Human cerebrovascular and ventilatory CO_2 reactivity to end-tidal, arterial and internal jugular vein P_{CO_2}

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This study examined cerebrovascular reactivity and ventilation during step changes in CO_2 in humans. We hypothesized that: (1) end-tidal P_{CO_2} (P_{ET,CO_2}) would overestimate arterial P_{CO_2} (P_{a,CO_2}) during step variations in P_{ET,CO_2} and thus underestimate cerebrovascular CO_2 reactivity; and (2) since P_{CO_2} from the internal jugular vein (P_{jv,CO_2}) better represents brain tissue P_{CO_2}, cerebrovascular CO_2 reactivity would be higher when expressed against P_{jv,CO_2} than with P_{a,CO_2}, and would be related to the degree of ventilatory change during hypercapnia. Incremental hypercapnia was achieved through 4 min administrations of 4% and 8% CO_2. Incremental hypocapnia involved two 4 min steps of hyperventilation to change P_{ET,CO_2}, in an equal and opposite direction, to that incurred during hypercapnia. Arterial and internal jugular venous blood was sampled simultaneously at baseline and during each CO_2 step. Cerebrovascular reactivity to CO_2 was expressed as the percentage change in blood flow velocity in the middle cerebral artery (MCAv) per mmHg change in P_{a,CO_2} and P_{jv,CO_2}. During hypercapnia, but not hypocapnia, P_{ET,CO_2} overestimated P_{a,CO_2} by $+2.4 \pm 3.4$ mmHg and underestimated MCAv-CO_2 reactivity ($P < 0.05$). The hypercapnic and hypocapnic MCAv-CO_2 reactivity was higher ($\sim$97% and $\sim$24%, respectively) when expressed with P_{jv,CO_2} than P_{a,CO_2} ($P < 0.05$). The hypercapnic MCAv–P_{jv,CO_2} reactivity was inversely related to the increase in ventilatory change ($R^2 = 0.43$; $P < 0.05$), indicating that a reduced reactivity results in less central CO_2 washout and greater ventilatory stimulus. Differences in the P_{ET,CO_2}, P_{a,CO_2} and P_{jv,CO_2}–MCAv relationships have implications for the true representation and physiological interpretation of cerebrovascular CO_2 reactivity.

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Measurement of cerebrovascular reactivity to CO_2 has been widely applied in clinical practice to evaluate cerebral vascular function – e.g. in patients with carotid artery stenosis (Widder *et al.* 1994), hypertension (Serrador *et al.* 2005), stroke (Wijnhoud *et al.* 2006) and heart failure (Xie *et al.* 2005), and a related impairment has been linked to cerebral ischaemic events (Cosentino & Volpe, 2005; Wijnhoud *et al.* 2006). The acute manipulation of P_{a,CO_2} through hyperventilation has been used as an intervention to rapidly reduce intracranial pressure or to adjust cerebral blood flow (CBF) to metabolic needs. Furthermore, changes in cerebrovascular CO_2 reactivity affect stability of the ventilatory responsiveness to CO_2 via alterations in the degree of washout in central chemoreceptor hydrogen $[H^+]$; these changes have been documented in a range of physiological (Cummings *et al.* 2007) and pathophysiological (Xie *et al.* 2005; Ainslie *et al.* 2007) conditions.

In most instances CBF reactivity is expressed as the percentage change in CBF per mmHg change in P_{a,CO_2}, or end-tidal CO_2 (P_{ET,CO_2}) obviating the more invasive P_{a,CO_2} measurement. The tight correlation between the percentage of change in middle cerebral artery blood flow velocity (MCAv) measured by transcranial Doppler ultrasonography during P_{ET,CO_2} variations (Markwalder *et al.* 1984; Ide *et al.* 2003) has encouraged the use of transcranial Doppler (TCD) ultrasonography to measure CO_2 cerebrovascular reactivity. However, several considerations are important when using P_{a,CO_2} (or P_{ET,CO_2}) to investigate cerebral blood flow reactivity. First, P_{ET,CO_2} has been shown to underestimate P_{a,CO_2} at rest (Robbins *et al.* 1990) and to overestimate P_{a,CO_2} during exercise (Jones *et al.* 1979; Robbins *et al.* 1990). Similarly, a positive $P_{ET,CO_2}-P_{a,CO_2}$ gradient is seen in animals exposed to increased CO_2 (Jennings & Chen, 1975; Oliven *et al.* 1985; Tojima *et al.* 1988). Such alterations in the

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P_{a,CO_2}–P_{ET,CO_2} relationship may have implications for the true representation and physiological interpretation of cerebrovascular reactivity to CO_2. In humans, it is not known how the P_{a,CO_2}–P_{ET,CO_2} relationship is altered throughout the hypercapnic and hypocapnic range. To address these inaccuracies, Jones *et al.* (1979) developed a regression equation using P_{ET,CO_2} and tidal volume to provide an estimate of P_{a,CO_2} (eP_{a,CO_2}) during exercise to compensate for the overestimation of P_{a,CO_2} by P_{ET,CO_2}. Thus, we reasoned that since hypercapnia evokes an increase in ventilation (and tidal volume), this empirical equation could also be used to estimate P_{a,CO_2} during step changes in P_{ET,CO_2}. Therefore, cerebrovascular CO_2 reactivity during hypercapnia and hypocapnia could be expressed in three different ways (P_{a,CO_2}, eP_{a,CO_2} and P_{ET,CO_2}) and compared accordingly.

The second consideration when investigating CBF reactivity is whether cerebrovascular reactivity to CO_2 might be even better expressed as a percentage of brain tissue P_{CO_2}. This idea evolves from a study by Shapiro and colleagues who showed that changes in CBF corelated more closely with jugular venous CO_2 tension (P_{jv,CO_2}) than P_{a,CO_2} ($r = 0.83$ *versus* $r = 0.72$, respectively), suggesting that P_{a,CO_2} is not the effective stimulus for cerebral vasodilatation (Shapiro *et al.* 1966). In contrast, Severinghaus & Lassen (1967) provided data to indicate that brain tissue P_{CO_2} (based on P_{jv,CO_2}) was not the ultimate determinant of CBF during a single step of hypocapnia, and that P_{a,CO_2} – or arterial wall P_{CO_2} – may have an important role. Whether there is a differential control of CBF via brain tissue P_{CO_2} or P_{a,CO_2} during hypercapnia and hypocapnia remains to be established. With respect to ventilation, however, it is clear that the central contribution to the ventilatory response to CO_2 is determined not by P_{a,CO_2} but by changes in brain tissue P_{CO_2} and $[H^+]$ (Ahmad & Loeschcke, 1982; Smith *et al.* 2006). Thus, the stimulus at the central chemoreceptor level might also be better represented by the P_{CO_2} of the venous cerebral outflow (Fencl, 1986; Xie *et al.* 2006).

Given the outlined literature, it would appear that potential difference in cerebrovascular reactivity when expressed against P_{a,CO_2} or P_{jv,CO_2}, and the potential relationship to CO_2 ventilatory drive, has not been fully described in humans. Therefore the aims of this study were (1) to compare the accuracy of P_{ET,CO_2} and eP_{a,CO_2} for predicting P_{a,CO_2} during step hyper- and hypocapnia changes, and (2) to compare CBF and ventilatory sensitivities to P_{a,CO_2}, P_{jv,CO_2}, eP_{a,CO_2} and P_{ET,CO_2}. Based on the aforementioned studies, we tested two original hypotheses: first, that P_{ET,CO_2} but not eP_{a,CO_2} would overestimate P_{a,CO_2} during step changes in P_{CO_2}, and therefore result in apparent lower cerebrovascular CO_2 reactivity; and second that because the changes in cerebral vascular tone occurring with changes in CO_2 tension serve to limit changes in brain tissue P_{CO_2} and thus P_{jv,CO_2}, both P_{ET,CO_2} and P_{a,CO_2} reactivities would be lower when compared with P_{jv,CO_2}. We also reasoned that, if MCAv–P_{jv,CO_2} reactivity determines brain P_{CO_2}, in those individuals with a low MCAv–P_{jv,CO_2} reactivity there would theoretically be less CO_2 washout at the level of the central chemoreceptors and therefore a greater ventilatory stimulus.

Methods

Subjects

Twelve healthy individuals (aged 27 ± 4 years (mean ± S.D.), body mass index $24 \pm 4\,kg\,m^{-2}$, 10 male, 2 female) volunteered for this study, which was approved by the Lower South Regional Ethics committee and conformed to the standards set by the Declaration of Helsinki. All participants received verbal and written explanations of the experimental procedures, including risks involved in the study and written informed consent was obtained. Participants were non-smokers and not on any medication and none had a known history of cardiovascular, neurological or respiratory disease.

Experimental design

Following full familiarization of each subject to the experimental protocol (excluding cannulation), participants arrived at the laboratory (> 1 week) having abstained from exercise and alcohol for 24 h, and having not consumed items containing caffeine, or a heavy meal for 4 h.

Cannulation of internal jugular vein and radial artery

Following placement of three-lead ECG and peripheral O_2 saturation monitor, participants were positioned in the Trendelenberg position for placement of the internal jugular vein catheter under local anaesthesia (1% lidocaine). A 16-gauge, 5 inch catheter (Arrow International) was advanced to the right jugular bulb using the Seldinger technique and the position was confirmed by ultrasound. Another catheter (20 gauge BD Insyte) was placed, under local anaesthesia into the radial artery. Both catheters were regularly infused with normal saline (0.9% NaCl) to maintain patency. After cannulation participants rested quietly in the supine position, breathing room air, for at least 30 min to allow the setup of monitoring equipment (see below). During this time the participants also acclimatized to the breathing apparatus, after which baseline measurements were obtained. Participants then underwent the tests of cerebrovascular reactivity to CO_2 described below.

Experimental protocol

CO_2 vasoreactivity. Incremental hypercapnia was induced by switching the inspired gas from room air to 4% CO_2 (in 21% O_2 with the balance N_2; Hypercapnia I) for 4 min, then 8% CO_2 (in 21% O_2 with the balance N_2; Hypercapnia 2) for 4 min. The P_{ET,CO_2} was recorded during the final 30 s of each hypercapnic exposure. Following the incremental hypercapnia, subjects breathed room air to ensure that parameters returned to baseline values. Participants were then instructed to increase their rate and depth of breathing to generate two levels (4 min each level) of decremental hypocapnia to match, in an equal and opposite direction, the rise in P_{ET,CO_2} incurred during the incremental hypercapnia steps. Training for the voluntary hyperventilation was performed during the familiarization session, during which verbal feedback was provided to assist subjects to reach and maintain the target levels of hyperventilation. In the current study, the hypercapnia steps were conducted first to allow the individual changes in P_{ET,CO_2} to be recorded in order to determine the required P_{ET,CO_2} changes needed for the two hypocapnia steps. This order was important since previous reports indicate that prior hypocapnia (but not prior hypercapnia) may cause persistent cerebral vasoconstriction, thus influencing the normal MCAv-CO_2 response to hypercapnia (Ide *et al.* 2003). Although it has been reported that prior hypercapnia does not influence the CBF response to hypocapnia (Ide *et al.* 2003), full recovery was permitted between the hyper- and hypocapnia tests to rule out any prior influence of the hypercapnia. Cerebrovascular reactivity to CO_2 was expressed as the percentage (%) change in MCAv per mmHg change in P_{ET,CO_2}, P_{a,CO_2}, an empirical equation involving P_{ET,CO_2} and tidal volume (eP_{a,CO_2}) or the P_{jv,CO_2}. As used in other studies, MCAv was expressed at the percentage change from the baseline to allow between-study comparisons and to reduce inter-individual variability that is unrelated to the experimental manipulation (Ide *et al.* 2003; Xie *et al.* 2005; Ainslie *et al.* 2007; Cummings *et al.* 2007). The eP_{a,CO_2} was calculated by using the regression equation developed by Jones *et al.* (1979):

$$eP_{a,CO_2} = 5.5 + 0.9P_{ET,CO_2} - 2.1V_T$$

where tidal volume (V_T) is in litres.

Monitoring equipment

Blood flow velocity in the right middle cerebral artery was measured using a 2 MHz pulsed Doppler ultrasound system (DWL Doppler, Sterling, VA, USA) using search techniques described elsewhere (Aaslid *et al.* 1982). Beat-to-beat arterial blood pressure and heart rate were monitored using finger photoplethysmography (Finometer, TPD Biomedical Instrumentation, the Netherlands) and ECG, respectively. End-tidal CO_2 was sampled from a leak-free mask and measured by a gas analyser (model CD-3A CO_2 analyser, AEI Technologies, Pittsburgh, PA, USA). All data were acquired continuously at 200 Hz using an analog-to-digital converter (Powerlab/16SP ML795; ADInstruments, Colorado Springs, CO, USA) interfaced with a computer and were subsequently analysed using commercially available software (Chart version 5.02, ADInstruments).

Analytical measurements

Blood samples were drawn simultaneously from the arterial and jugular catheters. Timed collections were drawn twice (separated by 10 min) during baseline air breathing and during the final 30 s of each of the hypercapnic and hypocapnic challenges. Before procuring samples 1–2 ml of arterial and venous blood was aspirated from the catheter's dead space and disregarded. Arterial and venous blood was then drawn slowly over a 20 s period through the 20 G cannulas to ensure adequate flow and to avoid haemolysis. Immediately after acquisition, the arterial and venous blood samples were transferred to capillary tubes for measurement of arterial and venous blood gases (NPT7 Series, Radiometer, Copenhagen). Commercial standards were used to calibrate the blood gas analyser before starting the tests. The reproducibility of the blood gas measurements (and P_{ET,CO_2}) at rest was assessed from the coefficient of variation of the difference between the two baseline collections divided by $\sqrt{2}$ (Atkinson & Nevill, 1998). The within-test coefficient of variation for P_{ET,CO_2}, P_{a,CO_2} and P_{jv,CO_2} was $< 0.5\%$.

Statistical analysis

All data were analysed using the SPSS statistics package (SPSS Inc., Chicago, IL, USA). End-tidal CO_2 and eP_{a,CO_2} were averaged over the 20 s period during the simultaneous arterial and venous blood draws. Agreement between the indirect estimates of P_{a,CO_2} (P_{ET,CO_2}, eP_{a,CO_2}) with actual P_{a,CO_2} during the step changes in CO_2 was assessed in a number of ways. (1) Using the final data points for each incremental change in CO_2, a one-way ANOVA was used to assess the statistical differences to compare absolute change in P_{a,CO_2} with the indirect estimates (Table 1). The Bonferroni–Dunn test was used for *post hoc* analysis when a significant effect was found. (2) The bias (mean absolute difference between P_{a,CO_2}) (Bland & Altman, 1986) and relative bias (bias/P_{a,CO_2} value $\times$ 100%) of the end-tidal and eP_{a,CO_2} values at baseline and during the last 30 s of each step change in CO_2. Additionally, the 95% limits of agreement were calculated as $\pm 1.96 \times$ S.D. of the bias (Bland &

Table 1. Haemodynamic and respiratory changes during step changes in P_{CO_2}

	Baseline	+ 4%	+ 8%	Hypo 1	Hypo 2
MCAv (cm s^{-1})	61 ± 15	71 ± 16	107 ± 23*†	52 ± 15*	40 ± 12*†
P_{a,CO_2} (mmHg)	40 ± 4	44 ± 4	54 ± 4*†	34 ± 3.1*	23.0 ± 4.3*†
P_{ET,CO_2} (mmHg)	42 ± 3	47 ± 3	60 ± 3.0*†	36 + 4*	23 + 4.0*†
eP_{a,CO_2} (mmHg)	42 ± 3	46 ± 3*	55 ± 3*†	36 ± 4*	24 ± 4*†
P_{jv,CO_2} (mmHg)	50 ± 6	52 ± 4	58 ± 5*†	44 ± 4*	41 ± 7*†
T_V (l)	0.6 ± 0.3	0.9 ± 0.4*	2 ± 0.3*†	0.9 ± 0.5*	1.2 ± 0.5*
Ventilation (l min^{-1})	7 ± 2	12 ± 4	33 ± 13*†	9 ± 3	24 ± 9*†
MAP (mmHg)	88 ± 9	89 ± 9	98 ± 11	86 ± 9	80 ± 15
HR (b min^{-1})	59 ± 10	62 ± 9	74 ± 8	59 ± 7	69 ± 8

Values are mean ± S.D. P_{ET,CO_2}, end-tidal CO_2; eP_{a,CO_2}, regression equation using both end-tidal P_{CO_2} and tidal volume to estimate P_{a,CO_2}; P_{a,CO_2}, arterial P_{CO_2}. *Different from baseline ($P < 0.05$); †different from + 4 and hypo 1 ($P < 0.05$).

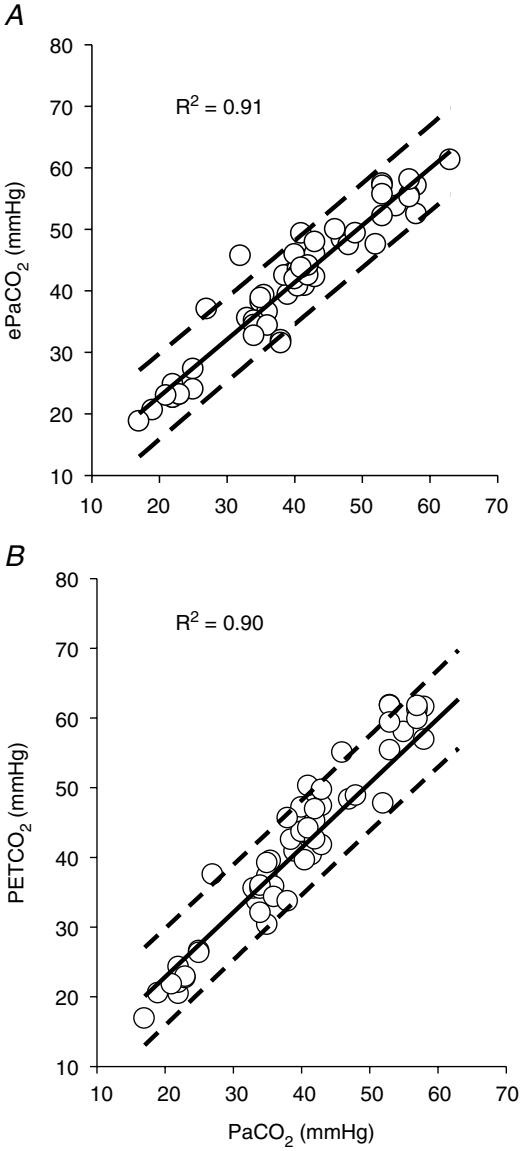

Figure 1. Pooled linear regressions for eP_{a,CO_2} (*A*) and end-tidal CO_2 (*B*) against P_{a,CO_2}
Data points were obtained at baseline and during the last 30 s of each step change in P_{CO_2}. Dotted lines represent 95% confidence intervals.

Altman, 1986). The slope of MCAv with P_{a,CO_2}, P_{ET,CO_2} and eP_{a,CO_2} was determined by the least squares linear regression analysis for each subject in the hyper- and hypocapnic range. Independent analysis confirmed that, in the current study, the relationship between MCAv–P_{CO_2} change (end-tidal, arterial or jugular vein) was better described as a linear response ($R^2 > 0.89$), rather than exponential ($R^2 < 0.80$) or sigmoidal ($R^2 < 0.85$). Stepwise linear regression equations were constructed with P_{a,CO_2} or P_{jv,CO_2} as the dependant variables to determine how well these correlated with P_{ET,CO_2}. When each P_{ET,CO_2} value was regressed against their corresponding P_{a,CO_2} or P_{jv,CO_2} tensions, correlation coefficients of $R^2 = 0.92$ and $R^2 = 0.79$, respectively, were found ($P < 0.05$). It was revealed that when multiple regression analysis was performed, addition of any other physiological variable (e.g. frequency, ventilation, tidal volume) as a second independent variable did not improve the correlation. Thus, based upon the P_{ET,CO_2} responses, a multiple regression equation was constructed to predict P_{a,CO_2} and P_{jv,CO_2}. Previous data ($n = 14$; Ainslie *et al.* unpublished data) from our laboratory in which P_{a,CO_2} and P_{ET,CO_2} were sampled (using the same experimental set-up in the supine posture) over a range of P_{a,CO_2} values (23–63 mmHg; 69 sample points), was used to validate the regression equation. All group data are expressed as a means ± standard deviation (S.D.). Significance was established at an alpha level of $P < 0.05$.

Results

Changes during hyper- and hypocapnia

Incremental hypercapnia caused a progressive increase in P_{a,CO_2} and MCAv whereas decremental hypocapnia caused a progressive decline in P_{a,CO_2} and MCAv (Table 1). The P_{jv,CO_2} was higher than P_{a,CO_2}, P_{ET,CO_2} and eP_{a,CO_2} at baseline and during the step changes in P_{CO_2} (Table 1). There were no significant changes in either mean blood

pressure or heart rate during incremental hypercapnia or hypocapnia (Table 1).

Agreement of P_{ET,CO_2} and eP_{a,CO_2} with P_{a,CO_2} during changes in P_{CO_2}

Pooled linear regressions for P_{ET,CO_2} and eP_{a,CO_2} with P_{a,CO_2} during hyper- and hypocapnia using the individual values obtained for each step change in P_{CO_2} are shown in Fig. 1. Bland and Altman analyses were carried out to compare the estimates (P_{ET,CO_2} and eP_{a,CO_2}) of P_{a,CO_2} the data throughout the entire range of changes in P_{CO_2} for all the subjects. The Bland and Altman plot is presented for each indirect method in Fig. 2. The $P_{ET,CO_2}-P_{a,CO_2}$ difference was greater than the e$P_{a,CO_2}-P_{a,CO_2}$ difference (2.4 ± 3.4 mmHg *versus* 1.4 ± 4.5 mmHg; $P < 0.0.5$), indicating an overestimation of P_{a,CO_2} by P_{ET,CO_2}. This overestimation occurred at baseline and during hypercapnia, but not during hypocapnia (Table 2). At baseline, the limits of agreement (± 1.96 s.d. of the difference between estimated and actual P_{a,CO_2}) were not different between eP_{a,CO_2} (2.01 ± 3.72 mmHg) and P_{ET,CO_2} (1.95 ± 4.1 mmHg). Similarly, at baseline the relative bias for eP_{a,CO_2} and P_{ET,CO_2} was $4.9 \pm 2.1\%$ and $5.0 \pm 3.27\%$, respectively. However, during incremental hypercapnia, in contrast to hypocapnia, there was a progressive increase in both absolute and relative bias of P_{ET,CO_2} which was not seen with eP_{a,CO_2} (Table 2). Stepwise multiple regression yielded the following equations to better predict P_{a,CO_2} or P_{jv,CO_2} by P_{ET,CO_2}:

$$P_{a,CO_2} = 2.367 + 0.884 P_{ET,CO_2} \quad (1)$$

$$P_{jv,CO_2} = 19.576 + 0.705 P_{ET,CO_2} \quad (2)$$

Addition of another physiological variable (e.g. frequency, ventilation, tidal volume) as a second independent variable did not increase the precision of

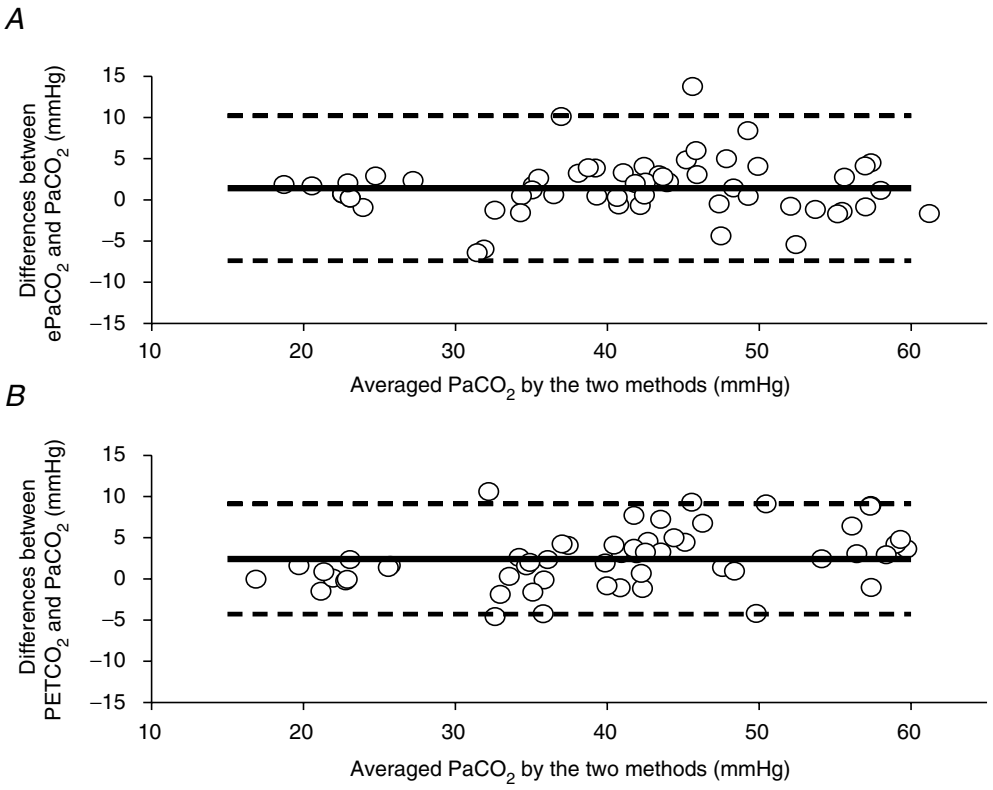

Figure 2. Bland and Altman plot of differences between estimates (eP_{a,CO_2}, *A*; end-tidal CO_2, *B*) and measured P_{a,CO_2} for the whole group of subjects
Dotted lines represent 95% confidence intervals and continuous line represents the mean bias. Individual points in the hypocapnic range are clustered near zero illustrating that there is little difference between eP_{a,CO_2} (*A*) or P_{ET,CO_2} (*B*) and P_{a,CO_2} in this range. However, in the hypercapnic range the spread of values increases, indicating the values of the estimates vary further from measured P_{a,CO_2} values. This is clearly seen in the increase in bias in the hypercapnic steps in Table 1. These plots as well as the values in Table 1 also show that in the hypocapnic range P_{ET,CO_2} seems to better agree with measured P_{a,CO_2} values (lower bias), whereas in the hypercapnic range eP_{a,CO_2} agrees better with measured P_{a,CO_2} values than P_{ET,CO_2}.

Table 2. Comparison of absolute and relative bias of estimate of P_{CO_2} at baseline and during step changes in P_{CO_2}

	P_{a,CO_2}	P_{ET,CO_2}	eP_{a,CO_2}
Baseline			
Mean ± s.d. (mmHg)	40 ± 4	42 ± 3*	42 ± 3*
B ± lA (mmHg)	—	2 ± 4	2 ± 4
RB ± s.d. (%)	—	5 ± 2	5 ± 3
4% CO_2			
Mean ± s.d. (mmHg)	43 ± 4	47 ± 3*	46 ± 3
B ± lA (mmHg)	—	4 ± 7	2 ± 6
RB ± s.d. (%)	—	8 ± 4	5 ± 3
8% CO_2			
Mean ± s.d. (mmHg)	55 ± 4	60 ± 3*†	56 ± 3
B ± lA (mmHg)	—	5 ± 6	0.2 ± 6
RB ± s.d. (%)	—	9 ± 3	0.4 ± 3
Hypo 1			
Mean ± s.d. (mmHg)	35 ± 3	36 ± 4	34 ± 3
B ± lA (mmHg)	—	2 ± 9	2 ± 12
RB ± s.d. (%)	—	5 ± 5	5 ± 6
Hypo 2			
Mean ± s.d. (mmHg)	23 ± 4	23 ± 4	24 ± 4
B ± lA (mmHg)	—	0.3 ± 3	0.9 ± 3
RB ± s.d. (%)	—	1 ± 1	4 ± 1

Values are mean ± s.d. P_{ET,CO_2}, end-tidal CO_2; eP_{a,CO_2}, regression equation using both end-tidal P_{CO_2} and tidal volume to estimate P_{a,CO_2}; P_{a,CO_2}, arterial P_{CO_2}; s.d., standard deviation; RB, relative bias; B, bias; LA, 95% limits of agreement (±1.96 × s.d. of the bias). *Different from P_{a,CO_2} ($P < 0.05$); †different from eP_{a,CO_2} ($P < 0.05$).

either regression equation. The developed regression equation was used to predict P_{a,CO_2} from P_{ET,CO_2} from previously collected data in our laboratory using the same experimental set-up in the supine posture. Predicted values for P_{a,CO_2} were close to the measured values ($R^2 = 0.97$; $P < 0.05$): the comparison of the predicted and measured values for P_{a,CO_2} showed a s.d. of 0.87 Torr.

Comparison of cerebrovascular and ventilatory CO_2 reactivity determined with end-tidal, arterial P_{CO_2} or internal jugular vein P_{CO_2}

The MCAv-CO_2 reactivity in the hyper- and hypocapnic range as determined with P_{a,CO_2}, P_{ET,CO_2}, eP_{a,CO_2} or P_{jv,CO_2} is displayed in Fig. 3. In both the hyper- and hypocapnia range, MCAv–P_{jv,CO_2} reactivity was higher than with all other methods. Moreover, this reactivity was ∼97% greater in the hypercapnia range when compared with a ∼24% increase in the hypocapnia range. During hypercapnia, in contrast to hypocapnia, the slope of the MCAv–P_{ET,CO_2} relationship was smaller than the slopes of both the MCAv–eP_{a,CO_2} and MCAv–P_{a,CO_2} relationships ($P < 0.05$; Fig. 3). The slope of the ventilatory response to hypercapnia was also steeper when presented against P_{jv,CO_2} than P_{ET,CO_2} or P_{a,CO_2} ($3.0 \pm 1.8\,l\,min^{-1}\,mmHg^{-1}$, $1.6 \pm 0.8\,l\,min^{-1}\,mmHg^{-1}$ and $1.7 \pm 0.9\,l\,min^{-1}\,mmHg^{-1}$, respectively; $P < 0.05$; Fig. 4). In addition to the elevation in the slope of MCAv–P_{jv,CO_2}, as expected there was a significant rightward shift in the x-axis intercept of the ventilation–P_{jv,CO_2} plot when compared with the ventilation–P_{a,CO_2} and ventilation–P_{ET,CO_2} plot (46.3 ± 6.8 mmHg, 34.6 ± 4.9 mmHg and 37.0 ± 4.8 mmHg, respectively; $P < 0.05$). During hypercapnia, the MCAv–P_{jv,CO_2} reactivity was related to the degree of ventilatory change ($R^2 = 0.43$; $P < 0.05$). No relationships were evident between MCAv–P_{a,CO_2} reactivity and the degree of ventilatory change.

Discussion

The novel integration of ventilation, CBF, and end-tidal, arterial and jugular vein P_{CO_2} during step steady-state changes in CO_2 has produced three new findings: (1) there is a systemic overestimation of P_{a,CO_2} by P_{ET,CO_2} during hypercapnia but not hypocapnia; (2) cerebrovascular and ventilatory CO_2 reactivity to hypercapnia are underestimated by P_{ET,CO_2} when compared with P_{a,CO_2}, whereas both P_{ET,CO_2} and P_{a,CO_2} reactivities are lower when compared with P_{jv,CO_2}; and (3) during hypercapnia, the slope of the MCAv–P_{jv,CO_2} relationship was inversely related to the degree of ventilatory change, possibly because of the reduced CO_2 washout occurring with decreasing MCAv reactivity, leading ultimately a greater ventilatory stimulus at the central chemoreceptors. Collectively, these findings suggest that knowledge of the changes in cerebral tissue or venous P_{CO_2} is critical for the correct interpretation of data related to the sensitivities of both cerebral blood flow and ventilation to CO_2, as well as their corelationship.

Arterial versus end-tidal CO_2 monitoring

Our data indicate that caution is required when assuming that P_{ET,CO_2} accurately reflects P_{a,CO_2} during hypercapnia. To circumvent the problem that P_{ET,CO_2} significantly overestimates P_{a,CO_2} during exercise, a regression equation using both P_{ET,CO_2} and tidal volume to estimate P_{a,CO_2} was developed (Jones *et al.* 1979). It should be noted that the P_{ET,CO_2}–P_{a,CO_2} gradient is relatively fixed in one body position with constancy of respiratory rate, minute volume, and circulatory stability. A change in any of these variables affects the P_{ET,CO_2}–P_{a,CO_2} gradient; this has been quantified for changes in pulmonary dead-space (Bjurstedt *et al.* 1962) and with ventilation–perfusion ratio changes induced by standing (Immink *et al.* 2006), whereas the relationship between dynamic changes in pulmonary and circulatory variables and P_{ET,CO_2} is nonlinear and complex (Gisolf *et al.* 2004). Based on our data set, limited to the supine posture with controlled changes in CO_2,

we developed a new regression equation to provide a better estimate of P_{a,CO_2} during supine resting conditions ($P_{a,CO_2} = 2.367 + 0.884\ P_{ET,CO_2}$). In contrast to exercise (Jones *et al.* 1979), tidal volume was not required as a second independent variable to increase the precision of either regression equation. A possible reason for this difference is that the magnitude of the tidal volume change evoked by hypercapnia in the supine posture is lower than that during upright exercise and insufficient to affect the $P_{ET,CO_2}-P_{a,CO_2}$ difference.

Our observation that P_{ET,CO_2} overestimates P_{a,CO_2} during steady state increases in CO_2, is consistent with several human studies using CO_2 rebreathing (Denison *et al.* 1969*a*,*b*; Laszlo *et al.* 1971). Although this $P_{ET,CO_2}-P_{a,CO_2}$ overestimation was present at comparable time points to the current study, Laszlo *et al.* (1971) considered this to be a transient phenomenon since the P_{ET,CO_2} and P_{a,CO_2} were similar after 10 min. Also, in animals P_{ET,CO_2} overestimates P_{a,CO_2} during rebreathing (Guyatt *et al.* 1973) and steady-state methods (Jennings & Chen, 1975; Tojima *et al.* 1988) of CO_2 administration. Whilst it appears that a definitive mechanism for the $P_{ET,CO_2}-P_{a,CO_2}$ gradient has not been established, Gurtner & Traystman (1979) proposed the 'charged membrane hypothesis', which ultimately results in the production of CO_2 in the region of the negatively charged alveolar capillary wall and in turn creates a positive gradient for CO_2 between the alveolar gas and pulmonary arterial blood.

One noteworthy finding of the present study was that P_{ET,CO_2} was in good agreement with P_{a,CO_2} during hypocapnia. The physiological basis for this effect is unclear. Based on previous literature (Domino *et al.* 1993; Dorrington & Talbot, 2004), hypocapnia might be expected to increase the $P_{ET,CO_2}-P_{a,CO_2}$ gradient by increasing the venous admixture, an effect owing to

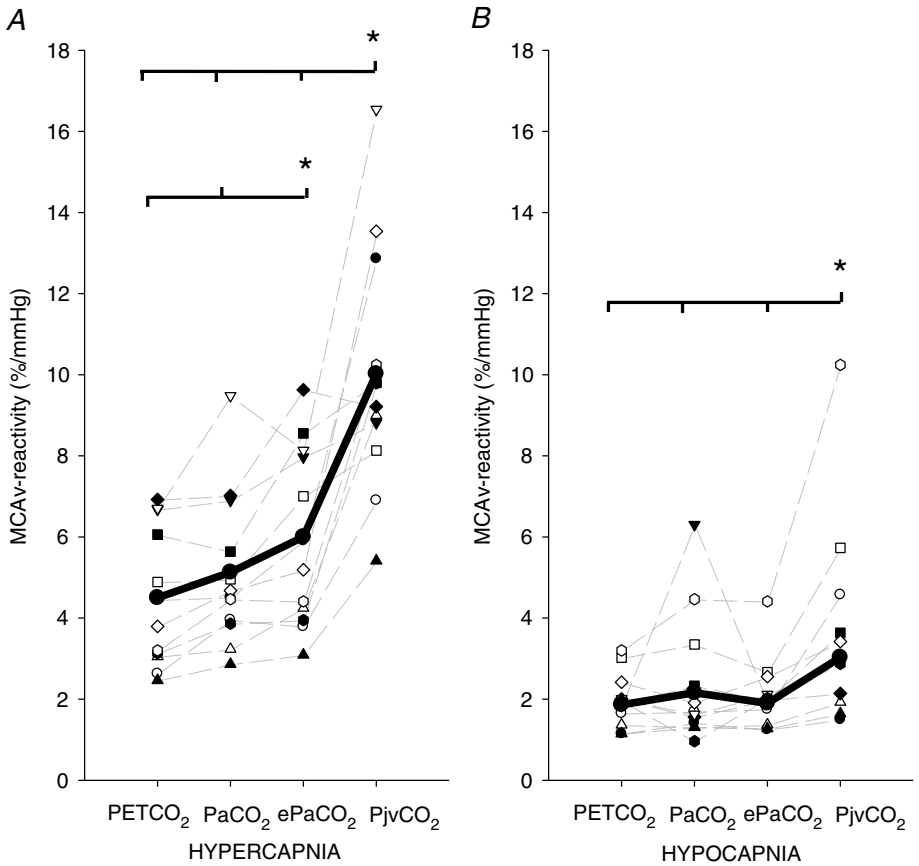

Figure 3. Calculation of cerebrovascular CO_2 reactivity using P_{ET,CO_2}, P_{a,CO_2}, eP_{a,CO_2} and P_{jv,CO_2} during hypercapnia (*A*) and hypocapnia (*B*)

Each faint line represents an individual reactivity. Bold lines represent group average. Note: P_{jv,CO_2}–MCAv reactivity was higher during both hypercapnia (~97%, *A*) and hypocapnia (~24%, *B*) when compared with P_{a,CO_2}–MCAv reactivity. There seemed to be three visual outliers in both *A* and *B*; however, upon closer examination, these data points are within 2 S.D. of the sample mean and therefore not classed as statistical outliers. Further, these data points do not reflect any non-compliance or technical error, and all other data (i.e. heart rate, MCAv, BP, end-tidal CO_2) were normal during each test.

hypocapnia-induced bronchoconstriction (Oliven *et al.* 1985) and pulmonary vasodilatation (Dorrington & Talbot, 2004). From our current data, we cannot provide further mechanistic insight to explain this disparity; however, because P_{a,CO_2} was highly reproducible at baseline, and selective changes occurred between hypercapnia (increase in $P_{ET,CO_2}-P_{a,CO_2}$ gradient) and hypocapnia (decrease in $P_{ET,CO_2}-P_{a,CO_2}$ gradient), it seems unlikely that this finding is due to technical artifact.

Arterial or internal jugular vein P_{CO_2} as the independant variable for cerebrovascular reactivity testing?

Our data are consistent with earlier human-based reports which have indicated that CO_2 inhalation causes an increase in internal jugular venous P_{CO_2} to a lesser degree than the corresponding increase in systemic arterial blood (Kety & Schmidt, 1948; Fencl, 1986). As mentioned, Shapiro *et al.* (1966) showed a higher correlation of jugular venous CO_2 tension, when compared with arterial P_{CO_2}, with the associated changes in CBF during elevations in CO_2, indicating that brain tissue CO_2 tension, rather than P_{a,CO_2}, provides a better correlate of the physiological stimulus. Conversely, during hypocapnia experimental data indicate that P_{a,CO_2} (or arterial wall P_{CO_2}) may have a more important role than P_{jv,CO_2} (Severinghaus & Lassen, 1967). In the current study, cerebrovascular CO_2 reactivity was higher in both the hyper- and hypocapnic range when presented against P_{jv,CO_2} when compared with P_{a,CO_2}. It is noteworthy that this increase in P_{jv,CO_2}–CO_2 reactivity was highest in the hypercapnic range (∼97%) compared with the hypocapnic range (∼24%). It is tempting to speculate that these hyper-/hypocapnic reactivity differences may reflect a differential control of CBF via brain tissue P_{CO_2} or P_{a,CO_2} during hypercapnia (Shapiro *et al.* 1966) and hypocapnia (Severinghaus & Lassen, 1967); however, it should be acknowledged that, whilst P_{jv,CO_2} is likely to be a closer index of brain tissue P_{CO_2} than P_{a,CO_2} (Fencl, 1986; Xie *et al.* 2006), clarifying the mechanisms involved is difficult knowing only arterial–venous P_{CO_2} gradients and not information related to the CO_2 flux across the brain.

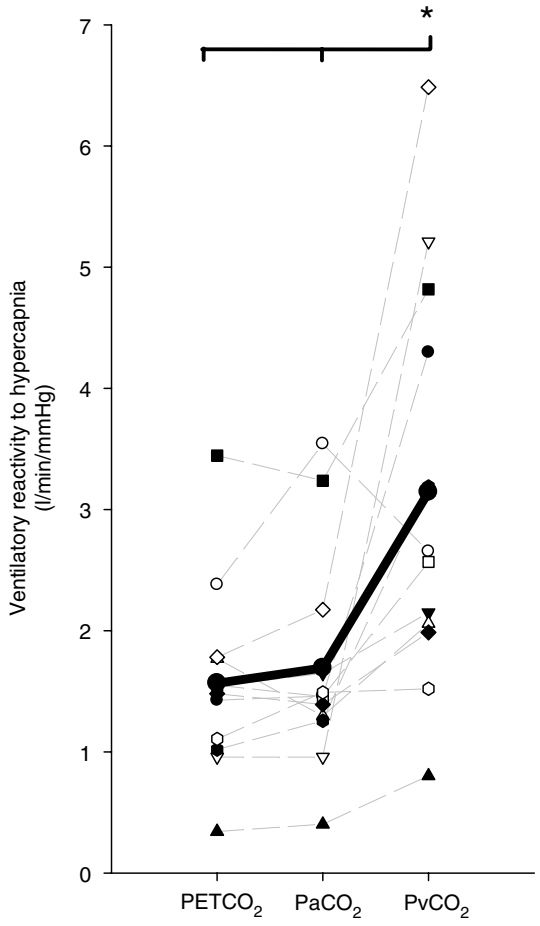

Figure 4. Calculation of ventilatory CO_2 reactivity using P_{ET,CO_2}, P_{a,CO_2}, eP_{a,CO_2} and P_{jv,CO_2} during hypercapnia
Each faint line represents an individual reactivity. Bold line represents group average.

Consistent with previous reports (Ide *et al.* 2003; Xie *et al.* 2005, 2006; Cummings *et al.* 2007), MCAv-CO_2 reactivity was greater in the hypercapnic range than the hypocapnic range (Fig. 4). One logical possibility is that the greater CBF response with hypercapnia limits the change in brain tissue P_{CO_2} and thus P_{jv,CO_2}. In the hypocapnic range, a reduced CBF reactivity suggests a greater change in P_{jv,CO_2} with changes in P_{a,CO_2} and thus a lesser slope in the P_{jv,CO_2}–CBF response (i.e. the denominator ($\Delta P_{jv,CO_2}$) for $\Delta MCAv/\Delta P_{jv,CO_2}$ gets higher). 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).

Integration of ventilation and cerebral blood flow

Changes in cerebrovascular CO_2 reactivity affect stability of the ventilatory responsiveness to CO_2 via alterations in the degree of washout in central chemoreceptor hydrogen $[H^+]$ (Xie *et al.* 2006; Ainslie *et al.* 2007; Cummings *et al.* 2007). In humans, to avoid the need for invasive procedures, these ventilatory and cerebrovascular responses to CO_2 are typically estimated using a change P_{a,CO_2} as an index. One important consideration is that the medullary central chemoreceptors are not stimulated directly by P_{a,CO_2}; rather, they are simulated by $[H^+]$ via alterations in brain tissue CO_2 tension. This stimulus at the central

chemoreceptor level is therefore more likely to be better represented by the P_{CO_2} of the venous cerebral outflow or by a mean of arterial and cerebral venous P_{CO_2} (Fencl, 1986; Xie *et al.* 2006). In further support of this, during hypercapnia, MCAv–P_{jv,CO_2} reactivity (but not MCAv–P_{a,CO_2} reactivity) was inversely related to the increase in ventilation ($R^2 = 0.43$; $P < 0.05$). Whilst not establishing cause-and-effect, we interpret these findings to indicate that, in those individuals with a reduced MCAv–P_{jv,CO_2}, there is less CO_2 washout at the level of the central chemoreceptors and therefore a greater ventilatory stimulus. If the changes in MCAv reflect the changes in the posterior circulation supplying the medulla (see Methodological considerations), then it seems reasonable to assume that differences in CBF reactivity and related CO_2 washout may cause changes in central chemoreceptor stimulation. These findings support the conclusion that cerebrovascular responsiveness to CO_2 is an important determinant of eupnoeic ventilation and of hypercapnic ventilatory responsiveness in humans, primarily via its effects at the level of the central chemoreceptors (Xie *et al.* 2006).

Methodological considerations

Although transcranial Doppler ultrasound measures flow velocity, rather than blood flow, in the MCA, the majority of research suggests that MCAv is a reliable index of regional CBF due to little or no change in the diameter of the MCA (Giller *et al.* 1993; Valdueza *et al.* 1997; Serrador *et al.* 2000). Our reported values for cerebrovascular CO_2 reactivity are consistent with those reported previously in healthy humans using MRI (van der Zande *et al.* 2005; Wise *et al.* 2007). An important consideration is whether steady-state MCAv was reached during the last minute of each of the 4 min hypo- hypercapnia steps. The MCAv changes and reactivity reported in this study are comparable with those of previous studies using 90 s increments in end-tidal CO_2 (Ide *et al.* 2003) or using 5 min incremental steps of inspired CO_2 (Xie *et al.* 2006), suggesting that the 240 s steps of hypercapnia is sufficiently long for the MCAv responses to unfold. With transcranial Doppler, it is possible to assess only the major changes in regional CBF-CO_2 reactivity; however, it is known that the cerebrovascular responses to CO_2 are qualitatively similar throughout the brain, although more pronounced in grey *versus* white matter and in cerebellar and brainstem regions *versus* cortical areas (Ramsay *et al.* 1993; van der Zande *et al.* 2005). It seems reasonable to suggest that changes in regional MCAv reactivity should reflect blood flow in other region of the brain, including the posterior circulation supplying the medulla, where the change of CO_2 and pH is directly sensed by the central chemoreceptors and thereby affects the respiratory neurons (Kawai *et al.* 1996). Previous data from our laboratory indicate that during step changes in P_{CO_2}, there is a close relationship ($R^2 = 0.82$; Ainslie *et al.* unpublished data) between the changes in MCAv (regional) and global CBF as determined using the Fick equation (CBF = cerebral metabolic oxygen consumption/arterio-venous O_2 content difference), assuming that cerebral metabolic oxygen consumption was unchanged during changes in P_{a,CO_2}. This technique and related assumptions has been reported in detail previously (Wasserman & Patterson, 1961; Shapiro *et al.* 1966; Severinghaus & Lassen, 1967). Therefore, in the present study, it seems reasonable to assume that changes in MCAv adequately reflect global changes in CBF.

In summary, there is a systematic overestimation of P_{a,CO_2} by P_{ET,CO_2} during step changes in P_{CO_2}; this overestimation results in a lower MCAv-CO_2 reactivity. Both P_{ET,CO_2} and P_{a,CO_2} reactivities, however, are lower when compared with MCAv–P_{jv,CO_2} reactivity. During hypercapnia, a lower MCAv–P_{jv,CO_2} reactivity may result in less CO_2 washout and greater ventilatory stimulus and the level of the central chemoreceptors. Differences in these relationships have implications for the true representation and interpretation of cerebrovascular CO_2 reactivity. These results may serve as the point of reference for 'normal' responses for comparison against pathological disorders.

References

Aaslid R, Markwalder TM & Nornes H (1982). Noninvasive transcranial Doppler ultrasound recording of flow velocity in basal cerebral arteries. *J Neurosurg* **57**, 769–774.

Ahmad HR & Loeschcke HH (1982). Transient and steady state responses of pulmonary ventilation to the medullary extracellular pH after approximately rectangular changes in alveolar PCO_2. *Pflugers Arch* **395**, 285–292.

Ainslie PN, Burgess K, Subedi P & Burgess KR (2007). Alterations in cerebral dynamics at high altitude following partial acclimatization in humans: wakefulness and sleep. *J Appl Physiol* **102**, 658–664.

Atkinson G & Nevill AM (1998). Statistical methods for assessing measurement error (reliability) in variables relevant to sports medicine. *Sports Med* **26**, 217–238.

Bjurstedt H, Hesser CM, Liljestrand G & Matell G (1962). Effects of posture on alveolar-arterial CO_2 and O_2 differences and on alveolar dead space in man. *Acta Physiol Scand* **54**, 65–82.

Bland JM & Altman DG (1986). Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* **1**, 307–310.

Cosentino F & Volpe M (2005). Hypertension, stroke, and endothelium. *Curr Hypertens Rep* **7**, 68–71.

Cummings KJ, Swart M & Ainslie PN (2007). Morning attenuation in cerebrovascular CO_2 reactivity in humans is associated with a lowered cerebral oxygenation and an augmented ventilatory response to CO_2. *J Appl Physiol* **102**, 1891–1898.

Denison D, Edwards R, Jones G & Pope H (1969*a*). Comparison of rebreathing estimates with direct measurements of mixed venous PCO_2 and PO_2 in man. *J Physiol* **202**, 75–76.

Denison D, Edwards R, Jones G & Pope H (1969*b*). Direct and rebreathing estimates of the O_2 and CO_2 pressures in mixed venous blood. *Respir Physiol* **7**, 326–334.

Domino K, Swenson E, Polissar N, Lu Y, Eisenstein B & Hlastala M (1993). Effect of inspired CO_2 on ventilation and perfusion heterogeneity in hyperventilated dogs. *J Appl Physiol* **75**, 1306–1314.

Dorrington K & Talbot N (2004). Human pulmonary vascular responses to hypoxia and hypercapnia. *Pflugers Arch* **449**, 1–15.

Fencl V (1986). *Acid–Base Blance in Cerebral Fluids*. Weaverly Press, Baltimore, MD, USA.

Giller CA, Bowman G, Dyer H, Mootz L & Krippner W (1993). Cerebral arterial diameters during changes in blood pressure and carbon dioxide during craniotomy. *Neurosurgery* **32**, 737–741.

Gisolf J, Wilders R, Immink R, Van Lieshout J & Karemaker J (2004). Tidal volume, cardiac output and functional residual capacity determine end-tidal CO_2 transient during standing up in humans. *J Physiol* **554**, 579–590.

Gurtner G & Traystman R (1979). Gas-to-blood PCO_2 differences during severe hypercapnia. *J Appl Physiol* **47**, 67–71.

Guyatt A, Yu C, Lutherer B & Otis A (1973). Studies on alveolar-mixed venous CO_2 and O_2 gradients in the rebreathing dog lung. *Respir Physiol* **17**, 178–194.

Ide K, Eliasziw M & Poulin MJ (2003). Relationship between middle cerebral artery blood velocity and end-tidal PCO_2 in the hypocapnic-hypercapnic range in humans. *J Appl Physiol* **95**, 129–137.

Immink RV, Secher NH, Roos CM, Pott F, van Madsen PL & Lieshout JJ (2006). The postural reduction in middle cerebral artery blood velocity is not explained by $PaCO_2$. *Eur J Appl Physiol* **96**, 609–614.

Jennings D & Chen C (1975). Negative arterial-mixed expired PCO_2 gradient during acute and chronic hypercapnia. *J Appl Physiol* **38**, 382–388.

Jones NL, Robertson DG & Kane JW (1979). Difference between end-tidal and arterial PCO_2 in exercise. *J Appl Physiol* **47**, 954–960.

Kawai A, Ballantyne D, Muckenhoff K & Scheid P (1996). Chemosensitive medullary neurones in the brainstem–spinal cord preparation of the neonatal rat. *J Physiol* **492**, 277–292.

Kety SS & Schmidt CF (1948). The nitrous oxide method for the quantitative determination of cerebral blood flow in man: theory, procedure and normal values. *J Clin Invest* **27**, 476–483.

Laszlo G, Clark T, Pope H & Campbell E (1971). Differences between alveolar and arterial PCO_2 during rebreathing experiments in resting human subjects. *Respir Physiol* **12**, 36–52.

Markwalder TM, Grolimund P, Seiler RW, Roth F & Aaslid R (1984). Dependency of blood flow velocity in the middle cerebral artery on end-tidal carbon dioxide partial pressure – a transcranial ultrasound Doppler study. *J Cereb Blood Flow Metab* **4**, 368–372.

Oliven A, Cherniack N, Deal E & Kelsen S (1985). The effects of acute bronchoconstriction on respiratory activity in patients with chronic obstructive pulmonary disease. *Am Rev Respir Dis* **131**, 236–241.

Ramsay SC, Murphy K, Shea SA, Friston KJ, Lammertsma AA, Clark JC, Adams L, Guz A & Frackowiak RS (1993). Changes in global cerebral blood flow in humans: effect on regional cerebral blood flow during a neural activation task. *J Physiol* **471**, 521–534.

Robbins PA, Conway J, Cunningham DA, Khamnei S & Paterson DJ (1990). A comparison of indirect methods for continuous estimation of arterial PCO_2 in men. *J Appl Physiol* **68**, 1727–1731.

Serrador JM, Picot PA, Rutt BK, Shoemaker JK & Bondar RL (2000). MRI measures of middle cerebral artery diameter in conscious humans during simulated orthostasis. *Stroke* **31**, 1672–1678.

Serrador JM, Sorond FA, Vyas M, Gagnon M, Iloputaife ID & Lipsitz LA (2005). Cerebral pressure-flow relations in hypertensive elderly humans: transfer gain in different frequency domains. *J Appl Physiol* **98**, 151–159.

Severinghaus JW & Lassen N (1967). Step hypocapnia to separate arterial from tissue PCO_2 in the regulation of cerebral blood flow. *Circ Res* **20**, 272–278.

Shapiro W, Wasserman AJ & Patterson JJ (1966). Mechanism and pattern of human cerebrovascular regulation after rapid changes in blood CO_2 tension. *J Clin Invest* **45**, 913–922.

Smith C, Rodman J, Chenuel B, Henderson K & Dempsey JA (2006). Response time and sensitivity of the ventilatory response to CO_2 in unanesthetized intact dogs: central vs. peripheral chemoreceptors. *J Appl Physiol* **100**, 13–19.

Toda N & Okamura T (1998). Cerebral vasodilators. *Jpn J Pharmacol* **76**, 349–367.

Tojima H, Kuriyama T & Fukuda Y (1988). Arterial to end-tidal PCO_2 difference varies with different ventilatory conditions during steady state hypercapnia in the rat. *Jpn J Physiol* **38**, 445–457.

Valdueza JM, Balzer JO, Villringer A, Vogl TJ, Kutter R & Einhaupl KM (1997). Changes in blood flow velocity and diameter of the middle cerebral artery during hyperventilation: assessment with MR and transcranial Doppler sonography. *AJNR Am J Neuroradiol* **18**, 1929–1934.

van der Zande FH, Hofman PA & Backes WH (2005). Mapping hypercapnia-induced cerebrovascular reactivity using BOLD MRI. *Neuroradiology* **47**, 114–120.

Wasserman AJ & Patterson JL Jr (1961). The cerebral vascular response to reduction in arterial carbon dioxide tension. *J Clin Invest* **40**, 1297–1303.

Widder B, Kleiser B & Krapf H (1994). Course of cerebrovascular reactivity in patients with carotid artery occlusions. *Stroke* **25**, 1963–1967.

Wijnhoud AD, Koudstaal PJ & Dippel DW (2006). Relationships of transcranial blood flow Doppler parameters with major vascular risk factors: TCD study in patients with a recent TIA or nondisabling ischemic stroke. *J Clin Ultrasound* **34**, 70–76.

Wise RG, Pattinson KT, Bulte DP, Chiarelli PA, Mayhew SD, Balanos GM, O'Connor DF, Pragnell TR, Robbins PA, Tracey I & Jezzard P (2007). Dynamic forcing of end-tidal carbon dioxide and oxygen applied to functional magnetic resonance imaging. *J Cereb Blood Flow Metab* **27**, 1521–1532.

Xie A, Skatrud JB, Khayat R, Dempsey JA, Morgan B & Russell D (2005). Cerebrovascular response to carbon dioxide in patients with congestive heart failure. *Am J Respir Crit Care Med* **172**, 371–378.

Xie A, Skatrud JB, Morgan BJ, Chenuel B, Khayat R, Reichmuth K, Lin J & Dempsey JA (2006). Influence of cerebrovascular function on the hypercapnic ventilatory response in healthy humans. *J Physiol* **577**, 319–329.

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