

Regional brain blood flow in man during acute changes in arterial blood gases

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Key points

- The partial pressures of arterial carbon dioxide (P_{aCO_2}) and oxygen (P_{aO_2}) has a marked influence on brain blood flow.
- It is unclear if the larger brain arteries are also sensitive to changing P_{aCO_2} and P_{aO_2} and if different areas of the brain possess different sensitivities.
- We separately altered P_{aCO_2} and P_{aO_2} and measured the diameter and blood flow in the main arteries delivering blood to the cortex and brainstem.
- During alterations in P_{aCO_2} and P_{aO_2} , the large arteries changed diameter and blood flow to the brainstem changed more than that to the cortex.
- These findings change the basis of our understanding of brain blood flow control in humans.

Abstract Despite the importance of blood flow on brainstem control of respiratory and autonomic function, little is known about regional cerebral blood flow (CBF) during changes in arterial blood gases. We quantified: (1) anterior and posterior CBF and reactivity through a wide range of steady-state changes in the partial pressures of CO_2 (P_{aCO_2}) and O_2 (P_{aO_2}) in arterial blood, and (2) determined if the internal carotid artery (ICA) and vertebral artery (VA) change diameter through the same range. We used near-concurrent vascular ultrasound measures of flow through the ICA and VA, and blood velocity in their downstream arteries (the middle (MCA) and posterior (PCA) cerebral arteries). Part A ($n = 16$) examined iso-oxic changes in P_{aCO_2} , consisting of three hypocapnic stages ($P_{\text{aCO}_2} = \sim 15, \sim 20$ and ~ 30 mmHg) and four hypercapnic stages ($P_{\text{aCO}_2} = \sim 50, \sim 55, \sim 60$ and ~ 65 mmHg). In Part B ($n = 10$), during isocapnia, P_{aO_2} was decreased to $\sim 60, \sim 44$, and ~ 35 mmHg and increased to ~ 320 mmHg and ~ 430 mmHg. Stages lasted ~ 15 min. Intra-arterial pressure was measured continuously; arterial blood gases were sampled at the end of each stage. There were three principal findings. (1) Regional reactivity: the VA reactivity to hypocapnia was larger than the ICA, MCA and PCA; hypercapnic reactivity was similar. With profound hypoxia (35 mmHg) the relative increase in VA flow was 50% greater than the other vessels. (2) Neck vessel diameters: changes in diameter ($\sim 25\%$) of the ICA was positively related to changes in P_{aCO_2} ($R^2, 0.63 \pm 0.26$; $P < 0.05$); VA diameter was unaltered in response to changed P_{aCO_2} but yielded a diameter increase of $+9\%$ with severe hypoxia. (3) Intra- vs. extra-cerebral measures: MCA and PCA blood velocities yielded smaller reactivities and estimates of flow than VA and ICA flow. The findings respectively indicate: (1) disparate

blood flow regulation to the brainstem and cortex; (2) cerebrovascular resistance is not solely modulated at the level of the arteriolar pial vessels; and (3) transcranial Doppler ultrasound may underestimate measurements of CBF during extreme hypoxia and/or hypercapnia.

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Abbreviations BA_v, basilar artery blood velocities; CBF, cerebral blood flow; CBV, cerebral blood velocity; CVC, cerebrovascular conductance; HR, heart rate; ICA, internal carotid artery; MAP, mean arterial pressure; MCA, middle cerebral artery; MCA_v, middle cerebral artery blood velocity; PCA, posterior cerebral artery; PCA_v, posterior cerebral artery blood velocity; TCD, transcranial Doppler ultrasound; VA, vertebral artery.

Introduction

Brain perfusion is highly sensitive to changes in the partial pressure of carbon dioxide in arterial blood (P_{aCO_2}) and, to a lesser degree, the partial pressure of oxygen in arterial blood (P_{aO_2}) (Kety & Schmidt, 1948). These high sensitivities, especially to P_{aCO_2} , are unique to the cerebrovasculature when compared with the peripheral vasculature (Lennox & Gibbs, 1932; Ainslie *et al.* 2005). Elevations in P_{aCO_2} (hypercapnia) cause a decrease in cerebrovascular resistance and consequent increases in cerebral blood flow (CBF), whereas hypocapnia causes increased cerebrovascular resistance and related decreased CBF (Kety & Schmidt, 1948; Wasserman & Patterson, 1961). This response of the brain's circulation to P_{aCO_2} partly serves to stabilize respiratory and cardiovascular autonomic control, which are regulated by specific regions of the brainstem sensitive to the pH of surrounding tissue (Ainslie & Duffin, 2009; Xie *et al.* 2009). Thus, the increase in CBF with hypercapnia mitigates rising tissue CO_2 , and the decreased CBF with hypocapnia attenuates falling tissue CO_2 . Under normal physiological circumstances, hypoxia-induced increases in ventilation result in hypocapnia; changes in P_{aO_2} and P_{aCO_2} therefore influence both ventilation and CBF (Ainslie & Ogoh, 2010; Lucas *et al.* 2010*b*). Despite the importance of this reciprocal regulation at the level of the brainstem to central respiratory and autonomic control, most studies to date have measured the velocity response of the middle cerebral artery (MCA_v), assuming blood gas reactivity is similar for different brain regions. But there has been limited study of regional differences in brain blood flow regulation with which to verify this assumption.

Jansen *et al.* (2002) reported greater increases in middle cerebral artery blood velocity (MCA_v) than basilar artery blood velocities (BA_v) during 7% O_2 gas exposure; however, these data were collected under dynamic poikilocapnic conditions, precluding analysis of cerebrovascular CO_2 and O_2 sensitivities independently of each other. Conversely, with changes in P_{aCO_2} , previous studies have found a comparable cerebrovascular

sensitivity between the MCA_v and BA_v (Ogawa *et al.* 1988; Hida *et al.* 1996). Hauge *et al.* (1980) also reported similar CO_2 reactivity differences in velocity between the vertebral artery (VA) and internal carotid artery (ICA). However, all previous studies are limited by their measurement of blood velocity (rather than flow) and may consequently have underestimated changes and/or differences in CO_2 sensitivities, and no study has assessed reactivity in the posterior cerebral artery (PCA). Recent data indicate that within 3 h of hypoxic exposure (12% O_2), the MCA may dilate (Wilson *et al.* 2011). Moreover, MCA diameter may change by 4–18% during a ~15 mmHg range in P_{aCO_2} (Giller *et al.* 1993). It is unknown if these diameter changes are more pronounced through a broader P_{aCO_2} range, but if so may lead to a marked error in estimations of flow. Sato & Sadamoto (2010) reported different regional blood flow changes through the ICA and VA during progressive exercise (and concomitant changes in P_{aCO_2}); interpretation with respect to sensitivities to blood gases *per se* is confounded by the myriad other changes manifest during exercise. Moreover, if neck arteries are *directly* sensitive to changes in arterial blood gases, estimation of cerebrovascular reactivity, and hence physiological interpretations, could be broadly inaccurate as well.

The current model of cerebrovascular function is a single-resistor model, with changes in cerebrovascular resistance occurring at the precapillary arterioles of the pia mater – the pial vessels (Forbes & Wolff, 1928; Wolff & Lennox, 1930; Harper & Glass, 1965; Kontos *et al.* 1978). There are several animal studies, however, demonstrating that the large cerebral arteries, and distal arteries of the neck (ICA and VA) contribute significantly to total changes in cerebrovascular resistance during alterations of blood pressure and arterial gases (Heistad *et al.* 1978; Faraci & Heistad, 1990), but to our knowledge, the relative sensitivities of ICA and VA to altered blood gases is not known, and moreover, this concept has never been intentionally explored in humans.

We investigated the regional CBF distribution through a wide range of steady state progressive changes in P_{aCO_2} and P_{aO_2} near the limits of tolerance in conscious humans.

Second, we aimed to determine if the large extra-cerebral arteries (ICA and VA) change diameter through the same range of arterial blood gases, indicating their potential role in cerebrovascular regulation. Based on the different ICA and VA flow responses reported by Sato & Sadamoto (2010) during exercise, we hypothesized that VA and ICA flow would exhibit disparate reactivity to changes in arterial blood gases. Based on highly controlled animal studies (Heistad *et al.* 1978; Faraci & Heistad, 1990), we further speculated that the ICA and VA would dilate in hypoxia, and hypercapnia, and constrict with hypocapnia.

Methods

Participants

Twenty-six adults volunteered for the study and gave written informed consent. Part A (carbon dioxide investigations) included 16 adults (5 female) with a mean age of 22 ± 3.2 years (mean \pm SD), and body mass index of 23.8 ± 1.8 kg m⁻². Eleven subjects were studied between 07.00 and 12.00 h, four subjects between 12.00 and 17.00 h; there were no statistical differences found for time of day, and data were therefore pooled. Part B (oxygen investigations) included 10 adults (5 female) with a mean age of 28 ± 4.5 years, and body mass index of 24 ± 2.2 kg m⁻². Parts A and B were completed at different centres on a different group of participants. Participants were non-smokers, had no previous history of cardiovascular, cerebrovascular or respiratory diseases, and were not taking any medications other than contraception; females were studied during the follicular phase. The study was approved by the Clinical Research Ethics Board of the University of British Columbia (Study A) and the Institutional Review Board of Duke University Medical Center (Study B) and conformed to the standards set by the *Declaration of Helsinki*.

Experimental design

After familiarization with the experimental procedures outlined below, each participant was studied on one occasion. Before each experimental session, participants were required to abstain from exercise and alcohol for 24 h, caffeine for 12 h and a heavy meal for 4 h prior. After local anaesthesia (1% lidocaine), a 20-gauge catheter (Arrow, Markham, Ontario, Canada) was placed into the radial artery and attached to a pressure transducer (ADI Instruments, Colorado Springs, CO, USA) positioned at the level of the right atrium in the midaxillary line for the measurement of beat-to-beat arterial blood pressure and arterial blood gases. Following cannulation, subjects rested quietly in the supine position, breathing room air, for ≥ 30 min to allow the setting up of monitoring equipment,

which included calibration of the pressure transducer. An automated gas blender adjusted the composition and flow to a sequential gas delivery mask and breathing circuit according to the method described by Slessarev *et al.* (2007) (RespirAct; Thornhill Research, Toronto, Canada). This apparatus enables prospective control of the individual's end-tidal CO₂ and O₂ (P_{ETCO_2} and P_{ETO_2}), and minute ventilation independently of one another. At least during modest changes in P_{aCO_2} (35–55 mmHg), the breathing circuit has been shown to operate such that the P_{ETCO_2} is equal to P_{aCO_2} (Slessarev *et al.* 2007). Two protocols were conducted, described below.

Part A (carbon dioxide investigations). Following a 15 min baseline at 40 mmHg P_{aCO_2} , either progressive hypocapnia or hypercapnia was randomly begun. In either case, sequential iso-oxic ~ 15 min steps approximating 15 and 20 mmHg (hypocapnia), and 50, 55, 60 and 65 mmHg (hypercapnia) were conducted. Due to technical limitations, the maximal hyperventilation stage ($P_{aCO_2} \sim 15$ mmHg) was carried out *without oxygen clamping*. Each step was separated by a recovery period long enough to allow P_{aCO_2} to return to baseline values – typically ~ 15 –30 min. Arterial blood gases were sampled at the end of each stage, the exact duration of which depended on subject tolerance, the time needed to stabilize at the desired P_{aCO_2} level, and the time needed to make neck blood flow measurements; in most cases stages lasted 12–15 min.

Part B (oxygen investigations). Following 15 min breathing room air, baseline measurements were made before P_{aO_2} was sequentially decreased to 60, 44 and 35 mmHg P_{aO_2} for exactly 15 min each. Subjects then breathed room air again for 15 min, before P_{aO_2} was increased to ~ 320 mmHg and ~ 430 mmHg. P_{aCO_2} was clamped at room air levels throughout all tests.

Measurements

Cerebral blood velocity. The left middle cerebral artery blood velocity (MCAv) and right posterior cerebral artery blood velocity (PCAv) were measured using a 2 MHz pulsed transcranial Doppler ultrasound (TCD) system (Spencer Technologies, Seattle, WA, USA). The left MCA was measured through the temporal window, at a depth 1 cm distal to the MCA–anterior cerebral artery bifurcation. The right P1 segment of the PCA was insonated from the right anterior temporal window. The probes were fixed and held in place using a specialized headband fixation device (M600 Bilateral Head frame, Spencer Technologies, Seattle, WA, USA). Standardized search techniques were used to optimize signal quality as detailed previously (Willie *et al.* 2011). Our test–retest

reliability for baseline measures of MCAv and PCAv are approximately 3% and 2%, respectively.

Extracranial ultrasound of blood flow in conduit vessels.

Continuous diameter and blood flow recordings in the left internal carotid artery and right vertebral artery were obtained using a 10 MHz multifrequency linear array probe attached to a high-resolution ultrasound machine (Terason 3000, Teratech, Burlington, MA, USA). Imaging of the extracranial arteries commenced when end-tidal clamping was stable for at least 1 min. One sonographer on the left side of the participant conducted all left ICA measures, while another sonographer on the right side performed imaging of the right VA. The left ICA was measured at least 2 cm from the carotid bifurcation, whilst ensuring there was no evidence of turbulent or retrograde flow. The right VA was measured between the transverse process of C4 and the subclavian artery, but always at the same location within each subject. Average diameter and blood flow recordings were made from a minimum of 10 cardiac cycles (see below), and care was taken to ensure probe position was stable so that the angle of insonation did not vary from 60 deg. The sample volume was positioned in the centre of the vessel and adjusted to cover the width of the vessel diameter. Measurement settings for each extracranial artery within an individual were standardized for all gas stages. Test–retest reliability for baseline measures of ICA and VA flow are approximately 5% and 11%, respectively.

All extracranial vascular images were directly stored as an AVI file for offline analysis. As described in depth elsewhere, analysis involved continuous measurements of arterial diameter synchronous with measurements of blood velocity at 30 Hz performed using an off-line custom-designed edge-detection and wall-tracking software (Black *et al.* 2008). Mean blood flow was determined as half the time averaged maximum velocity (Evans, 1985) multiplied by the cross-sectional lumen area. This method is used instead of the intensity weighted mean because the latter is more susceptible to noise and other distorting influences; the maximum follower is only affected by noise above the maximum frequency, noise that is visually obvious. Reproducibility of diameter measurements using this software is significantly better than manual methods as it reduces observer error significantly, and possesses an intra-observer coefficient of variation of 6.7% (Black *et al.* 2008).

Blood pressure and heart rate. Electrocardiogram, non-invasive beat-to-beat blood pressure by finger photoplethysmography (Finometer, Amsterdam, the Netherlands), intra-radial artery pressure, and P_{ETCO_2} were sampled continuously at 1 kHz via an analogue to

digital data acquisition system (Powerlab/16SP ML795; ADInstruments).

Arterial blood gases. Arterial blood gas samples from the radial artery were drawn into a preheparinised syringe, and analysed immediately. Following standardised calibration, all blood samples were analysed using an arterial blood-gas analysing system (ABL-80 Co-Ox, Radiometer, Copenhagen, Denmark) for pH, P_{O_2} , P_{CO_2} and S_{aO_2} .

Calculations

Values for mean arterial pressure (MAP), MCAv, PCAv, heart rate (HR), and end-tidal gases were averaged over the last 3 min of each stage. Cerebrovascular conductance (CVC) indices were calculated by dividing MAP by the mean cerebral blood velocities (CBV; for MCAv and PCAv), or flow (for ICA and VA). Two forms of the hypoxic cerebrovascular reactivity were calculated for each individual as the slopes of the linear regressions between: (1) absolute CBF (CBV or flow) *vs.* S_{aO_2} ; and (2) the relative (percentage) change from baseline CBF *vs.* S_{aO_2} . S_{aO_2} is conventionally used in place of P_{aO_2} to calculate hypoxic cerebrovascular reactivity because (1) the former gives an approximately linear (rather than exponential) relationship with CBF, and (2) S_{aO_2} is clinically meaningful and less invasively obtained than P_{aO_2} . Cerebrovascular CO_2 reactivity was similarly calculated both in absolute and relative terms as the slopes of the linear regression between CBF *vs.* P_{aCO_2} and $\% \Delta \text{CBF}$ *vs.* P_{aCO_2} , for the entire P_{aCO_2} range. All R^2 values were greater than 0.65. Cerebrovascular CO_2 reactivity was also calculated separately for hypo- and hypercapnia. Arterial blood gas sampling was not possible in two subjects (one due to lack of catheter, one to technical issues with the gas analyser). In these individuals P_{aO_2} and P_{aCO_2} were estimated from their measured end-tidal gases using the group relationship between arterial and end-tidal gases that yielded the regression equations: $P_{\text{aCO}_2} = 0.882 (P_{\text{ETCO}_2}) + 2.47$ ($R^2 = 0.98$); $P_{\text{O}_2} = 0.806 (P_{\text{ETO}_2}) + 8.3$ ($R^2 = 0.724$). The relationship between arterial and end-tidal CO_2 is shown in Fig. 1.

Arterial oxygen delivery was calculated as the product of the arterial content of oxygen (C_{aO_2}) and flow through the ICA and VA. C_{aO_2} was calculated as:

$$C_{\text{aO}_2} \text{ (ml dl}^{-1}\text{)} = [\text{haemoglobin}] \text{ (g dl}^{-1}\text{)} \times 1.36 \text{ (ml O}_2 \text{ (g haemoglobin)}^{-1}\text{)} \times S_{\text{aO}_2} \text{ (\%)/100} + 0.003 \text{ (ml dl}^{-1}\text{)} \times P_{\text{O}_2} \text{ (mmHg)}.$$

Statistical analysis

Normal distribution of variables was confirmed with the Shapiro–Wilk normality test. One-way ANOVA was used to compare differences across blood gas conditions,

and where appropriate, *post hoc* comparisons to baseline blood gases were made using Dunnett's *post hoc* test. One-way repeated measures ANOVA was used to compare ICA diameter with P_{aCO_2} stage in the eight subjects who completed all stages between -20 mmHg and 55 mmHg P_{aCO_2} . To make comparisons between ICA and VA, and between MCA and PCA, for a given blood gas stage, Student's *t* test was performed with appropriate Bonferroni correction for multiple comparison. Relationships between selected variables were determined using Pearson's correlations. There were no statistical differences in any variable between males and females, and these data were therefore pooled. Values are presented as means \pm SD.

Results

Subjects

CO₂ investigations. There were marked variation in tolerances to the extremes of both hypo- and hypercapnia. For example, with hypercapnia, the 50, 55, 60 and 65 mmHg steps were completed by 16, 15, 9 and 5 individuals, respectively. With hypocapnia, the 30, 20 and maximal hypocapnic (15 mmHg) steps were completed by 16, 15 and 11 individuals, respectively. Inadequate quality in ICA images was obtained in four subjects. Three of these subjects' images lacked clearly demarcated vessel walls throughout the cardiac cycle, and in one subject large changes in the position of the vessel with respiration limited imaging of the vessel over more than a few concurrent cardiac cycles. Adequate images of the VA were not obtained in four subjects due in all cases to inadequate vessel wall sharpness; two of these subjects were amongst those excluded from ICA analysis. Those subjects with inadequate ICA and/or VA images were excluded

only from ICA and/or VA analysis. Thus, group means for MAP, HR, P_{ETCO_2} , MCAv and PCAv were all based on $n = 16$, whereas VA and ICA metrics were calculated on their respective $n = 12$. Data from the individuals who completed all stages showed the same blood flow and reactivity trends as that of the group means. Final sample sizes for each of the vessel measures at each stage are given in Table 1. In one subject a radial arterial catheter was not possible; blood pressure values from finger photoplethysmography were substituted in this subject only. Automated blood pressure measures were used to confirm these finger photoplethysmography values.

O₂ investigations. Ten subjects began the protocol. One subject could not tolerate the mask at the 70% S_{aO_2} stage; her data were excluded from analysis. In another subject, post-analysis revealed technical problems with the blood pressure recording; therefore these data were also excluded from analysis. Of the $n = 8$ (age 28.6 ± 4.5) remaining, in one subject poor ICA images were attained; ICA analysis was therefore based on $n = 7$.

Cardiorespiratory data

CO₂ investigations. There were no differences between the pre-hypocapnia and pre-hypercapnia baselines; thus full recovery was obtained between the progressive hypocapnic and hypercapnic steps. As expected, P_{aCO_2} was altered at each stage ($P < 0.05$; Table 1). Although MAP did not differ from baseline during hypocapnia, it was significantly increased during each stage of hypercapnia (Table 1). Heart rate was not different at 10 mmHg P_{aCO_2} above or below baseline, but was elevated at every other stage.

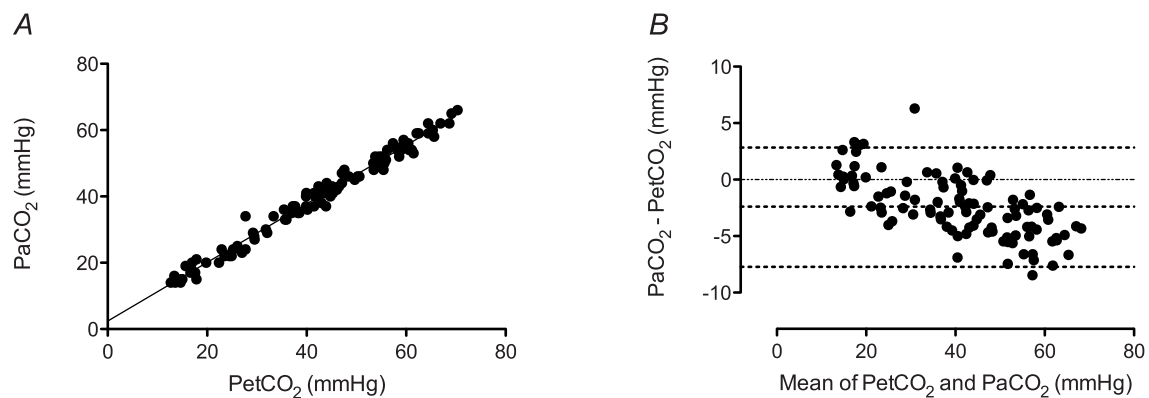


Figure 1. Relationship between arterial (P_{aCO_2}) and end-tidal (P_{ETCO_2}) partial pressures of carbon dioxide during targeted steady state changes in P_{ETCO_2} .

A, Regression equation: $P_{aCO_2} = 0.882 (P_{ETCO_2}) + 2.47$ ($R^2 = 0.98$). B, Bland-Altman plot of differences between P_{aCO_2} and P_{ETCO_2} , and the mean value of both. Dashed lines represent the 95% confidence intervals and the mean bias.

Table 1. Cardiorespiratory and cerebrovascular variables during iso-oxic P_{aCO_2} alterations

	15 mmHg	20 mmHg	30 mmHg	40 mmHg (baseline)	50 mmHg	55 mmHg	60 mmHg	65 mmHg
P_{aCO_2} †	16.6 ± 1.7*	20.0 ± 3.6*	29.2 ± 4.2*	39.2 ± 3.7	49.2 ± 3.7*	53.7 ± 3.5*	58.2 ± 4.7*	62.2 ± 3.8*
P_{aO_2}	109 ± 31	100 ± 25	92.6 ± 9	91.3 ± 17	95.9 ± 14	94.6 ± 14	93.2 ± 17	93.5 ± 21
HR (beats min ⁻¹)	89 ± 14*	79 ± 11*	68 ± 9	65 ± 1	72 ± 8.3	76 ± 11*	79 ± 5.8*	85 ± 6*
MAP (mmHg)	81.1 ± 3.6	82.1 ± 5.2	82.5 ± 4.5	82.4 ± 3.6	86.5 ± 5.3	91.7 ± 7.4*	93.9 ± 8.3*	100 ± 5.2*
\dot{Q}_{ICA} (ml min ⁻¹)	122 ± 30.0*	121 ± 24.3*	151 ± 25.0*	222 ± 47.6	341 ± 75.2*	418 ± 92.1*	482 ± 109*	489 ± 84.9*
D_{O_2} (ml O ₂ min ⁻¹)	26.3 ± 6.3*	26.4 ± 5.4*	32.4 ± 5.6	47.1 ± 11.0	72.5 ± 16.4*	87.4 ± 23.1*	102.7 ± 25.3*	108.3 ± 19.9*
Diameter (mm)	0.47 ± 0.05	0.48 ± 0.05	0.50 ± 0.07	0.52 ± 0.06	0.53 ± 0.07	0.55 ± 0.07	0.58 ± 0.06	0.59 ± 0.05
Δ% diameter	-6.6 ± 2.9	-6.0 ± 5.0	-3.2 ± 5.3	—	2.6 ± 4.3	5.8 ± 6.1	10.0 ± 6.0	11.5 ± 4.8
CVC (ml min ⁻¹ mmHg ⁻¹)	(1.5 ± 0.4)*	(1.5 ± 0.4)*	(1.8 ± 0.4)*	(2.6 ± 0.7)	(4.0 ± 1.2)*	(4.4 ± 1.0)*	(4.8 ± 1.3)*	(4.5 ± 1.3)*
Sample size	n = 7	n = 11	n = 12	n = 12	n = 12	n = 10	n = 7	n = 3
\dot{Q}_{VA} (ml min ⁻¹)	39.1 ± 25.4	39.9 ± 21.5	47.2 ± 22.7	71.4 ± 27.0	99.5 ± 35.3	118 ± 41.3*	158 ± 58.9*	187 ± 30.4*
D_{O_2} (ml O ₂ min ⁻¹)	8.4 ± 5.4	8.4 ± 4.1	8.7 ± 4.4	14.0 ± 5.4	19.6 ± 6.6	24.0 ± 8.3*	32.0 ± 10.7*	39.2 ± 3.2*
Diameter (mm)	0.40 ± 0.07	0.39 ± 0.07	0.40 ± 0.07	0.41 ± 0.07	0.41 ± 0.05	0.42 ± 0.04	0.43 ± 0.01	0.43 ± 0.04
Δ% diameter	-2.9 ± 9.7	-6.1 ± 8.8	0.3 ± 8.9	—	2.4 ± 6.3	3.1 ± 7.4	-0.5 ± 9.5	2.8 ± 6.7
CVC (ml min ⁻¹ mmHg ⁻¹)	(0.47 ± 0.3)	(0.49 ± 0.2)	(0.56 ± 0.27)	(0.87 ± 0.33)	(1.2 ± 0.45)	(1.3 ± 0.45)*	(1.8 ± 0.81)*	(2.0 ± 0.30)*
Sample size	n = 4	n = 6	n = 11	n = 12	n = 11	n = 9	n = 3	n = 2
MCAv (cm s ⁻¹)	37.9 ± 6.9*	40.5 ± 6.8*	46.6 ± 6.4*	62.4 ± 10.1	88.0 ± 14.5*	101 ± 14.3*	111 ± 18.7*	111 ± 19.6*
CVC (cm s ⁻¹ mmHg ⁻¹)	(0.47 ± 0.08)*	(0.50 ± 0.09)*	(0.56 ± 0.07)*	(0.76 ± 0.13)	(1.0 ± 0.18)*	(1.1 ± 0.18)*	(1.2 ± 0.20)*	(1.1 ± 0.16)*
Sample size	n = 11	n = 15	n = 16	n = 16	n = 16	n = 15	n = 9	n = 5
PCAv (cm s ⁻¹)	28.8 ± 6.4*	31.0 ± 7.1*	34.9 ± 7.1*	46.7 ± 11.5	67.4 ± 15.6*	76.8 ± 12.4*	84.3 ± 14.5*	86.3 ± 16.8*
CVC (cm s ⁻¹ mmHg ⁻¹)	(0.36 ± 0.09)*	(0.38 ± 0.10)*	(0.42 ± 0.09)*	(0.57 ± 0.15)	(0.78 ± 0.19)*	(0.84 ± 0.14)*	(0.90 ± 0.17)*	(0.86 ± 0.14)*
Sample size	n = 10	n = 14	n = 16	n = 16	n = 16	n = 15	n = 9	n = 5

† Values are arterial P_{aCO_2} and P_{aO_2} , except for six blood gas values distributed amongst the subjects, and in two subjects in whom arterial blood sampling was not possible. P_{aCO_2} and P_{aO_2} values were estimated in these cases from the regression equations between P_{aCO_2} , P_{aO_2} and P_{ETCO_2} for all subjects; see Methods. Sample sizes vary between measured vessels due to inadequate image quality for VA and ICA measures, and loss of PCA signal in one subject during hypocapnia. P_{aCO_2} and P_{aO_2} , partial pressures arterial carbon dioxide and oxygen; HR, heart rate; MAP, mean arterial pressure; \dot{Q}_{ICA} , internal carotid artery blood flow; D_{O_2} , Oxygen delivery through vessel; \dot{Q}_{VA} , vertebral artery blood flow; MCAv, middle cerebral artery blood velocity; PCAv, posterior cerebral artery blood velocity; CVC, cerebrovascular conductance (flow or velocity/MAP); * $P < 0.05$ vs. 40 mmHg (baseline).

O₂ investigations (Tables 2 and 3). There were no differences in any variable between pre-hypoxia and pre-hyperoxia trials. Full recovery was therefore achieved between the hypoxia and hyperoxia stages. Heart rate was elevated from baseline at each stage of hypoxia but was unaltered during hyperoxia. Mean arterial pressure did not change during any stage.

Cerebrovascular data: CO₂ investigations

Changes in flow and velocity with P_{aCO_2} (Figs 2 and 3; Table 1). \dot{Q}_{ICA} , MCAv and PCAv, and their respective CVC values were significantly increased during all stages of hypercapnia, and decreased during all stages of hypocapnia. \dot{Q}_{VA} and VA CVC were increased during hypercapnia; however, the decrease during hypocapnia did not reach statistical significance (Table 1). With the extreme level of hypercapnia (65 mmHg) the relative increases in CBF from baseline were: \dot{Q}_{ICA} : 146 ± 23.7%; \dot{Q}_{VA} : 158 ± 35%; MCAv: 99.1 ± 16.8%; and PCAv: 108 ± 22.7%. Conversely, at the extreme level

hypocapnia CBF decreases were: \dot{Q}_{ICA} : -41.0 ± 9.8%; \dot{Q}_{VA} : -49.8 ± 8.2%; MCAv: -39.1 ± 6.9%; PCAv: -39.5 ± 7.3%. Oxygen delivery through the ICA was decreased at 15 and 20 mmHg P_{aCO_2} , and increased at 50 mmHg P_{aCO_2} and above. Posterior cerebral (VA) oxygen delivery was increased above 50 mmHg P_{aCO_2} ; there was a trend for reduced VA oxygen delivery during hypocapnia (Table 1).

Comparisons between vessels (Fig. 3). Absolute flow/velocity values for each vessel for a given stage were different from each other ($P < 0.006$). Overall cerebrovascular reactivity to CO₂ based on percentage changes in CBF from baseline was ~25% greater ($P < 0.05$) for the neck arteries (ICA: 4.0 ± 0.38% mmHg⁻¹; VA 4.4 ± 2.1% mmHg⁻¹ versus that of the cerebral arteries (MCA: 2.9 ± 0.47% mmHg⁻¹; PCA: 3.0 ± 0.62% mmHg⁻¹). However, overall reactivity did not differ between ICA and VA, or between MCA and PCA. Reactivity in the hypercapnic range did not differ between vessels, whereas in the hypocapnic

Table 2. Cardiorespiratory and cerebrovascular variables during isocapnic hypoxaemia

	100% S_{aO_2}	90% S_{aO_2}	80% S_{aO_2}	70% S_{aO_2}
P_{aO_2} (mmHg)	106 ± 8.0	59 ± 4.0*	43 ± 2.8*	36 ± 4.3*
S_{aO_2} (%)	98.7 ± 0.39	92.4 ± 2.0*	80.3 ± 2.0*	70.1 ± 3.0*
P_{aCO_2} (mmHg)	40 ± 1.6	40 ± 2.4	41 ± 0.7	41 ± 1.7
HR (beats min ⁻¹)	65 ± 9	72 ± 11*	82 ± 10*	88 ± 10*
MAP (mmHg)	89 ± 5	91 ± 6	93 ± 10	95 ± 12
\dot{Q}_{ICA} (ml min ⁻¹)	230 ± 67.5	222 ± 66.9	284 ± 62.8	322 ± 95.9*
D_{O_2} (ml O ₂ min ⁻¹)	48.7 ± 16.2	42.4 ± 12.5	50.8 ± 17.4	46.2 ± 14.4
Diameter (mm)	0.54 ± 0.09	0.54 ± 0.09	0.55 ± 0.07	0.49 ± 0.21
Δ% diameter	—	-1.0 ± 8.1	1.4 ± 8.6	6.3 ± 8.1
CVC (ml min ⁻¹ mmHg ⁻¹)	2.7 ± 0.90	2.6 ± 0.86	3.3 ± 1.0	3.0 ± 1.8*
\dot{Q}_{VA} (ml min ⁻¹)	48.7 ± 16.5	52.6 ± 22.4	66.9 ± 30.8	92.4 ± 21.3*
D_{O_2} (ml O ₂ min ⁻¹)	9.4 ± 2.8	9.5 ± 4.2	10.4 ± 4.0	13.0 ± 2.3*
Diameter (mm)	0.33 ± 0.05	0.33 ± 0.06	0.35 ± 0.06	0.36 ± 0.06*
Δ% diameter	—	0.0 ± 4.8	5.3 ± 6.9	8.3 ± 9.2
CVC (ml min ⁻¹ mmHg ⁻¹)	0.55 ± 0.19	0.58 ± 0.24	0.75 ± 0.39	1.0 ± 0.31*
MCAv (cm s ⁻¹)	59.9 ± 10.8	66.3 ± 14.1	76.2 ± 16.6*	83.6 ± 18.4*
CVC (cm s ⁻¹ mmHg)	0.67 ± 0.12	0.73 ± 0.16	0.82 ± 0.17*	0.89 ± 0.18*
PCAv (cm s ⁻¹)	38.0 ± 8.8	40.6 ± 10.8	46.2 ± 10.7*	50.7 ± 12.0*
CVC (cm s ⁻¹ mmHg ⁻¹)	0.43 ± 0.12	0.45 ± 0.14	0.51 ± 0.16*	0.55 ± 0.18*

P_{aCO_2} and P_{aO_2} , partial pressures arterial carbon dioxide and oxygen; HR, heart rate; MAP, mean arterial pressure; \dot{Q}_{ICA} , internal carotid artery blood flow; D_{O_2} , Oxygen delivery through vessel; \dot{Q}_{VA} , vertebral artery blood flow; MCAv, middle cerebral artery blood velocity; PCAv, posterior cerebral artery blood velocity; CVC, cerebrovascular conductance (flow or velocity/MAP). * $P < 0.05$ vs. 100% S_{aO_2} (baseline).

Table 3. Cardiorespiratory and cerebrovascular variables during isocapnic hyperoxia

	Baseline	320 mmHg P_{O_2}	430 mmHg P_{O_2}
P_{aO_2} (mmHg)	104 ± 10.3	321 ± 7.8*	434 ± 33.2*
S_{aO_2} (%)	98 ± 0.74	99.6 ± 0.25*	99.6 ± 0.23*
P_{aCO_2} (mmHg)	40 ± 1.7	40 ± 2.6	41 ± 2.3
HR (beats min ⁻¹)	68 ± 10	66 ± 11	68 ± 11
MAP (mmHg)	91 ± 8	92 ± 8	92 ± 6
\dot{Q}_{ICA} (ml min ⁻¹)	242 ± 86.4	245 ± 110	243 ± 86.4
D_{O_2} (ml O ₂ min ⁻¹)	51.9 ± 15.6	54.5 ± 21.9	51.0 ± 20.0
Diameter (mm)	0.56 ± 0.08	0.53 ± 0.08	0.51 ± 0.06
CVC (ml min ⁻¹ mmHg ⁻¹)	2.9 ± 0.86	2.9 ± 1.2	2.7 ± 1.1
\dot{Q}_{VA} (ml min ⁻¹)	53.6 ± 15.4	52.1 ± 24.7	42.8 ± 12.3
D_{O_2} (ml O ₂ min ⁻¹)	10.8 ± 2.7	10.5 ± 5.1	8.9 ± 2.7
Diameter (mm)	0.36 ± 0.06	0.33 ± 0.08	0.32 ± 0.07
CVC (ml min ⁻¹ mmHg ⁻¹)	0.61 ± 0.18	0.56 ± 0.26	0.47 ± 0.15
MCAv (cm s ⁻¹)	64.0 ± 12.8	60.4 ± 11.8*	57.5 ± 10.7*
CVC (cm s ⁻¹ mmHg ⁻¹)	0.70 ± 0.12	0.66 ± 0.11*	0.62 ± 0.11*
PCAv (cm s ⁻¹)	41.4 ± 11.1	39.6 ± 10.3	38.8 ± 9.8*
CVC (cm s ⁻¹ mmHg ⁻¹)	0.44 ± 0.14	0.42 ± 0.13	0.41 ± 0.12*

P_{aCO_2} and P_{aO_2} , partial pressures arterial carbon dioxide and oxygen; HR, heart rate; MAP, mean arterial pressure; \dot{Q}_{ICA} , internal carotid artery blood flow; \dot{Q}_{VA} , vertebral artery blood flow; MCAv, middle cerebral artery blood velocity; PCAv, posterior cerebral artery blood velocity; CVC, cerebrovascular conductance (flow or velocity/MAP). *Differences from baseline ($P < 0.05$).

range VA reactivity was greater than the other vessels (Fig. 4). For comparison, Fig. 4 (bottom panel) also depicts CO₂ reactivity when calculated on absolute CBF values.

Relationships with P_{aCO_2} (Figs 5 and 6). Changes in P_{aCO_2} were reflected in significant changes in the diameter of the ICA (individual regression mean R^2 , 0.63 ± 0.26 ; Fig. 5). In the eight subjects who completed all P_{aCO_2} stages

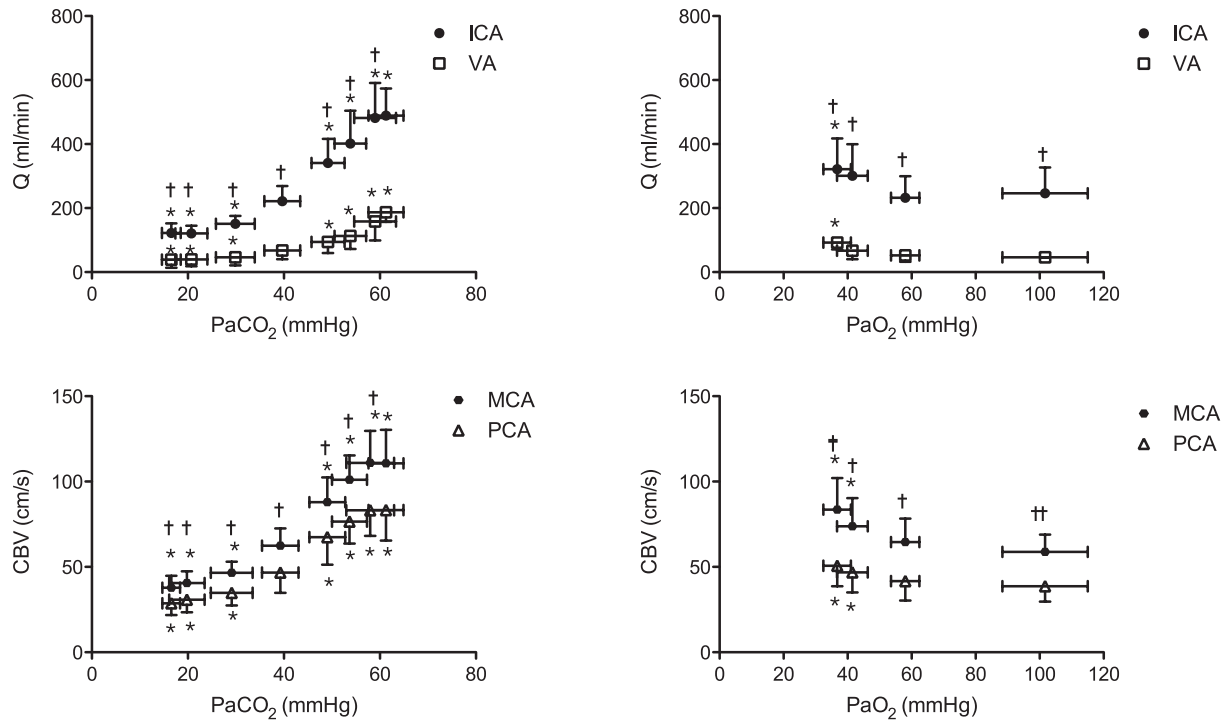


Figure 2. Blood flow (top panels; ICA and VA) and blood velocity (bottom panels; MCA and PCA) during steady state changes in arterial CO₂ (left panels) and oxygen (right panels)

*Difference from baseline (40 mmHg P_{aCO_2} or 100 P_{O_2}); $P < 0.05$. †Differences between vessel flow (ICA vs. VA) or velocity (MCA vs. PCA) at a given stage; P_{aCO_2} : $P < 0.006$; P_{O_2} : $P < 0.012$. All values are means \pm SD. ICA, internal carotid artery; VA, vertebral artery; MCA, middle cerebral artery; PCA, posterior cerebral artery. Note: the number of subjects comprising each mean value is different between stages and vessels. Please refer to Tables 1 and 2 for these values.

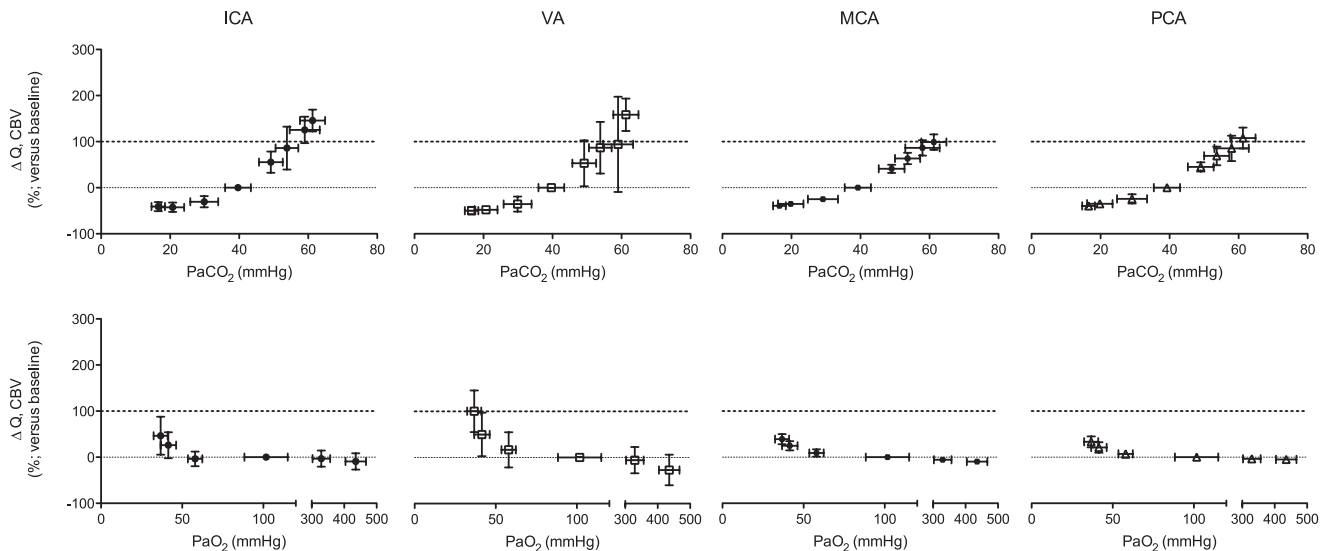


Figure 3. Percentage change from baseline in blood flow (Q; ICA and VA) and blood velocity (CBV; MCA and PCA) during steady state changes in arterial CO₂ (top panels) and oxygen (bottom panels)

All values are mean \pm SD. ICA, internal carotid artery; VA, vertebral artery; MCA, middle cerebral artery; PCA, posterior cerebral artery. The relative flow change in the neck arteries were \sim 50% greater than velocity change in the intracranial vessels during hypercapnia, suggesting either anatomical flow redistribution or dilatation of the MCA and PCA – or both. Note the much greater increase in VA flow during hypoxia compared to the other vessels; see Fig. 4 for comparative CO₂ and O₂ reactivities. Note: the number of subjects comprising each mean value is different between stages and vessels. Please refer to Tables 1 and 2 for these values.

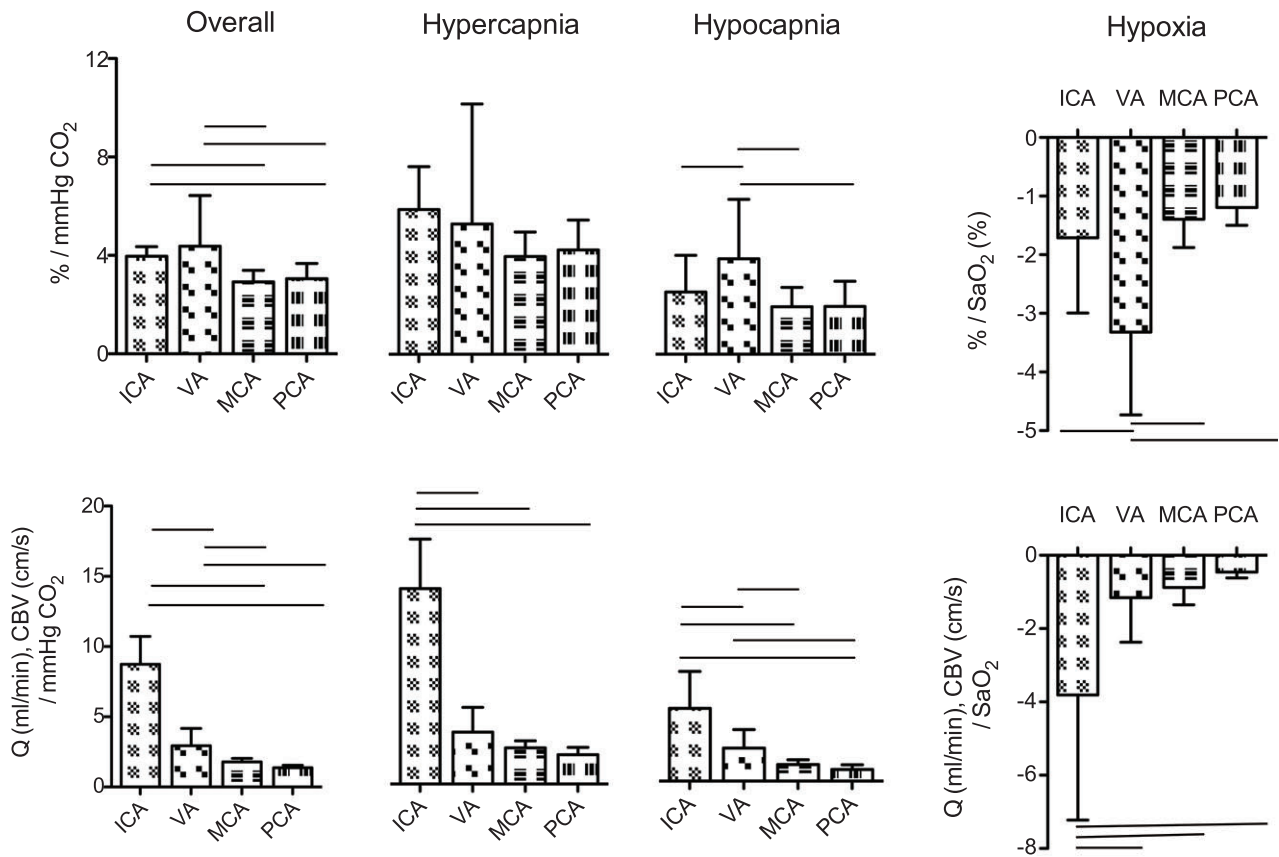


Figure 4. Mean (SD) cerebral blood flow responses to CO₂ and hypoxaemia
 The top row depicts the slope of the normalized CBF change (A%, relative to baseline) and P_{aCO₂} or S_{aO₂}; the bottom row depicts absolute flow (Q; ICA and VA) or blood velocity (CBV; MCA and PCA) and P_{aCO₂} or S_{aO₂}. Horizontal bars indicate significant relationships, P < 0.05.

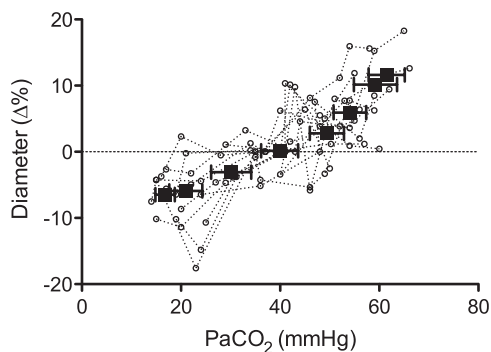


Figure 5. Mean (squares; ±SD) and individual (circles) percentage change in luminal diameters of the internal carotid artery during steady state change in P_{aCO₂}
 Linear regression of individual data $y = 3.6 \times 10^{-1} (P_{aCO_2}) - 13.3$; $R^2 = 0.63$. Through a large P_{aCO₂} range, internal carotid diameter changes by ~20%, indicating that cerebrovascular resistance is not solely modulated at cerebral arterioles with changes in the partial pressure of arterial blood gases.

from 20 mmHg to 55 mmHg, repeated measured ANOVA revealed that a >10 mmHg change in P_{aCO₂} produced a significant change in ICA diameter, in both the hyper- and hypocapnic ranges. There was no significant change in VA diameter. The hypercapnic-induced elevations in MAP were positively (P < 0.05) related to elevations in CBF or CBV in all arteries (R² 0.8–0.91; Fig. 6).

Cerebrovascular data: O₂ investigations

Changes in flow and velocity with S_{aO₂} (Figs 2 and 3). Q_{ICA} and Q_{VA} (and related CVC) were increased at 36 mmHg P_{aO₂} (S_{aO₂} ~70%) only. In contrast, MCAV and PCAV (and related CVC) were increased at both 44 mmHg (S_{aO₂} ~80%) and 36 mmHg P_{aO₂} (S_{aO₂} ~70%) stages. Both stages of hyperoxia decreased velocities and CVC of the MCA and PCA relative to baseline, but had no significant effect on the arteries of the neck (Table 3). Anterior cerebral (ICA) oxygen delivery was unchanged in hypoxia, whereas VA oxygen delivery was increased at 70% S_{aO₂} (Table 2). Hyperoxia elicited no change in oxygen delivery in either vessel.

Comparisons between vessels (Figs 2–4). Absolute values for each vessel at each P_{aO_2} stage were different from each other ($P < 0.006$). Cerebrovascular reactivity, expressed as the relative percentage change in CBF with S_{aO_2} , was $\sim 50\%$ greater in the VA (-3.3 ± 1.4) relative to the other arteries. Reactivities in these arteries were not different from one another (ICA: -1.71 ± 1.3 ; MCA -1.39 ± 0.5 ; PCA -1.19 ± 0.3). However, when expressed as the absolute value of flow (ICA and VA), or velocity (MCA and PCA), reactivity in the ICA was the highest; absolute reactivity was not different between the other three vessels.

Relationships with O_2 . The diameter of the VA increased at ~ 36 mmHg P_{aO_2} ($S_{aO_2} \sim 70\%$) whereas ICA diameter was unaffected by altered P_{aO_2} . In contrast to the P_{aCO_2} relationships, there was no relationship between the (non-significant) increase in MAP and CBF or CBV in any vessel (Fig. 6).

Proximal vs. distal measures of CBF

Figure 7 shows Bland–Altman plots of the difference in percentage change in CBF between VA and PCA, and ICA and MCA, plotted against their respective means. TCD provides an accurate estimate of CBF at lower CBF velocities but likely underestimates flow at the high CBF values during hypoxia or hypercapnia.

Discussion

This is the first study to examine regional cerebrovascular response differences over the tolerable extremes of acute arterial blood gas changes in humans. The novel findings of this study were: (1) VA reactivity to isocapnic hypoxia, and hypocapnia was greater than the other arteries (i.e. ICA, MCA and PCA); (2) overall cerebrovascular reactivity to P_{aCO_2} was greater in the neck arteries than the cerebral arteries (i.e. ICA and VA vs. MCA and PCA); (3) VA diameter increased during extreme hypoxaemia, whereas ICA diameter was sensitive to changes in P_{aCO_2} , indicating proximal resistance to the cerebral circulation is not constant during changes in blood gases; and (4) at high blood flows due to both isocapnic hypoxia and iso-oxic hypercapnia, TCD underestimates changes in global CBF. Collectively these findings indicate disparate blood gas sensitivity to the brainstem and cortex, challenge the dogma that cerebrovascular resistance is solely modulated at the level of the arteriolar pial vessels, and highlight limitations in the use of TCD for the measurement of CBF during extreme hypoxia and/or hypercapnia.

Cerebrovascular reactivity to P_{aCO_2} and P_{aO_2}

The cerebrovasculature is exquisitely sensitive to P_{aCO_2} and, to a lesser extent, P_{aO_2} . This sensitivity serves to buffer brain tissue pH, thereby stabilizing chemosensory and autonomic control. We examined the slope of the CBF– P_{aCO_2}/P_{aO_2} relationships, as well as the separate CBF reactivities to hypocapnia and hypercapnia. The neck arteries displayed greater overall CO_2 reactivity than the

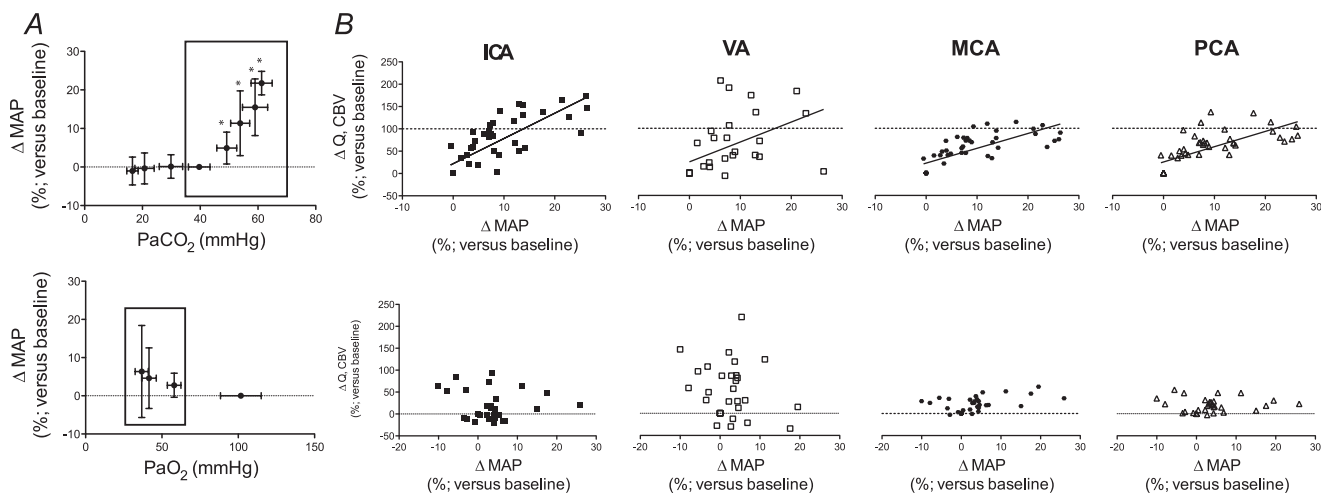


Figure 6. Percent change from baseline in MAP(A) and relationship between Δ MAP and Δ CBF or Δ CBV (B) during hypercapnia and hypoxia.

A, percentage change in (Δ MAP) relative to baseline for P_{aCO_2} (top row) and hypoxaemia (bottom row) trials. Right hand eight plots (B) depict Δ MAP in the hypercapnic or hypoxic ranges (shown boxed in A). ICA, internal carotid artery; VA, vertebral artery; MCA, middle cerebral artery; PCA, posterior cerebral artery. The hypercapnia related hypertension was positively related ($P < 0.05$) to elevations in CBF/CBV in all vessels; in contrast, hypoxia induced increases in MAP were not related to CBF/CBV.

intracranial vessels, and the VA displayed higher reactivity to hypoxia than both the ICA and intracranial vessels. The explanation for differences in reactivity between the neck vessels and their downstream arteries is likely to have either an anatomical or a technical basis – or both.

Anatomical. In addition to the MCA, the ICA also supplies the anterior cerebral artery. Thus the assumption of unity between ICA and MCA flow/velocity is contingent on a constant distributive relationship between the middle and anterior cerebral arteries. Similarly, the VAs feed a number of other vessels in addition to the PCA, which in regards to the vertebrobasilar system, has a higher degree of complexity. There are numerous anastomoses with the VA both extra- and intracranially. The VA communicates with branches of the deep cervical artery, and inferior thyroid artery extracranially, and upon entering the skull gives off multiple branches to the cerebellum, medulla and pons before joining to form the basilar artery (Edvinsson & Krause, 2002). A number of arteries project from the basilar artery to supply the cerebellum and pons before it ramifies to form the posterior circle of Willis and posterior

cerebral arteries (Willie *et al.* 2011). Thus, particularly in the case of the posterior vessels, the different reactivities in the neck *vs.* intracranial vessels could be a result of disparate changes in downstream resistance of regions *not* supplied by the MCA or PCA. In other words, for a given change in P_{aCO_2} or P_{aO_2} , blood may be diverted away from the MCA or PCA through intracranial vessels not directly measured in the current study.

There are no data, however, to suggest that this is the case, and given the similar differences in ICA/MCA and VA/PCA reactivities, we feel the following technical argument is more likely.

Technical. Diameter changes of the MCA and PCA themselves provide the second explanation for the difference in reactivity between neck and intracranial vessels. That the overall P_{aO_2} or P_{aCO_2} reactivity of the neck vessels was greater than the intracranial vessels could be evidence of vasodilatation at the site of insonation. Hypercapnic (or hypoxic)-induced dilatation would reduce blood velocity resulting in an underestimation of flow (see *Flow versus velocity with TCD*).

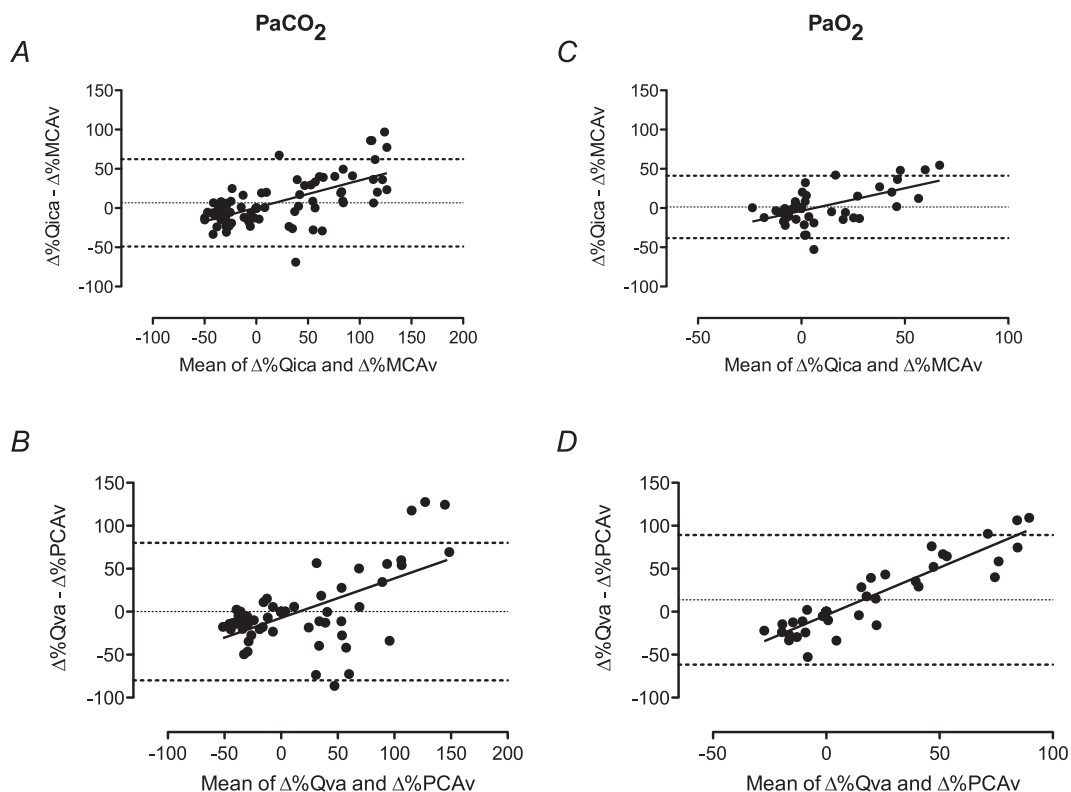


Figure 7. Bland–Altman plots of differences between neck artery blood flows and the respective downstream intracranial vessel, and the mean value of both

Values are percentage change from baseline. Dashed line represents the 95% confidence intervals, dotted line the mean bias. All plots show systematic error proportional to the increase in CBF; A and B suggest systematic and random error. Data show that at lower CBF values TCD estimates of intracranial blood velocity accurately reflect CBF for both the vertebral–PCA systems and ICA–MCA systems. With high CBF with hypoxia or hypercapnia MCAv and PCAv are likely to underestimate CBF.

We observed that in the hypercapnic range all vessels exhibit similar cerebrovascular reactivity, yet in the hypocapnic range the VA had greater CO₂ reactivity than the ICA, MCA and PCA, which were not different from one another. No previous study has reported reactivities for the ICA, VA or PCA but our MCA values are consistent with that previously reported (reviewed in: Ainslie & Duffin, 2009). Our values of P_{aCO_2} reactivity for MCA and PCA were similar to that reported for the basilar artery (Hida *et al.* 1996). As highlighted in the current study, it is known that steady-state cerebrovascular reactivity is more sensitive to increases in P_{aCO_2} (i.e. hypercapnic reactivity) than decreases (i.e. hypocapnic reactivity) (Ide *et al.* 2003; Xie *et al.* 2005; Peebles *et al.* 2007). The mechanisms underlying this greater reactivity to hypercapnia compared with hypocapnia may be related to a greater influence of vasodilator mediators on intracranial vascular tone compared with vasoconstrictive mediators (Toda & Okamura, 1998). It is also likely that increased MAP in the hypercapnic range influences reactivity, though there was no obvious relationship between MAP and CBF with hypoxia.

Brainstem blood flow regulation

Relative to all other vessels, the VA exhibited greater reactivity to hypoxia and to CO₂ in the hypocapnic range. Because of the complexity and distribution of the vertebrobasilar system, these results are difficult to interpret. There are also no values, to our knowledge, in the literature of VA reactivity to either CO₂ or hypoxia to compare our findings. Nevertheless, the greater reactivity of the VA, particularly to severe hypoxaemia and hypocapnia, is interesting in light of it being the principal blood conduit to the brainstem, and highlights the importance of regional blood flow distribution in the study of chemoreflex and autonomic control.

A proximal resistor for the human cerebral circulation?

We observed a 27% change in ICA diameter through a wide P_{aCO_2} range at the limits of our subjects' tolerance, and a 9% change in VA diameter with extreme hypoxaemia; to our knowledge this has not been previously reported in the literature. These data indicate that the large changes in CBF manifest through broad P_{aCO_2} and P_{aO_2} ranges may be partially affected by diameter modulation of the neck arteries themselves. That the regulation of brain blood flow is mediated by the pial arterioles has remained dogma for more than 70 years (Fog, 1934; Fog, 1938) despite evidence from animals suggesting that, unlike other vascular beds, the large arteries of the brain contribute significantly to total (cerebral) resistance. Indeed, this was proposed by Mchedlishvili 60 years ago, but his ideas did not reach

western scientific literature until much later (Mchedlishvili *et al.* 1973; Mchedlishvili, 1980). There are a number of studies demonstrating that the large cerebral vessels of cats and dogs contribute up to 30–45% of total cerebrovascular resistance (Heistad *et al.* 1978; Gross *et al.* 1980; Faraci & Heistad, 1990). The present data indicate that both the proximal cerebral arteries and distal cerebral arterioles dilate with hypercapnia and constrict with hypocapnia. Consistent with the results of the above animal studies, it is therefore likely that the entire cerebral arterial tree is sensitive to altered blood gases. Because the relative change in proximal and distal cerebral artery resistance ultimately determines cerebral microvasculature pressure, calculating the resistance changes based on the observed changes in diameter is not a meaningful quantification. But with union between small and large vessel vasomotion, microvascular pressure would be kept relatively constant despite large changes in flow. The ability of large cerebral arteries to act as 'protective' resistors (as opposed to static tubes) seems teleologically important. We acknowledge that it is possible that the increase in ICA diameter may not be due to the direct influence of P_{aCO_2} or hypoxia, but is rather a secondary endothelial-mediated response to changes in shear stress concomitant to flow changes, or passively during hypercapnia with increased MAP. At least at the level of brain arterioles in rats, however, vasodilatation with hypercapnia appears to be mediated through non-endothelial mechanisms (Wang *et al.* 1994), and we observed a significant decrease in ICA diameter during hypocapnia, when MAP did not change.

Our study was not designed to clarify the mechanism(s) or the specific site of P_{aCO_2} reactivity. Such a study will be difficult to design *in vivo*, however, given that subjecting only the arteries of the neck to changes in CO₂ but not their downstream associations is of obvious difficulty in humans. Regardless of the mechanism, however, our data clearly indicate that with severe changes in arterial blood gases the large vessels of the neck are not mere *conduit* vessels, and rather act as active resistance vessels. We speculate that these findings are not simply evidence that the proximal vessels are vasoactive, but rather that the entire arterial structure of the cerebrovasculature is responsive to changes in arterial blood gases. This finding requires validation with higher resolution imaging modalities through a normal range of physiological changes, but if true, represents a new paradigm in our understanding of human CBF regulation.

Methodological considerations

Assessment of reactivity: absolute versus relative comparisons. Our findings exemplify the marked differences in P_{aCO_2} and P_{aO_2} reactivity between vessels if absolute flow/velocity values are compared directly (Figs 2 and 3). Because different vessels have inherently

different baseline flow through them, the use of absolute values for reactivity calculation yields values that are largely dependent on the baseline flow. Because there is no analytical interpretational standard for comparison of vessel reactivities, we recommend both absolute and relative reactivities be shown.

Pressure–flow relationships across the cerebrovasculature

The brain is able to modulate its flow to some degree in the face of changes in arterial pressure (Ainslie & Tzeng, 2010), an ability conventionally termed cerebral autoregulation. However, it is likely that hypercapnia and hypoxia both impair cerebral autoregulation (Aaslid *et al.* 1989; Zhang *et al.* 1998; Subudhi *et al.* 2009). Figure 6 highlights this phenomenon, where CBF increases concomitantly with MAP during hypercapnia. Such findings are broadly consistent with a well-controlled experiment by Przybyowski and co-workers (2003) who reported that apnoea-induced elevations in MCAv were attenuated by ganglionic blockade that blunted increases in MAP. However, in this study, we cannot distinguish between the effects of P_{aCO_2} or elevations in MAP *per se* because they occur concurrently, hypertension being caused by the chemoreflex response to hypercapnia. Although the MAP increase during hypoxia is much lower than with hypercapnia, it is interesting to note the lack of obvious MAP–CBF relationship during hypoxia.

Flow versus velocity with TCD

During both hypercapnia and hypoxia, our findings clearly show a greater increase in flow in the ICA and VA flow compared with velocity changes in the MCA and PCA, respectively. Bland–Altman plots (Fig. 7) reveal a systematic underestimation of flow via TCD proportional to the higher ranges of CBF during hypercapnia and hypoxia. As we observed greater CO_2 reactivity in the ICA, and others have shown high CO_2 reactivity in the M2 segment of the MCA (Giller *et al.* 1993), we feel it is likely the diameter of the MCA changes with P_{aCO_2} . There are two salient points: there is now ample support in the literature to preclude the assumption of constant cerebral vessel diameter at extremes of blood gas changes (Giller *et al.* 1993; Wilson *et al.* 2011), and these cerebral vessel characteristics need to be directly characterized with the most sophisticated imaging modalities available.

End-tidal vs. arterial P_{O_2} and P_{CO_2}

A strength of our study is that we targeted desired P_{ETCO_2} and P_{ETO_2} and confirmed P_{aCO_2} and P_{aO_2} by arterial blood samples. Breathing via a sequential gas delivery circuit (Slessarev *et al.* 2007) reduces the CO_2 and O_2

alveolar–arterial difference in the lung (Ito *et al.* 2008), but this has not been assessed at the extreme values presented in this study. This validation is important, especially during curve fitting, since P_{ETCO_2} is known to underestimate P_{aCO_2} at rest (Robbins *et al.* 1990) and overestimate P_{aCO_2} during breathing of hypercapnic air in both animals (Tojima *et al.* 1988) and humans (Peebles *et al.* 2007). Our data indicate that P_{ETCO_2} overestimates P_{aCO_2} through the entire hypercapnic range (Fig. 1). Thus, arterial blood gas collection is necessary for the true representation and physiological interpretation of cerebrovascular reactivity to O_2 and CO_2 , at the extremes of physiological tolerance to hypoxia, alkalosis and acidosis presented here.

Assessment of blood flow

Although a number of previous studies have measured blood flow in the ICA and VA at rest and during exercise (Sato & Sadamoto, 2010; Sato *et al.* 2011), this is the first report to integrate novel edge detection and wall tracking software (Black *et al.* 2008). This software allows operator-independent simultaneous assessment of conduit artery diameter changes and velocity, and thus quantitative assessment of blood flow through the arteries of interest at high resolution (30 Hz). We were, however, limited to near-concurrent insonation of the right ICA and left VA, and cannot therefore account for side-to-side differences in flow between the arteries. Indeed, Schoning (1994) showed no difference between the right and left ICA, whereas the right VA (the vessel measured in the present study) was found to have a ~20% lower flow than the left VA. Regardless of potential differences in flows, we are unaware if any data suggesting there are bilateral differences in reactivity between the internal carotid arteries or vertebral arteries. Thus, we feel that our technological limitation of only being able to measure one ICA and one VA is unlikely to detract from our main study conclusions.

Curve fitting

As used extensively elsewhere for steady-state changes in P_{aCO_2} (Ainslie & Duffin, 2009) and S_{aO_2} (Jensen *et al.* 1996), we used linear regression to determine the relationship with CBF/CBV (R^2 mean: 0.86 ± 0.07). In the case of P_{aCO_2} , this approach allows both the determination of the full reactivity to P_{aCO_2} and for hypercapnia and hypocapnia separately. Because the cellular mechanisms are likely to differ between the hypercapnia vs. hypocapnia ranges (reviewed in Ainslie & Duffin, 2009), consideration of them separately is important, especially when changes in both ranges are not comparable in many aspects of pathophysiology (Xie *et al.* 2005),

physiology (e.g. with exercise (Ogoh *et al.* 2009), and high altitude (Lucas *et al.* 2010b)). However, our capacity for more complex curve fitting was consequently limited. Although technically challenging because of the prolonged vessel imaging necessitated, future studies are now needed to compare volumetric flow measures in the ICA and VA during Duffin-type rebreathing (Battisti-Charbonney *et al.* 2011).

In summary, we have characterized regional cerebrovascular responses through the widest range of P_{aCO_2} and P_{aO_2} reported in the literature to date. This is the first study in humans to implicate the large cerebral vessels of the neck in blood gas related modulation of CBF, and moreover identify regional differences in neck artery blood-gas reactivity. That the VA demonstrated greater sensitivity to hypoxia and to CO_2 in the hypocapnic range has significant implications for our understanding of brainstem control of respiratory and autonomic function. These data need to be considered in future studies of cerebrovascular function, as the long-held paradigm that blood flow regulation is modulated solely at the pial arterioles is likely to be an inadequately simplistic regulatory model.

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Author contributions

Part A of the study was completed at the University of British Columbia Okanagan, Part B at Duke University Medical Center. C.K.W. and P.N.A. were involved in the conception and design of the study, data collection, analysis and interpretation, and drafted and revised the manuscript. D.B.M., A.D.S., K.J.S., K.I., J.G., and N.C.L. were involved in data collection and interpretation, and revision of the manuscript. Y.C.T., N.D.E. and T.A.D. contributed to the intellectual content of the manuscript. All authors have approved the final version for publication.

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