in each case (Table 1), also demonstrate the appropriateness of using logistic curve models to describe the effects of $PaCO_2$ 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 $EtCO_2$ 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 (Dineen *et al* 2010). 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 (6x4=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 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 (Panerai 2014).

Importantly, although we have elected to use the ARI index to describe the dependence of dCA on $PaCO_2$, 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 $PaCO_2$. Mx is a mean index, based on continuous assessment of slow and spontaneous fluctuations of CBFV and cerebral perfusion pressure offering information on cerebral pressure reactivity (Czosnyka *et al* 1996). With different indices though, it is likely that the scatter of the four parameters describing the logistic model (Table 3) 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.

Conclusions

Expressing the influence of $PaCO_2$ on CBF and its regulatory mechanisms, with a logistic curve reflecting the dependence of ARI as a function of $EtCO_2$, represents a new paradigm for the simultaneous assessment of dCA and CO_2 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.

Competing interests

No competing interests.

Acknowledgements

JSM is a Dunhill Medical Trust Clinical Research Training Fellow (RTF97/0117) at the Department of Cardiovascular Sciences, University of Leicester. TGR is an NIHR Senior Investigator. We thank the subjects for their willingness to participate. This work falls under the portfolio of research conducted within the NIHR Leicester Biomedical Research Centre.

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Tables

Table 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; $EtCO_2$, 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.

Table 2. Population distribution values of logistic model parameters for CBFV, ARI, HR, ABP, CrCP and RAP as a function of $EtCO_2$.

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; $EtCO_{2ma}$x, 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; x_0 coefficient: $EtCO_2$ 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 Legends

Figure 1. Schematic representation of the four parameter logistic function demonstrating the shape and relationship between parameters. y can represent either mean CBFV, ABP, HR, CrCP, RAP or ARIt; x is the $EtCO_2$ level and k is the exponential gain coefficient. Fitting the model allows estimation of its four parameters, namely y_{max}, y_{min}, k and x0.

Figure 2. Representative recordings from a 21-year-old female study participant.
A. Normocapnia, B. Hypercapnia (8% CO_2), C. Hypocapnia (-10mmHg from baseline). Dotted vertical lines represent onset of respective manoeuvres.

Figure 3. Logistic model fitting for same subject as in Fig. 1 for (A) CBFV and (B) ARI as a function of $EtCO_2$.

Figure 4. Population average logistic model curves for the dependence of (a) CBFV and ARI, (b) HR, ABP, CrCP and RAP. Corresponding shaded areas represent the ±1 SEM boundaries.