Research Paper

Cerebral vasomotor reactivity: steady-state *versus* transient changes in carbon dioxide tension

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New Findings

- **What is the central question of this study?**
  The relationship between changes in cerebral blood flow and arterial carbon dioxide tension is used to assess cerebrovascular function. Hypercapnia is generally evoked by two methods, i.e. steady-state and transient increases in carbon dioxide tension. In some cases, the hypercapnia is immediately preceded by a period of hypocapnia. It is unknown whether the cerebrovascular response differs between these methods and whether a period of hypocapnia blunts the subsequent response to hypercapnia.
- **What is the main finding and its importance?**
  The cerebrovascular response is similar between steady-state and transient hypercapnia. However, hyperventilation-induced hypocapnia attenuates the cerebral vasodilatory responses during a subsequent period of rebreathing-induced hypercapnia.

Cerebral vasomotor reactivity (CVMR) to changes in arterial carbon dioxide tension (P_{aCO_2}) is assessed during steady-state or transient changes in P_{aCO_2}. This study tested the following two hypotheses: (i) that CVMR during steady-state changes differs from that during transient changes in P_{aCO_2}; and (ii) that CVMR during rebreathing-induced hypercapnia would be blunted when preceded by a period of hyperventilation. For each hypothesis, end-tidal carbon dioxide tension (P_{ET,CO_2}) middle cerebral artery blood velocity (CBFV), cerebrovascular conductance index (CVCI; CBFV/mean arterial pressure) and CVMR (slope of the linear regression between changes in CBFV and CVCI *versus* P_{ET,CO_2}) were assessed in eight individuals. To address the first hypothesis, measurements were made during the following two conditions (randomized): (i) steady-state increases in P_{ET,CO_2} of 5 and 10 Torr above baseline; and (ii) rebreathing-induced transient breath-by-breath increases in P_{ET,CO_2}. The linear regression for CBFV *versus* P_{ET,CO_2} ($P = 0.65$) and CVCI *versus* P_{ET,CO_2} ($P = 0.44$) was similar between methods; however, individual variability in CBFV or CVCI responses existed among subjects. To address the second hypothesis, the same measurements were made during the following two conditions (randomized): (i) immediately following a brief period of hypocapnia induced by hyperventilation for 1 min followed by rebreathing; and (ii) during rebreathing only. The slope of the linear regression for CBFV *versus* P_{ET,CO_2} ($P < 0.01$) and CVCI *versus* P_{ET,CO_2} ($P < 0.01$) was reduced during hyperventilation plus rebreathing relative to rebreathing only. These results indicate that cerebral vasomotor reactivity to changes in P_{aCO_2} is similar regardless of the employed methodology to

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induce changes in P_{aCO_2} and that hyperventilation-induced hypocapnia attenuates the cerebral vasodilatory responses during a subsequent period of rebreathing-induced hypercapnia.

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Introduction

The cerebral circulation is tightly regulated by arterial carbon dioxide tension (P_{aCO_2}; Serrador *et al.* 2000; Ide *et al.* 2003). In physiological conditions, hypercapnia increases while hypocapnia decreases cerebral blood flow (CBF; Serrador *et al.* 2000; Ide *et al.* 2003). The relationship between changes in CBF and P_{aCO_2} has been termed cerebral vasomotor reactivity (CVMR) in previous studies and is often assessed in research and clinical settings to evaluate cerebrovascular function (Ringelstein *et al.* 1988; Kleiser & Widder, 1992; Blaser *et al.* 2002).

In most studies, the end-tidal tension of CO_2 (P_{ET,CO_2}) is measured as a non-invasive proxy for P_{aCO_2} (Claassen *et al.* 2007; Low *et al.* 2008; Ogoh *et al.* 2009; Brothers *et al.* 2011; Nelson *et al.* 2011). There are generally two different methods used to assess CVMR. The first method involves measuring CBF responses to steady-state changes in P_{ET,CO_2} (Ide *et al.* 2003; Xie *et al.* 2005; Peebles *et al.* 2007). In this method, CVMR in the hypocapnic range is assessed by having the subject hyperventilate at a predetermined frequency for a brief period (typically 30 s to several minutes) to reduce P_{ET,CO_2} (Claassen *et al.* 2007), while in the hypercapnic range CVMR is often assessed by the subject breathing predetermined levels of elevated carbon dioxide gas concentrations for several minutes (Ide *et al.* 2003; Xie *et al.* 2005; Peebles *et al.* 2007). Another approach to investigate CVMR is to assess CBF responses during non-steady-state (i.e. transient) changes in P_{ET,CO_2} (Read & Leigh, 1967; Duffin & McAvoy, 1988; Mohan & Duffin, 1997; Claassen *et al.* 2007). In this method, a rebreathing procedure, developed originally by Read and Leigh to assess ventilatory responses, is used to induce a ramp-like breath-by-breath increase in P_{ET,CO_2} (Read & Leigh, 1967).

Recently, a modified rebreathing method was developed to assess CVMR (Casey *et al.* 1987; Duffin & McAvoy, 1988; Claassen *et al.* 2007). This method includes a brief period of hyperventilation immediately followed by several minutes of rebreathing to induce a wider range of changes in P_{ET,CO_2} (Casey *et al.* 1987; Duffin & McAvoy, 1988; Mohan & Duffin, 1997; Claassen *et al.* 2007). Using this method, CBF responses to changes in P_{ET,CO_2} could be fitted reasonably well by a sigmoidal model over a range of changes in P_{ET,CO_2} from ∼20 to 65 Torr (Casey *et al.* 1987; Duffin & McAvoy, 1988; Mohan & Duffin, 1997; Claassen *et al.* 2007).

However, several studies have observed that assessment of CVMR during transient changes in P_{ET,CO_2} using either the original Read and Leigh or the modified rebreathing methods is ∼30% lower than those obtained in steady-state conditions (Pandit *et al.* 2003, 2007). These observations raised concerns for CVMR measurements using these different methods. Several possibilities have been proposed to explain differences in CVMR between these methods. With the modified rebreathing method, cerebral vasoconstriction as a result of the hyperventilation immediately before rebreathing may attenuate cerebral vasodilatation and thus, CBF responses during the subsequent hypercapnic rebreathing process (Pandit *et al.* 2007). In addition, both the Read and Leigh and the modified rebreathing methods use a rebreathing bag prefilled with a gas mixture of high CO_2 concentration (∼7%), balanced with 100% oxygen (Read & Leigh, 1967; Pandit *et al.* 2003, 2007). Thus, an elevated initial oxygen concentration or a reduction in oxygen concentration during prolonged rebreathing may influence CBF responses to increases in P_{aCO_2} (Kety & Schmidt, 1948; Watson *et al.* 2000; Ogoh *et al.* 2014). Finally, CO_2-induced changes in mean arterial pressure (MAP), heart rate (HR), cardiac output and autonomic neural activity have been well documented (Dumville *et al.* 1998; Panerai *et al.* 1999; Shoemaker *et al.* 2002) and thus, potential differences in these systemic responses between transient and steady-state methods may affect CBF responses, hence CVMR (Pandit *et al.* 2003, 2007).

Given these considerations, CVMR was assessed using two different experimental designs. In the first experiment, the CVMR response using a new simplified rebreathing method was compared against CVMR obtained in steady-steady conditions (experiment 1). This approach allowed for comparisons between steady-state and rebreathing-induced hypercapnia. For this comparison, hyperventilation prior to the simplified rebreathing method was not performed. The second experiment assessed the impact of a brief period of hyperventilation, resulting in hypocapnia, on the subsequent response of the cerebral vasculature to hypercapnia induced by the new simplified rebreathing method (experiment 2). This approach allowed for the assessment of a brief

period of hypocapnia on the subsequent hypercapnic response. For both experiments, the simplified rebreathing method involved the subjects rebreathing their own expired air to increase P_{ET,CO_2} gradually, rather than prefilling the rebreathing bag with a gas mixture of high CO_2 concentration, while arterial oxygen saturation (S_{aO_2}) was maintained by bleeding a small amount of oxygen into the rebreathing bag (Claassen *et al.* 2007). For experiment 1, we hypothesized that CVMR measured during steady-state changes in P_{aCO_2} would differ from CVMR measured during transient changes in P_{aCO_2} associated with rebreathing. For experiment 2, it was hypothesized that CVMR would be blunted when the rebreathing protocol was preceded by a period of hyperventilation.

Methods

Eight healthy young subjects participated in experiment 1 (four males). Average (mean ± SD) subject characteristics were as follows: age, 28 ± 6 years; height, 166 ± 8 cm; and weight, 68 ± 8 kg. A separate group of eight healthy young subjects participated in experiment 2 (six males). Average (mean ± SD) subject characteristics were as follows: age, 27 ± 2 years; height, 176 ± 5 cm; and weight, 74 ± 6 kg. None of the subjects, for either experiment, was taking medications, and all were free of any known cardiovascular, cerebrovascular, metabolic or neurological diseases. For each experiment, subjects were informed of the purpose and risks of the study prior to providing their informed written consent. The consent and procedures for experiment 1 were approved by the University of Texas Southwestern Medical Center at Dallas and Texas Health Presbyterian Hospital Dallas, while the consent and procedures for experiment 2 were approved by the University of Texas at Austin. For each experiment, subjects refrained from alcohol, caffeine and exercise for 24 h prior to either of the studies.

Instrumentation and measurements

Instrumentation for each experiment was identical. Heart rate was continuously obtained from an electrocardiogram (HP Patient Monitor, Agilent, Santa Clara, CA, USA) interfaced with a cardiotachometer (CWE, Ardmore, PA, USA). Continuous beat-by-beat MAP was recorded from a finger using the Penaz method [Finometer; Finapres Medical Systems, Amsterdam, The Netherlands (experiment 1) and the CNAP, Biopac Monitor 500, Bruck an der Mur Austria (experiment 2)] and was corrected according to intermittent blood pressure measurements obtained by auscultation of the brachial artery via electrosphygmomanometry (SunTech, Raleigh, NC, USA). Blood flow in the middle cerebral artery (CBFV) was continuously measured using transcranial Doppler ultrasonography. A 2 MHz Doppler probe (Multi-flow; DWL Elektronische Systeme, Singen, Germany) was adjusted over the temporal window of the right middle cerebral artery until an optimal signal was identified. The probe was then fixed and held in place using a headband strap to prevent subtle movement of the Doppler probe. An index of cerebrovascular conductance (CVCI) was calculated from the ratio of CBFV to MAP. The P_{ET,CO_2} was measured continuously using a capnograph (VitalCap Capnograph Monitor; Oridion, Needham, MA, USA).

Protocol for experiment 1: comparison of the cerebrovascular response to steady-state *versus* transient increases in carbon dioxide tension

Transient increases in carbon dioxide tension. Following instrumentation, subjects rested quietly in the supine position. After a 20 min controlled rest period, subjects were fitted with a nose-clip and breathed room air through a mouthpiece attached to a Y-valve. One end of the Y-valve was connected to a 5 litre bag and the other end was open to room air. Subjects breathed room air for 5 min while haemodynamic data, including CBFV, MAP, HR and P_{ET,CO_2}, were collected. Following this baseline period, subjects were exposed to a rebreathing protocol. This was accomplished by having the subjects first perform a deep inspiration and then exhale into an empty rebreathing bag via a Y-valve. The subjects continued to rebreathe their own expired gas mixture continuously for a period of 5 min before switching the valve back to room air for a recovery period. During the rebreathing period, S_{aO_2} was maintained constant by bleeding a small amount of oxygen into the rebreathing bag (Claassen *et al.* 2007).

Steady-state changes in carbon dioxide tension. Following a 10 min controlled rest period, baseline measures were obtained during 5 min of spontaneous respiration. This period was immediately followed by steady-state increases in P_{ET,CO_2} of 5 or 10 Torr (randomized), which were accomplished using a P_{ET,CO_2} clamping protocol. This clamping protocol uses a computer-controlled gas blender, sequential gas delivery and rebreathing circuit (RespirAct™; Thornhill Research, Toronto, ON, Canada) which has been described in detail elsewhere (Ito *et al.* 2008; Mandell *et al.* 2008*a*,*b*; Prisman *et al.* 2008; Brothers *et al.* 2009). The Respiract™ device was programmed to achieve the desired level of hypercapnia (i.e. 5 and 10 Torr above baseline values) while maintaining normoxia via administration of a mixture of nitrogen, oxygen and carbon dioxide gases into a rebreathing circuit. Following 5 min of steady-state hypercapnic breathing, the procedure was terminated and

a port on the device was opened, which enabled the subject to breathe room air for a recovery period. The order of the levels of hypercapnia was counterbalanced and randomized, with a minimum of 15 min between levels to allow the measured variables to return to baseline. The transient and steady-state increase in P_{ET,CO_2} protocols were also performed in a counterbalanced and randomized manner, with a minimum of 15 min elapsing between the protocols to allow the measured variables to return to baseline.

Data analysis and statistics for experiment 1. During baseline conditions (i.e. eucapnia), haemodynamic data were sampled at 100 Hz via a data-acquisition system (Biopac System, Santa Barbara, CA, USA). Mean arterial blood pressure, CBFV and P_{ET,CO_2} were measured and CVCI (CBFV/MAP) was subsequently calculated on a breath-by breath basis. These variables were averaged during the last 60 s of the 5 min baseline periods. The exception is for the second level of steady-state hypercapnia, when these variables were obtained during the last 60 s of the 15 min recovery period in between hypercapnic stages to account for any shift in baseline values.

During the rebreathing protocol, breath-by-breath MAP, CBFV, P_{ET,CO_2} and CVCI were measured. Maximal changes in these variables at the end of rebreathing were determined as the average from the last 45 s of rebreathing. In the steady-state protocol, breath-by-breath MAP, CBFV, P_{ET,CO_2} and CVCI were averaged during the last 45 s of each of the steady-state hypercapnic stimuli. For each method, the percentage change in CBFV and CVCI during the hypercapnic stimuli with respect to baseline values was determined, while the absolute change in P_{ET,CO_2} with respect to baseline was determined. For each method, CVMR was calculated as the slope of the linear regression between changes in CBFV and CVCI *versus* P_{ET,CO_2}. For steady-state hypercapnia, CVMR assessment was accomplished via linear regression of the baseline (i.e. normocapnic) value and the values obtained for each of the subsequent two stages of hypercapnia (i.e. regression analysis from three data points).

Comparison of the baseline (i.e. prehypercapnic stimuli) MAP and HR values was performed via one-way repeated-measures ANOVA. Comparison of the effect of hypercapnia relative to the respective baseline value within each stimulus was performed via Student's paired *t* tests. Comparison of maximal changes in haemodynamic variables during the different procedures was performed via a two-way repeated-measures ANOVA. The CVMR slopes obtained by the two different methods to induce hypercapnia were compared via Student's paired *t* tests. All values are presented as means ± SD, and the level of significance was $P < 0.05$.

Protocol for experiment 2: impact of a brief period of hyperventilation on the subsequent response of the cerebral vasculature to transient increases in carbon dioxide tension induced by rebreathing. All instrumentation and data collection were similar to those described for experiment 1. The differences were as follows: (i) the steady-state procedure to induce hypercapnia was not performed; (ii) the cerebrovascular response to a simplified rebreathing procedure was performed twice, once immediately following a brief period of hyperventilation (hyperventilation + rebreathe) and once during a period of only rebreathing (rebreathe); and (iii) each of the rebreathe portions was performed for 4 min as opposed to 5 min. The two trials were performed in a counterbalanced and randomized manner, with a minimum of 15 min elapsing between the protocols to allow the measured variables to return to baseline.

Following instrumentation and the aforementioned baseline period, the hyperventilation + rebreathe protocol was performed. Subjects first conducted metronome-paced hyperventilation at a rate of 30 breaths min^{-1} for 1 min. All subjects were familiarized with this procedure during initial instrumentation (prior to data collection). Subjects were instructed to alternate forceful inhalations and exhalations on each beat of the metronome. This procedure consistently produces 15–20 Torr reductions in P_{ET,CO_2} (Low *et al.* 2008; Sato *et al.* 2012). After this period of hyperventilation, the subjects were immediately exposed to the aforementioned rebreathing protocol for a period of 4 min before switching the valve back to room air for a recovery period. Following the 15 min break period, the rebreathe protocol was performed. This was similar to the hyperventilation + rebreathe protocol with the exception that the subjects did not hyperventilate prior to the rebreathing protocol. During both rebreathing periods, S_{aO_2} was maintained constant by bleeding a small amount of oxygen into the rebreathing bag (Claassen *et al.* 2007).

Data analysis and statistics for experiment 2. During baseline conditions (i.e. eucapnia), haemodynamic data were sampled at 100 Hz via a data-acquisition system (Biopac System). Mean arterial blood pressure, CBFV and P_{ET,CO_2} were measured and CVCI (CBFV/MAP) was subsequently calculated on a breath-by-breath basis. These variables were averaged during the last 60 s of the 5 min baseline periods. During the rebreathing protocols, breath-by-breath MAP, CBFV, P_{ET,CO_2} and CVCI were measured. For each method (i.e. hyperventilation + rebreathe and rebreathe only), the percentage change in CBFV and CVCI during the hypercapnic stimuli with respect to eucapnic baseline was determined while the absolute change in P_{ET,CO_2} with respect to eucapnic baseline was determined. For each method, CVMR was

calculated as the slope of the linear regression between changes in CBFV and CVCI *versus* P_{ET,CO_2}. In order to compare the impact of hyperventilation on the ensuing hypercapnic response directly, for the hyperventilation + rebreathe protocol we chose to analyse only the portion of the slope beginning when P_{ET,CO_2} returned to eucapnia (i.e. value measured immediately prior to hyperventilation).

Comparison of the baseline (i.e. prehypercapnic stimuli) values was performed via Student's paired *t* tests. The primary focus of this experiment was on the impact of a brief period of hyperventilation on the subsequent response of the cerebral vasculature to transient increases in carbon dioxide tension induced by rebreathing; therefore, the main analysis considered the CVMR slopes obtained by the hyperventilation + rebreathe and rebreathe-only procedures, which were compared via Student's paired *t* tests. All values are presented as means ± SD, and the level of significance was $P < 0.05$.

Results

Experiment 1: comparison of the cerebrovascular response to steady-state *versus* transient increases in carbon dioxide tension

Baseline values of MAP, HR, CBVF and P_{ET,CO_2} were similar prior to the hypercapnic stimuli between protocols ($P > 0.05$ for all comparisons). There was a hypercapnia-dependent increase in CBFV, MAP and P_{ET,CO_2} for each method (Fig. 1).

By design, P_{ET,CO_2} was elevated by 5.5 ± 1.2 and 9.8 ± 0.7 Torr during the two phases of the steady-state protocol. These stimuli evoked significant increases in CBFV (+5 Torr, 18 ± 10%; and +10 Torr, 41 ± 17%) and CVCI (+5 Torr, 12 ± 9%; and +10 Torr, 28 ± 11%). At the end of the rebreathing protocol, P_{ET,CO_2} was elevated by 15.9 ± 2.1 Torr while CBFV and CVCI were elevated by 54 ± 8 and 31 ± 12%, respectively, relative to baseline conditions.

Of note, each of the hypercapnic stimuli elicited increases in MAP (steady-state +5 Torr, 4.5 ± 5.1 mmHg; steady-state +10 Torr, 7.3 ± 8.5 mmHg; and rebreathe, 15.8 ± 8.5 mmHg; $P < 0.05$ for all conditions) and HR (steady-state +5 Torr, 3.7 ± 4.3 beats min^{-1}; steady-state +10 Torr, 5.5 ± 4.7 beats min^{-1}; and rebreathe, 7.4 ± 3.0 beats min^{-1}; $P < 0.05$ for all conditions) relative to the respective baseline value for each condition (Table 1). Large interindividual variability in both MAP and HR responses was observed during each of the hypercapnic stimuli (Table 1).

Representative examples of the relationship between CBFV *versus* P_{ET,CO_2} and CVCI *versus* P_{ET,CO_2} are illustrated in Fig. 2*A* and *B*, respectively. Similar results were observed in all subjects such that the slope of the linear regression for CBFV *versus* P_{ET,CO_2} was similar between the two methods of hypercapnia (Fig. 2*C*; steady state, 4.1 ± 1.5% Torr^{-1}, $r = 0.97 \pm 0.05$; and rebreathe, 4.4 ± 1.9% Torr^{-1}, $r = 0.87 \pm 0.13$; comparison between the methods, $P = 0.65$). Likewise, the slope of the linear regression for CVCI *versus* P_{ET,CO_2} was similar between the two methods (Fig. 2*D*; steady state, 2.8 ± 1.3% Torr^{-1}, $r = 0.91 \pm 0.13$; and rebreathe, 2.4 ± 1.8% Torr^{-1}, $r = 0.73 \pm 0.25$; comparison between the methods, $P = 0.44$). Notably, the slope of CBFV *versus* P_{ET,CO_2} was significantly steeper than that of CVCI *versus* P_{ET,CO_2} ($P < 0.05$), regardless of the method used to induce changes in P_{ET,CO_2}.

Experiment 2: impact of a brief period of hyperventilation on the subsequent response of the cerebral vasculature to transient increases in carbon dioxide tension induced by rebreathing

Baseline values of MAP, HR, CBFV, CVCi and P_{ET,CO_2} were similar between protocols ($P > 0.05$ for all comparisons).

Representative examples of the relationship between CBFV *versus* P_{ET,CO_2} and CVCI *versus* P_{ET,CO_2} during the two procedures are illustrated in Fig. 3*A* and *B*, respectively. Similar results were observed in all subjects such that the slope of the linear regression for CBFV *versus* P_{ET,CO_2} was reduced during hyperventilation + rebreathe relative to the rebreathe-only protocol (Fig. 3*C*; hyperventilation + rebreathe, 3.2 ± 1.0% Torr^{-1}, $r = 0.93 \pm 0.05$; and rebreathe, 5.0 ± 1.1% Torr^{-1}, $r = 0.83 \pm 0.25$; comparison between the methods, $P < 0.01$). Likewise, the slope of the linear regression for CVCI *versus* P_{ET,CO_2} was reduced during hyperventilation + rebreathe (Fig. 3*D*; hyperventilation + rebreathe, 2.6 ± 0.9% Torr^{-1}, $r = 0.88 \pm 0.09$; and rebreathe, 4.3 ± 1.1% Torr^{-1}, $r = 0.89 \pm 0.08$; comparison between the methods, $P < 0.01$). Notably, the slope of CBFV *versus* P_{ET,CO_2} was significantly steeper than that of CVCI *versus* P_{ET,CO_2} ($P < 0.05$), regardless of the procedure used.

Discussion

The primary findings of the present study are twofold. First, we demonstrated that assessment of CVMR was similar during transient and steady-state changes in P_{ET,CO_2}; however, individual variability between the methods existed among subjects. Second, hyperventilation-induced hypocapnia attenuated the cerebral vasodilatory responses during a subsequent period of rebreathing-induced hypercapnia.

Methodological considerations

This study employed a simplified rebreathing method to assess CVMR. There are several important methodological differences between this and previous studies that deserve further discussion (Casey *et al.* 1987; Duffin & McAvoy, 1988; Mohan & Duffin, 1997; Pandit *et al.* 2003, 2007, 2008; Claassen *et al.* 2007). First, in contrast to the original Read and Leigh and the modified rebreathing methods, in which the rebreathing bag is prefilled with a high concentration of CO_2 balanced with 100% oxygen (Read & Leigh, 1967; Pandit *et al.* 2003, 2007), during rebreathing in both experiments of the present protocol subjects breathed into an initially empty bag, and a small amount of oxygen was added to the rebreathed gas throughout to maintain S_{aO_2} at the baseline level (Claassen *et al.* 2007). This was confirmed by S_{aO_2} of 97.4 ± 0.5% at baseline (i.e. prerebreathing) and 97.3 ± 0.5% immediately prior to termination of rebreathing in experiment 1 ($P = 0.40$) and S_{aO_2} values of 97.7 ± 0.3 and 97.1 ± 0.2% at baseline (hyperventilation + rebreathe and rebreathe, respectively) and 97.3 ± 0.6 and 97.4 ± 0.3% immediately prior to termination of rebreathing (hyperventilation + rebreathe and rebreathe, respectively) in experiment 2. This approach eliminated potential effects of changes in oxygen gas concentration during prolonged rebreathing on CBF responses (Kety & Schmidt, 1948; Watson *et al.* 2000; Ogoh *et al.* 2014). This is further supported by recently published data indicating that CVMR during hypercapnia (assessed as the velocity of blood in the middle cerebral artery) was significantly blunted during hypoxia (breathing 12% O_2) relative to CVMR during normoxia (Ogoh *et al.* 2014). In addition, it should be noted that the Read and Leigh method was designed originally in an attempt to accelerate CO_2 equilibrium between the lung, arterial blood and brain tissue to improve assessment

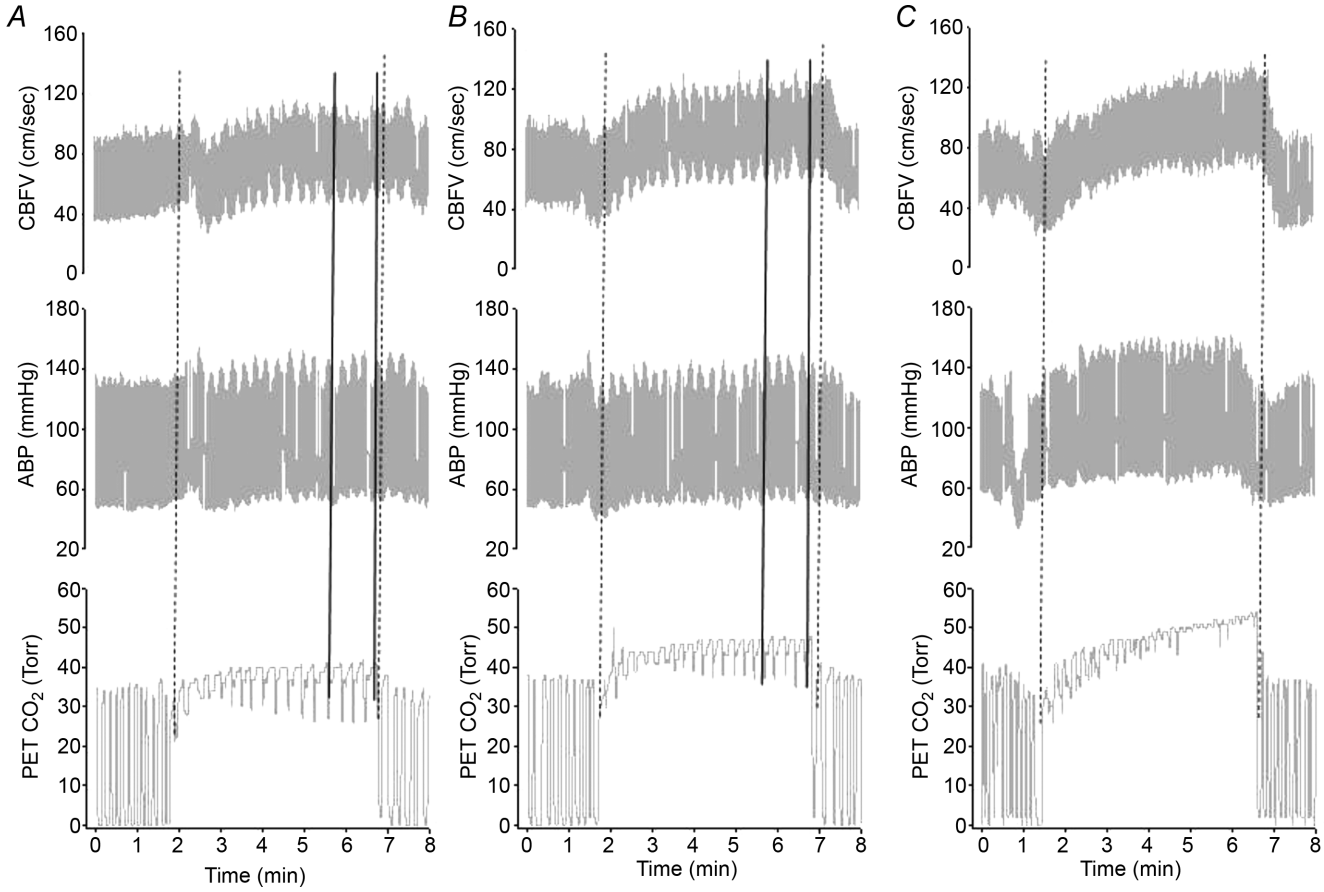

Figure 1. Representative illustration of middle cerebral artery blood velocity (CBFV), mean arterial blood pressure (MAP) and end-tidal carbon dioxide (P_{ET,CO_2}) during steady-state and simplified rebreathing-induced hypercapnia from one subject
A and *B* depict CBFV, MAP and P_{ET,CO_2} responses during steady-state elevations in P_{ET,CO_2} of 5 and 10 Torr, respectively, while *C* depicts the responses during the modified rebreathing protocol. The continuous vertical lines in *A* and *B* show the period (45 s) when the steady-state measures were obtained, while the dashed vertical lines in *A*–*C* show the start and end of the hypercapnic periods.

Table 1. Changes in mean arterial pressure (MAP) and heart rate during the hypercapnic stimuli

	Steady state +5 Torr				Steady state +10 Torr				Rebreathe			
	MAP (mmHg)		Heart rate (beats min^{-1})		MAP (mmHg)		Heart rate (beats min^{-1})		MAP (mmHg)		Heart rate (beats min^{-1})	
Subject	Baseline	Hypercapnia	Baseline	Hypercapnia	Baseline	Hypercapnia	Baseline	Hypercapnia	Baseline	Hypercapnia	Baseline	Hypercapnia
1	102	99	79	81	101	106	80	79	103	121	77	81
2	86	96	69	71	92	98	63	68	91	102	66	71
3	79	81	55	55	82	84	54	53	81	105	57	66
4	91	102	59	58	87	115	58	67	97	123	60	66
5	66	76	54	64	67	75	56	67	71	92	57	65
6	74	79	54	62	79	85	55	61	86	96	54	59
7	103	106	52	60	99	102	55	65	97	105	54	61
8	79	78	58	59	76	76	50	55	91	101	56	69
Mean	85	90*	60	64*	85	93*	59	64*	90	106*,†	60	67*
SD	13	12	9	8	12	15	9	8	10	11	8	7

Values are means ± SD from all of the subjects ($n = 8$). *Significantly different ($P < 0.05$) relative to respective baseline (i.e. normocapnic) value. †Effect of hypercapnia relative to respective baseline value *versus* the effect of hypercapnia in +5 and +10 Torr stages ($P < 0.05$).

of ventilatory responses to central CO_2 stimuli (Read & Leigh, 1967). Of particular relevance to the present study, previous studies have shown that CBF responded to arterial rather than brain tissue CO_2 concentration (Severinghaus & Lassen, 1967; Pandit *et al.* 2003). Thus, if changes in P_{ET,CO_2} reflect changes in P_{aCO_2}, initiation of rebreathing with a high CO_2 concentration, as used in the Read and Leigh and the modified rebreathing methods, may not be necessary for the assessment of CVMR.

Cerebral vasomotor reactivity during transient and steady-state hypercapnia

Our value of CVMR as CBFV *versus* P_{ET,CO_2} during steady-state hypercapnia (4.1% Torr^{-1}) is remarkably consistent with previous studies using different modalities for measuring CBF and for assessing CVMR (Kety & Schmidt, 1948; Olesen *et al.* 1971; Pandit *et al.* 2003, 2007, 2008; Battisti-Charbonney *et al.* 2011). The CBFV *versus* P_{ET,CO_2} relationship during transient hypercapnia (4.4% Torr^{-1}) is also similar to those reported previously. Most importantly, we did not observe statistically significant differences in CVMR between transient and steady-state hypercapnia assessed using either the CBFV *versus* P_{ET,CO_2} or the CVCI *versus* P_{ET,CO_2} relationship despite the presence of variability in the individual responses to these procedures (Fig. 2*C* and *D*).

However, several studies have shown differences in assessments of CVMR between the transient and steady-state measures using either the modified or Read and Leigh rebreathing method (Pandit *et al.* 2003, 2007). For example, Pandit *et al.* (2003, 2007) showed that CVMR, when expressed as CBFV *versus* P_{ET,CO_2}, is lower during transient increases in P_{ET,CO_2} relative to steady-state increases in P_{ET,CO_2} (transient, ~2.5–2.9% Torr^{-1}; and steady state, ~4.8% Torr^{-1}). The underlying mechanism(s) leading to these differences are not clear but could be related to the potential effects of a prolonged hypocapnic hyperventilation immediately prior to rebreathing and/or changes in S_{aO_2} during rebreathing on assessment of CVMR (Read & Leigh, 1967; Pandit *et al.* 2003, 2007; Ogoh *et al.* 2014). In this respect, the present findings from experiment 2 confirm this hypothesis of an attenuated cerebrovascular response to hypercapnia when it is immediately preceded by a period of hyperventilation-induced hypocapnia (Fig. 3*A*–*D*). It is interesting to note that the CVMR slopes during rebreathe for CBFV and CVCI differed somewhat between experiment 1 and experiment 2 (Figs 2 and 3) despite the use of identical rebreathing protocols. The reason for these somewhat differing responses is unknown but is likely to be related to different subject cohorts between protocols or different durations of rebreathing (5 min in experiment 1 and 4 min in experiment 2). That being said, the statistical

comparisons for each experiment were made within the same subject cohort.

Another possibility is that these previous studies evoked steady-state hypercapnia using an end-tidal forcing system, which may induce a gradient between P_{ET,CO_2} and P_{aCO_2} (Ainslie & Duffin, 2009). The present study evoked steady-state hypercapnia using the RespirAct^{TM} (Thornhill Research), which can achieve a desired level of hypercapnia by using a closed-loop rebreathing circuit, thereby eliminating the potential influence of a gradient between P_{ET,CO_2} and P_{aCO_2} on CVMR (Ito *et al.* 2008; Mandell *et al.* 2008*a*,*b*; Prisman *et al.* 2008; Ainslie & Duffin, 2009; Brothers *et al.* 2009). Thus, the findings of the present study argue against the possibility that effective CO_2 stimuli to the cerebral vasculature may be smaller during transient *versus* steady-state conditions (Berkenbosch *et al.* 1986).

Another important factor is the assessment of CVMR via the changes in CVCI in response to P_{ET,CO_2}. Of note, the maximal increase in CBFV was by 18, 41 and 54% during the steady-state elevations of P_{ET,CO_2} by 5 and 10 Torr and at the end of the rebreathing, respectively. However, assessment of CVMR via changes in CBFV ignores the potential influence of hypercapnia-related increases in MAP on CBF. This is apparent when comparing the CBFV data with the CVCI data, where the maximal increase in

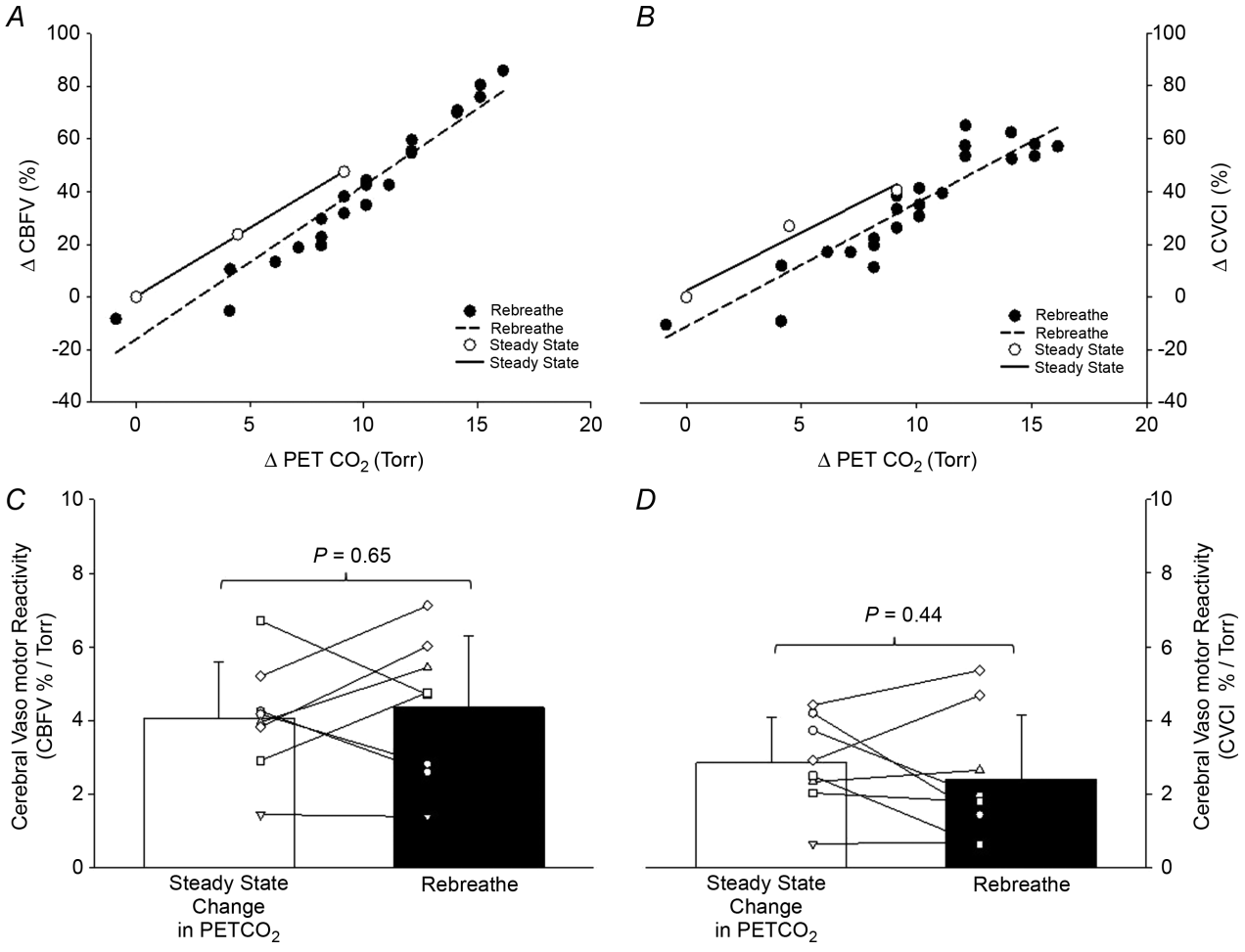

Figure 2. Cerebral vasomotor reactivity during steady-state and simplified rebreathing-induced hypercapnia
A illustrates a representative relationship between CBFV and P_{ET,CO_2}, while *B* illustrates a representative relationship between cerebrovascular conductance index (CVCI) and P_{ET,CO_2} from one subject. *C* depicts the individual (lines) and the group-averaged relationship (vertical bars) between CBFV and P_{ET,CO_2} (i.e. cerebral vasomotor reactivity) during steady-state and rebreathing-induced increases in P_{ET,CO_2}. *D* depicts the individual (lines) and the group-averaged relationship (vertical bars) between CVCI and changes in P_{ET,CO_2} (i.e. cerebral vasomotor reactivity) during steady-state and rebreathing-induced increases in P_{ET,CO_2}. The cerebrovascular response to hypercapnia was similar regardless of the method (i.e. steady state and modified rebreathing) of inducing hypercapnia or the analysis approach (i.e. CBFV and CVCI).

CVCI was by only 12, 28 and 31% to the aforementioned CO_2 challenges, respectively. Consistently, the slope of CBFV *versus* P_{ET,CO_2} was significantly steeper than that of CVCI *versus* P_{ET,CO_2}. The same pattern of a blunted slope of CVCI *versus* P_{ET,CO_2} relative to the slope of CBFV *versus* P_{ET,CO_2} was also observed in experiment 2. Collectively, these observations suggest that CVMR is likely to be overestimated when using CBFV, relative to CVCI (Figs 2 and 3), demonstrating the importance of accounting for CO_2-induced changes in MAP when assessing CVMR and thus the importance of analysing the data as CVCI. To facilitate comparisons with previous studies, both the CBFV *versus* P_{ET,CO_2} and the CVCI *versus* P_{ET,CO_2} relationships were used to assess CVMR in the present study.

Cardiovascular responses during hypercapnia: implications for CVMR

Changes in P_{aCO_2} induce prominent autonomic and cardiovascular responses, which can potentially modulate CBF (Richardson *et al.* 1961; Kontos *et al.* 1967; Claassen *et al.* 2007). For example, increases in P_{aCO_2} activate peripheral as well as central chemoreceptors (Bruce & Cherniack, 1987; Simmons *et al.* 2007), resulting in elevated sympathetic neural activity. Enhanced sympathetic activity may constrain increases in CBF to hypercapnic stimuli (Panerai *et al.* 1999; Edvinsson & Hamel, 2002; Zhang *et al.* 2011), although others have reported conflicting findings (Przybyłowski *et al.* 2003; Peebles *et al.* 2012). In addition, as discussed above, increases in MAP can contribute to increases

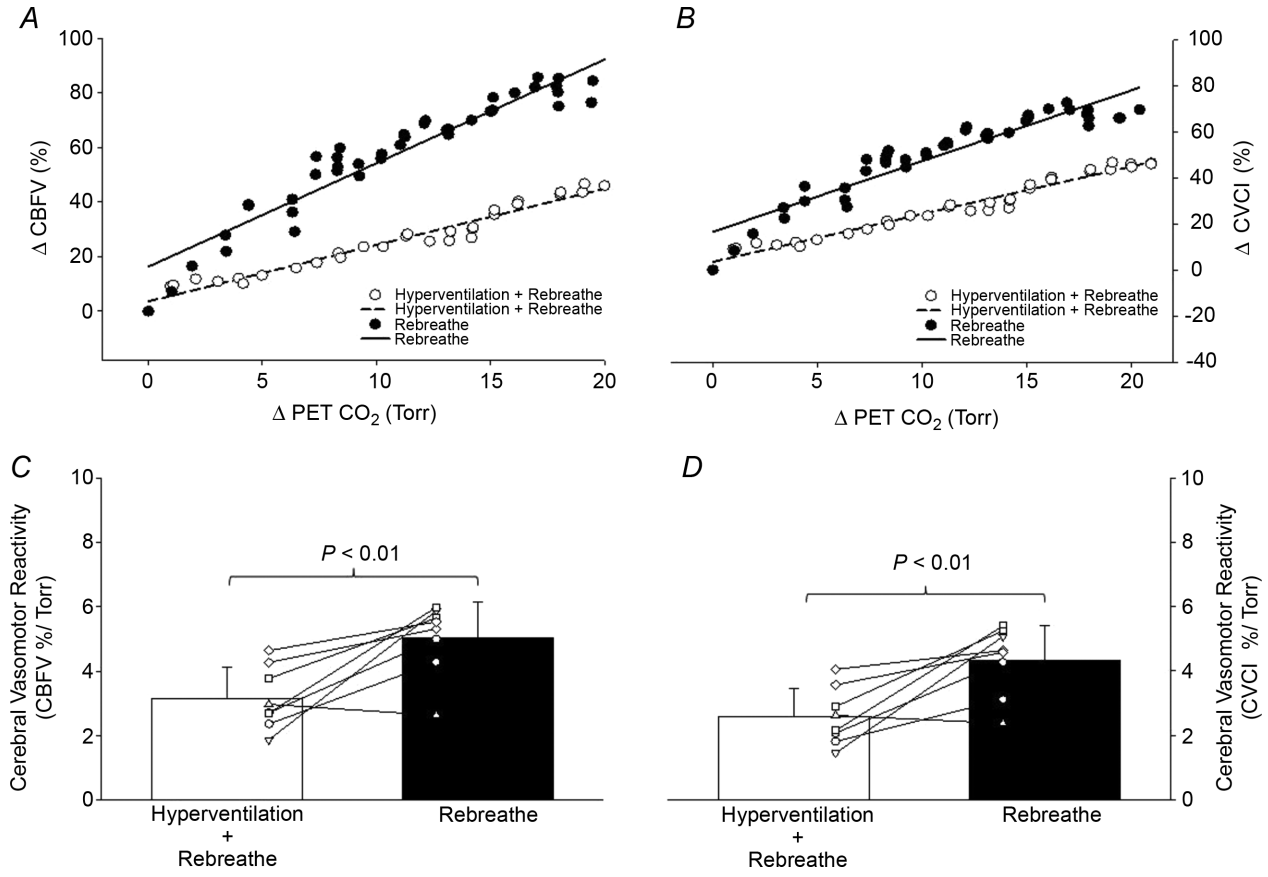

Figure 3. Cerebral vasomotor reactivity during simplified rebreathing-induced hypercapnia with (hyperventilation + rebreathe) and without a prior period of hyperventilation (rebreathe)
A illustrates a representative relationship between CBFV and P_{ET,CO_2}, while *B* illustrates a representative relationship between CVCI and P_{ET,CO_2} from one subject. *C* depicts the individual (lines) and the group-averaged relationship (vertical bars) between CBFV and P_{ET,CO_2} (i.e. cerebral vasomotor reactivity) during hyperventilation + rebreathe and rebreathe only. *D* depicts the individual (lines) and the group-averaged relationship (vertical bars) between CVCI and changes in P_{ET,CO_2} (i.e. cerebral vasomotor reactivity) during hyperventilation + rebreathe and rebreathe only. The cerebrovascular response to hypercapnia was significantly blunted when the rebreathing was preceded by a brief period of hyperventilation-induced hypocapnia (hyperventilation + rebreathe).

in CBF during hypercapnia, most probably because of compromised cerebral autoregulation in these conditions (Dumville *et al.* 1998; Panerai *et al.* 1999; Shoemaker *et al.* 2002).

In experiment 1 of the present study, with the exception of MAP during +5 Torr hypercapnia, the group-averaged MAP and HR were increased significantly during both transient and steady-state hypercapnic stimuli with respect to each respective baseline (i.e. normocapnic) value (Table 1). The simultaneous elevations in MAP and HR indicate that chemoreflex stimulation during hypercapnia is capable of either overriding baroreflex control of HR or resetting the baroreflex operating point to a higher level of MAP and HR (Bristow *et al.* 1971; Henry *et al.* 1998). However, the potential influences of increases in HR via effects on the stroke volume and cardiac output during hypercapnia on CVMR cannot be determined in the present study (Ogoh *et al.* 2005).

It is important to note that large interindividual variability in MAP and HR responses presented during both transient and steady-state hypercapnic stimuli (Table 1). The exact mechanism(s) resulting in these variabilites remain unknown and are likely to be multifactorial. It is possible that individual differences in or time-varying properties of chemoreflex sensitivity and/or its interaction with baroreflex function in buffing changes in MAP and HR during hypercapnia may play an important role (Ainslie & Duffin, 2009).

Limitations

By design, in experiment 1, the increases in P_{ET,CO_2} were ∼5 and ∼10 Torr for each phase of the steady-state protocol. These values were smaller than those achieved by the rebreathing protocol, which resulted in gradual increases in P_{ET,CO_2}, ultimately reaching elevations of ∼16 Torr by the end of rebreathing. This difference in the magnitude of hypercapnic stimuli, however, is unlikely to affect the interpretation of the present findings because CBFV as well as CVCI responses to changes in P_{ET,CO_2} were approximately linear and CMVR is calculated as a linear regression slope between these variables (Figs 2 and 3).

Another possible limitation is that we used only two steady-state levels of hypercapnia in experiment 1, which yielded a total of three data points (including the baseline normocapnic data point) in our linear regression analysis (Fig. 2). However, when all of the data points from each subject were pooled together for linear regression analysis, the similarity of slopes of CBFV *versus* P_{ET,CO_2} and CVCI *versus* P_{ET,CO_2} between the steady-state and transient changes in P_{ET,CO_2} were confirmed (data not shown).

While experiment 1 used a traditional 5 min rebreathing period, the rebreathing period in experiment 2 was only 4 min in duration. We feel that this shorter period in experiment 2 does not cloud the results for two main reasons: (i) 4 min of rebreathing still yielded significantly large increases in P_{ET,CO_2}; and (ii) the degree of hypercapnia for both protocols in experiment 2 was similar. Thus, all statistical comparisons for experiment 2 were made relative to a similar hypercapnic stimulus. Lastly, this change was made to reduce the possibility of discomfort associated with prolonged CO_2 rebreathing. Moreover, both CBF and CVCI responses to moderate changes in CO_2 can be approximated by a linear relationship to simplify the data analysis, as shown in the present study.

Cerebral perfusion was indexed from CBFV. Although CBFV is not the same as cerebral blood flow, a linear relationship exists between changes in flow and CBFV if the diameter of the insonated vessel does not change. Of particular relevance to the present study, the diameters of large cerebral vessels, such as the middle cerebral artery, do not change significantly during moderate changes in arterial pressure and CO_2 (Schreiber *et al.* 2000; Serrador *et al.* 2000; Willie *et al.* 2012). Thus, changes in CBFV in these conditions reflect changes in blood flow (Bishop *et al.* 1986; Dahl *et al.* 1992).

Perspectives and significance

In conclusion, using a simplified rebreathing method, we found that assessment of CVMR was similar between transient and steady-state hypercapnic stimuli; however, there was some individual variability in the responses. Mean arterial pressure was elevated in both conditions, even with moderate hypercapnia, emphasizing the importance of taking these changes into account when assessing CVMR. We also observed that a brief period of hyperventilation blunts the cerebral vasodilatory responses during a subsequent period of hypercapnia. These findings indicate that the simplified rebreathing method has a potential to provide a reliable and easily applicable approach for assessment of CVMR.

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Additional information

Competing interests

None declared.

Author contributions

R.M.B. contributed to the study design, data analysis and data interpretation and drafted this manuscript. R.A.I.L., C.G.C. and R.Z. contributed to the study design, data collection, data analysis, data interpretation and editorial process of the manuscript. Y.-S.Z. contributed to the study design, data collection, data interpretation and editorial process of the manuscript. All authors approved the final version of this manuscript. All experiments of the present study were either conducted in the Cerebrovascular Laboratory at The Institute for Exercise and Environmental Medicine or in the Environmental and Autonomic Physiology Laboratory at the University of Texas at Austin.

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